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**Hoon Choi. Transfer factor calculated using dermal exposure and dislodgeable foliar residue and exposure assessment for reentry worker after pesticide application in cucumber field. (2023) Appl. Biol. Chem. 66: 1**

This study aimed to determine the transfer factor (TF) of methidathion for cucumber harvesters in greenhouses using the dermal exposure rates (DERs) and dislodgeable foliar residues (DFRs) measured simultaneously in my previous works. The DERs recalculated using the reference body surface area for the Korean adult males were 31.5–1281.1 μg/h, and the DFR values were 12.1–222.5 ng/cm2 over 7 d after application. A strong correlation between the DERs and DFRs was observed, with a regression coefficient of 0.9982. The TF for cucumber harvesters in greenhouses was determined to be 6020.4 cm2/h, which was five times higher than that proposed by the US Environmental Protection Agency (EPA). Additionally, based on TF value of methidathion, the reentry intervals (REIs) with or without personal protective equipment (PPE) were estimated for 82 pesticides registered on cucumber. The REIs with PPE, obtained from acceptable operator exposure levels and TF value, were less than 0 d, indicating the lowest risk possibility. However, REIs without PPE were estimated between 0.04 and 4.4 d for seven pesticides, including chlorothalonil, emamectin benzoate, flubendiamide, fluquinconazole, iminoctadine tris(albesilate), propineb, and pyridaben. In conclusion, cucumber harvesters should wear PPE for health safety when they reenter the greenhouse to harvest cucumbers following application of pesticides.

**Hosien Hashemi Moghaddam, Ali Ashraf Jafari, Fatemeh Sefidkon, Sepideh Kalate Jari. Influence of climatic factors on essential oil content and composition of 20 populations of*Nepeta binaludensis* Jamzad from Iran. (2023) Appl. Biol. Chem. 66: 2**

*Nepeta binaludensis* Jamzad is an endemic and rare perennial plant belonging to the Lamiaceae family, which grows in a limited area in Binaloud Mountain in northeast of Iran. In this study to evaluate the diversity of 20 populations (localities) of *N. binaludensis* and the influence of environmental factors on essential oil (EO) content and composition, the plant aerial parts were collected at the full flowering stage. The plant materials dried in shade and subjected to hydro-distillation for obtaining their EOs. Analysis of the EO was carried out using GC and GC/MS. The oil yields were varied from 1.2 to 4.9%. Classification of populations was made based on EO compounds. The results of mean comparison between populations belong to different habitats showed that the populations of Darood and Friezy with average values of 4.91 and 1.2% had the highest and lowest EO yield, respectively. Twenty-two compounds were identified in the oils with 1,8-cineol (25.4–59.0%), 4aα,7α,7aα-nepetalactone (13.8–55.1%), myrcene (2.3–5.5%) and p-cymene (1.1–5.7%) as the main components. Result of correlation analysis showed that the oil yield was positively correlated with precipitation and negatively with temperature. In addition, 1,8-cineole was positively and nepetalactone was negatively correlated with altitude. Cluster analysis by Ward method categorized the populations into two groups. The major compound of the oils in cluster 1 was nepetalactone (with an average of 37.9%), while the oils in cluster 2, contained higher percentage of 1,8-cineole (52–59%). Most of the populations in cluster 2, were originated from high, cold, rainy, and steep areas.

**Hyang-Yeol Lee, Jun-Sub Kim. Cherry fruit anthocyanins cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside protect against blue light-induced cytotoxicity in HaCaT cells. (2023) Appl. Biol. Chem. 66: 3**

Blue light derived from multiple sources, including sunlight, generates reactive oxygen species (ROS) and negatively affects the skin in a manner similar to that of ultraviolet light. Cyanidin-3-*O*-glucoside (C3OG) and cyanidin-3-*O*-rutinoside (C3OR) are anthocyanin antioxidants that have protective effects on various tissues and cell types. However, the effects of anthocyanins on blue light-mediated changes remain unconfirmed. In this study, we determined the protective effects of C3OG and C3OR isolated and purified from waste cherry fruits (*Prunus serrulata* L. var. *tomentella* Nakai) against the blue light-induced ROS formation and inflammatory responses in HaCaT cells. It is showed that the treatment of C3OG and C3OR significantly reduced the blue light-induced cytotoxicity and ROS production in a dose dependent manner. Furthermore, we found that focal adhesion kinase (FAK) is a major upstream of blue light-induced expression of inflammatory cytokines (TNF-α, IL-6 and IL-8), and these effects were attenuated by C3OG or C3OR treatment. In the initial reaction, blue lights increased the phosphorylation of inhibitory-κB Kinase α (IKKα), c-jun N-terminal kinase (JNK), and p38. The phosphorylation of these intracellular proteins was reduced via FAK inhibitor, NAC (ROS scavenger), and anthocyanin treatments. After 24 h of blue light irradiation, C3OG or C3OR treatment markedly inhibited caspase-3-mediated apoptosis and cleaved-FAK-mediated anoikis, which is cell detachment-induced apoptosis. Therefore, our results indicate that C3OG and C3OR effectively protected human keratinocytes from harmful blue light-induced cytotoxicity and inflammation.

**Jwakyung Sung, Woojin Kim, Taek-Keun Oh, Yoon-Sup So. Nitrogen (N) use efficiency and yield in rice under varying types and rates of N source: chemical fertilizer, livestock manure compost and food waste-livestock manure compost. (2023) Appl. Biol. Chem. 66: 4**

An optimal use of organic composts derived from animal and food wastes could provide an opportunity to achieve both sustainable crop production and soil quality, and a lot of research has provided the evidence. The nitrogen use efficiencies (NUEs) is a definition to evaluate the interaction between crop and nitrogen (N), and, due to this reason, widely used in agriculture. The current work tried to evaluate NUEs as an indicator of N acquisition capacity and physiological responses of rice grown under varying N levels. To do this, we employed different types and rates of nitrogen source, chemical fertilizer, livestock manure-based compost and food waste and livestock manure-containing compost. Despite of the enhanced rice growth and yield by fertilization, a difference by types and rates of fertilization was not observed. Net photosynthetic rate was significantly higher in the treatments of 90–317 N kg ha-1. The NUE (N uptake efficiency × N utilization efficiency) was the highest in lower N application groups, and sharply reduced with an increase in fertilization rates. In contrast, the nitrogen harvest index (NHI, grain N/total biomass N, kg kg-1) showed higher (0.71– 0.76 kg kg-1) in greater N application treatments (≤ 317 N kg ha-1). Accordingly, in terms of NUE, our result suggest that rice may be affordable of the application of less than 300 kg N ha-1 (combination with chemical fertilizer and organic compost). Nevertheless, it should be investigated how excess N application affects soil quality, and how long rice plant and soil can accept excess N without an environmental load.

**Minsu Park, Yujin Kweon, Dowhan Lee, Chanseok Shin. Suppression of *Phytophthora capsici* using double-stranded RNAs targeting *NLP* effector genes in *Nicotiana benthamiana***

**. (2023) Appl. Biol. Chem. 66: 5**

RNA interference (RNAi) is a gene regulatory mechanism that involves the interaction of small interfering RNAs (siRNAs) and RNA-induced silencing complex (RISC). Dicer cleaves exogenous double-stranded RNA (dsRNA) into siRNAs, which get incorporated into RISC and bind to complementary sequences on the target mRNA to induce its degradation. In this study, we adopted RNAi technology using dsRNAs to suppress *Phytophthora capsici*, which causes diseases in solanaceous crops, including pepper. We designed and synthesized dsRNAs targeting the *P*. *capsici* effector genes *PcNLP2* and *PcNLP6*, respectively. These genes encode necrosis and ethylene-inducing peptide 1-like proteins in *P*. *capsici*, which are known to promote oomycete infection. *Nicotiana benthamiana* leaves were first infiltrated with dsRNAs and inoculated with *P*. *capsici* 2 days later. We confirmed significant suppression of *P*. *capsici* and *PcNLP2*, *PcNLP6* expression in dsRNA-treated leaves. In addition, we found that downregulation of *PcNLP2* and *PcNLP6* distinctly affected the expression of some defense-related genes. These results suggest that dsRNA mediated RNAi technology can be used to suppress various pathogens, and may contribute toward crop protection.

**Dong Ho Jung, Hyun Yang, Joo Tae Hwang, Byoung-Seob Ko. Effect of traditional herbal medicine, danggui-yukhwang-tang, on post-menopausal weight gain in ovariectomized high-fat diet rats. (2023) Appl. Biol. Chem. 66: 6**

The decrease in estrogen due to menopause leads to impaired lipid metabolism and is closely related to the increase in metabolic syndrome due to weight gain. Hormone replacement therapy is effective for menopause, but with an increased risk of side effects. Danggui-yukhwang-tang (DYT) is a traditional drug, comprising seven herbs, used to treat diseases like slight fever with sweating, blood-flow disorders, and neurasthenia. However, the effect on menopausal obesity has not been reported. This study aimed to investigate the inhibitory effect of DYT on weight gain in female rats fed a high-fat diet after ovariectomy. Adipocyte differentiation was effectively reduced by DYT in 3T3-L1 cells, and the mRNAs of *PPARγ*, *C/EBPα*, and *FABP4*, which are adipogenesis-related genes, were reduced. In the in vivo study, OVX and HFD elevated body weight; however, its induction significantly decreased in the DYT-treated groups. The serum lipid profile was also examined, and DYT treatment significantly decreased LDL-cholesterol, triglyceride, and total cholesterol levels compared with the OVX and OVX + HFD groups. DYT treatment effectively reduced the temperature(s) of the tail and body in the rats. The study demonstrates that DYT inhibits adipogenic differentiation, hypercholesterolemia, and weight gain in a post-menopausal rat model by regulating adipogenic markers (PPAR*γ*, C/EBP*α*, FABP4) and the serum lipid profile in OVX + HFD rats.

**Xian-Tao Yan, Ziqi Zhang, Yubao Wang, Wenmiao Zhang, Longfei Zhang, Yang Liu, Dawei Chen, Wenqiong Wang, Wenlong Ma, Jian-Ya Qian, Ruixia Gu. Antioxidant capacity, flavor and physicochemical properties of FH06 functional beverage fermented by lactic acid bacteria: a promising method to improve antioxidant activity and flavor of plant functional beverage. (2023) Appl. Biol. Chem. 66: 7**

The ability of natural plants to treat chronic diseases is closely related to their antioxidant function. Lactic acid bacteria (LAB) fermentation is an effective way to improve the nutritional value, biological activity and flavor of food. This study investigated the pH, titratable acidity, total polysaccharide, total flavone, total saponin, total polyphenol, and antioxidant activity of the FH06 beverage before and after probiotic fermentation. Results: After fermentation, FH06 had lower contents of total polysaccharides, total flavonoids, total saponins and total polyphenols but higher titratable acidity. The antioxidant activity was tested by total antioxidant capacity (FRAP method) and DPPH· scavenging ability. The FRAP value significantly increased after fermentation (*P* < 0.05), and the maximum increase was observed for *Lactobacillus fermentum* grx08 at 25.87%. For DPPH· scavenging ability, the value of all fermentations decreased, and *L. fermentum* grx08 had the smallest reduction at 2.21% (*P* < 0.05). The results of GC–MS and sensory analysis showed that fermentation eliminated bad flavors, such as grass, cassia and bitterness, and highlighted the fruit aroma and soft sour taste. Conclusion: The FRAP value and sensory flavor of FH06 fermentation by *L. fermentum* grx08 were significantly improved, indicating its great potential as a functional food with both strong antioxidant activity and good flavor.

**Jung Eun Park, Hee Jun Kwon, Hwa Jin Lee, Hyung Seo Hwang. Anti-inflammatory effect of taxifolin in TNF-α/IL-17A/IFN-γ induced HaCaT human keratinocytes. (2023) Appl. Biol. Chem. 66: 8**

Taxifolin, a bioactive flavonoid, has been attracting attention as a beneficial and valuable phytochemical due to its antioxidant, anticancer, and anti-inflammatory properties. Recently, an improvement effect of taxifolin against psoriasis has been reported in an animal experimental model. However, its exact mechanism of action at molecular and cellular levels is not known. Thus, the purpose of this study was to verify the anti-inflammatory effect of taxifolin on psoriasis at cellular/molecular level using HaCaT human keratinocytes. First, a CCK-8 assay was performed to evaluate cytotoxicity of taxifolin. Results revealed that taxifolin was a relatively safe material, showing no cytotoxicity at concentrations up to 300 μg/mL. In TNF-α-induced HaCaT cells, taxifolin significantly inhibited mRNA expression levels of pro-inflammatory cytokines (IL-1α, IL-1-β, and IL-6) and chemokines (CXCL8 and CCL20). The ability of taxifolin to regulation expression of inflammatory cytokine genes was associated with phosphorylation of IκB/STAT3 protein. In addition, taxifolin inhibited expression levels of IL-1α/β, IL-6, CXCL8, and CCL20 by inhibiting IκB/STAT3 protein phosphorylation upon stimulation of TNF-α, IL-17A, and IFN-γ. These results show that taxifolin has the potential to be developed as a treatment for psoriasis and skin inflammation.

**Jin-Hyuk Choi, Youngmee Kim & Moonjae Cho** **Therapeutic effects of TMF and catechol in pulmonary fibrosis: in vitro and in vivo analysis. (2023) Appl. Biol. Chem. 66: 9**

Idiopathic pulmonary fibrosis is a fatal lung disorder characterized by abnormal deposition of extracellular matrix (ECM), which is secreted by activated myofibroblasts. While the origin of myofibroblasts has been discussed, epithelial-mesenchymal transition (EMT) is being noticed as one of the mechanisms of myofibroblast activation. Recent studies have shown that reactive oxygen species appear to induce not only EMT but also fibrotic progression and maintenance. Therefore, we tested chemicals that have antioxidant capacity as drugs for fibrosis. To evaluate the effects of 4′,6,7-trimethoxyisoflavone (TMF) and catechol (CAT) on EMT and fibrosis, we used an in vitro transforming growth factor (TGF)-β1 or bleomycin-induced model and an in vivo BLM-induced model. The results showed that the co-administration of TMF/CAT ameliorated pulmonary fibrosis by decreasing EMT and ECM accumulation by hindering both Smad and non-Smad TGF-β signalling cascades. Furthermore, significant increases in the number of total immune cells (especially lymphocytes) were observed in BLM-treated animals treated with TMF/CAT. Our findings suggest that co-intervention with TMF/CAT may be a potential treatment for fibrosis.

**Junjie Shen, Zhiwen Yang, Xinlin Wu, Guodong Yao, Mingxing Hou. Baicalein facilitates gastric cancer cell apoptosis by triggering endoplasmic reticulum stress via repression of the PI3K/AKT pathway. (2023) Appl. Biol. Chem. 66: 10**

Objective

Gastric cancer (GC) remains a prevailing threat to life. Baicalein exhibits anti-cancer properties. This study estimated the mechanism of baicalein in GC cell apoptosis by mediating endoplasmic reticulum stress (ERS) through the PI3K/AKT pathway.

Methods

After treatment with different concentrations of baicalein, GC cell (HGC-27 and AGS) viability was detected by MTT assay. AGS cells more sensitive to baicalein treatment were selected as study subjects. The IC50 of baicalein on AGS cells was determined. Colony formation, cell cycle, and apoptosis were detected using crystal violet staining and flow cytometry. Levels of ERS-related and BTG3/PI3K/AKT pathway-related proteins were determined by Western blot. Intracellular Ca2+ level was measured using Fluo-3 AM fluorescence working solution. GC mouse models were established by subcutaneously injecting AGS cells into the right rib and were intragastrically administrated with baicalein. Tumor volume and weight were recorded. Expression of Ki67 in tumor tissues and positive expression of apoptotic cells were detected by immunohistochemistry and TUNEL staining.

Results

Baicalein inhibited cell proliferation and induced G0/G1 arrest and apoptosis by regulating the cell cycle, and triggered ERS in GC cells. Baicalein impeded the PI3K/AKT pathway by activating BTG3, thereby triggering ERS and inducing apoptosis. BTG3 inhibition reversed baicalein-induced apoptosis and ERS. Baicalein regulated GC cells in a concentration-dependent manner. Moreover, in xenograft mice, baicalein prevented tumor growth, decreased Ki67-positive cells, activated BTG3, and inhibited the PI3K/AKT pathway, thus activating ERS and increasing apoptotic cells.

Conclusion

Baicalein facilitates GC cell apoptosis by triggering ERS via repression of the PI3K/AKT pathway.

**Vuong Vu, Young Mee Kim, Moonjae Cho. Effects of SCFAs and TMAO on non-alcoholic fatty liver disease indicating the therapeutic benefits of plant-based diet, and supplemental prebiotics, probiotics and synbiotics. (2023) Appl. Biol. Chem. 66: 11**

This review discusses the effects of short-chain fatty acids (SCFAs) and trimethylamine-N-oxide (TMAO) on metabolic diseases, focusing on non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease, and suggests dietary modification as a promising therapeutic strategy. SCFAs, a product of fiber fermentation by microbiota, foster intestinal cell populations, upregulate mucin production, and secure the gut barrier. In contrast, TMAO, a microbiota-produced metabolite from choline, phosphatidylcholine, and L-carnitine, induces atherosclerosis by decreasing cholesterol clearance. An unmanageable abundance of TMAO is potentially harmful to patients with NAFLD owing to its ability to regulate the synthesis and transport of bile acids. The production of SCFAs and TMAO is strongly dependent on the microbial community; therefore, dietary modifications, such as reduction in meat intake, and prebiotic and probiotic consumption that can shape the gut microbiome are considered as promissing therapeutic approaches. This review focuses on well-known prebiotics, such as inulin, fructooligosaccharides, and β-glucan, and probiotics, such as VSL#3 mixture, *Lactobacillus rhamnosus* GG, *Bifidobacterium*, and *Lactobacillus* spp. These additives facilitate microbiota modification, gut homeostasis, intestinal barrier maintenance, and promotion of cholesterol excretion, which may protect the liver from steatosis, inflammation, and fibrosis. Controversial results from previous studies suggest that personalized approaches should be used for dietary modifications.

**Su-Jin Kim, Dae-Seung Kim, Soo-Hyun Lee, Eun-Mi Ahn, Ji-Ye Kee, Seung-Heon Hong. Chelidonic acid ameliorates atopic dermatitis symptoms through suppression the inflammatory mediators in in vivo and in vitro. (2023) Appl. Biol. Chem. 66: 12**

Chelidonic acid (CA), a γ-pyrone compound, exerts various pharmacological functions, including anti-allergic and anti-colitis activities. However, the anti-atopic effect of CA and the mechanisms involved therein are not completely understood. The aim of the present study was to elucidate whether CA modulates atopic dermatitis (AD) in vitro and in vivo*.* We examined the pharmacological effects of CA on compound 48/80- or histamine-induced scratching behaviors and 2, 4-dinitrochlrobenzene-induced AD-like skin lesions in mice. Additionally, we evaluated the regulatory effects of CA on the expression of tumor necrosis factor -α, interleukin-6, cyclooxygenase -2 and inducible nitric oxide synthase and activation of nuclear factor-kappa B (NF-κB) in vivo and in vitro. The results showed that CA inhibited the symptoms of AD such as itching, eczema, erythema and dryness, and decreased the serum levels of IgE and histamine in mice. The inhibition rates of IgE and histamine levels by CA (2 mg/kg) were approximately 36.21 ± 4.19% and 28.93 ± 6.16%, respectively. Moreover, CA significantly attenuated the expression of inflammatory-related genes and NF-κB activation in AD-like skin lesions and mouse peritoneal macrophages. The maximal inhibition rates of NF-κB activation by CA were approximately 42.05 ± 2.12% (in AD-like skin lesions) and 37.17 ± 6.12% (in LPS-stimulated peritoneal macrophages), respectively. These results suggest that CA may be a useful therapeutic agent for skin inflammatory condition such as AD.

**Ulhas Sopanrao Kadam, Yuhan Cho, Tae Yoon Park, Jong Chan Hong. Aptamer-based CRISPR-Cas powered diagnostics of diverse biomarkers and small molecule targets. (2023) Appl. Biol. Chem. 66: 13**

CRISPR-Cas systems have been widely used in genome editing and transcriptional regulation. Recently, CRISPR-Cas effectors are adopted for biosensor construction due to its adjustable properties, such as simplicity of design, easy operation, collateral cleavage activity, and high biocompatibility. Aptamers’ excellent sensitivity, specificity, in vitro synthesis, base-pairing, labeling, modification, and programmability has made them an attractive molecular recognition element for inclusion in CRISPR-Cas systems. Here, we review current advances in aptamer-based CRISPR-Cas sensors. We briefly discuss aptamers and the knowledge of Cas effector proteins, crRNA, reporter probes, analytes, and applications of target-specific aptamers. Next, we provide fabrication strategies, molecular binding, and detection using fluorescence, electrochemical, colorimetric, nanomaterials, Rayleigh, and Raman scattering. The application of CRISPR-Cas systems in aptamer-based sensing of a wide range of biomarkers (disease and pathogens) and toxic contaminants is growing. This review provides an update and offers novel insights into developing CRISPR-Cas-based sensors using ssDNA aptamers with high efficiency and specificity for point-of-care setting diagnostics.

**Jieqiong Wang, Zegeng Li, Lili Zheng, Jiabing Tong, Chuanbo Wang. Knockdown of circ\_0006872 alleviates CSE-induced human bronchial epithelial cells injury in chronic obstructive pulmonary disease. (2023) Appl. Biol. Chem. 66: 14**

Circular RNAs (circRNAs) have been reported to be related to the initiation and progression of chronic obstructive pulmonary disease (COPD) by affecting the function of human bronchial epithelial cells (HBECs). Here, we aimed to investigate the function and mechanism of circ\_0006872 in regulating COPD process using cigarette smoke extract (CSE)-induced 16HBEC in vitro. The results showed that circ\_0006872 was increased in smokers without or with COPD, especially in smokers with COPD. Also, its expression was dose-dependently up-regulated by CSE exposure in 16HBECs. Functionally, circ\_0006872 knockdown dramatically attenuated CSE-evoked proliferation arrest, apoptosis, inflammatory response and oxidative stress in 16HBECs. Mechanistically, circ\_0006872/miR-485-3p/cyclin-dependent kinase inhibitor 1B (CDKN1B) formed a competitive endogenous RNA (ceRNA) network. CDKN1B was increased and miR-485-3p was decreased in COPD patients and CSE-induced 16HBECs. MiR-485-3p overexpression or CDKN1B knockdown protected 16HBEC against CSE-induced 16HBEC injury mentioned above. Moreover, rescue experiments showed that circ\_0006872 regulated CSE-induced 16HBEC injury via miR-485-3p/CDKN1B axis. Circ\_0006872 silencing protected against CSE-induced bronchial epithelial cell injury via miR-485-3p/CDKN1B axis, suggesting the potential application of circ\_0006872 in preventing cigarette smoke-induced COPD.

**Sung Jin Kim, Seung-Hoon Baek, Ki Sung Kang, Myoung-Sook Shin. Characterization of macrophage activation after treatment with polysaccharides from ginseng according to heat processing. (2023) Appl. Biol. Chem. 66: 15**

The worldwide persistence of infectious diseases is a significant public health issue. Consequently, studying immunomodulatory ingredients present in natural products, such as ginseng, is important for developing new treatment options. Here, we extracted three different types of polysaccharides from white (P-WG), red (P-RG), and heat-processed (P-HPG) ginseng and analyzed their chemical properties and immunostimulatory activity against RAW 264.7 murine macrophages. Carbohydrates were the main components of all three polysaccharide types, while uronic acid and protein levels were relatively low. Chemical analysis indicated that the content of carbohydrates (total sugar) increased with processing temperature, while that of uronic acid decreased. Treatment with P-WG, P-RG or P-HPG stimulated nitric oxide (NO) production and increased tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6 levels in RAW 264.7 macrophages, with P-WG showing the highest activity among the three polysaccharides. The expression of inducible NO synthase, which affects NO secretion, was highest in the macrophages treated with P-WG. Analysis of intracellular signaling pathways showed that mitogen-activated protein kinases (ERK, JNK, and p38) and NF-kB p65 were strongly phosphorylated by P-WG in macrophages but were only moderately phosphorylated by P-RG and P-HPG. Collectively, these results suggest that the polysaccharides isolated from ginseng undergo different changes in response to heat processing and display different chemical compositions and immune-enhancing activities.

**Padmanaban Mohanan, Tae-Jin Yang & Young Hun Song. Effect of far-red light on the production and diversity of ginsenosides in leaves of *Panax ginseng* Meyer. (2023) Appl. Biol. Chem. 66: 16**

Ginsenosides are the most valuable and pharmacologically active triterpenoid saponins found in *Panax ginseng*. Although light quality affects ginsenoside content, little is known about the underlying genetic and regulatory mechanisms. Additionally, the correlation between the adaptability of ginseng to shade and ginsenoside biosynthesis remains poorly understood. In the present study, transcriptome analysis of ginseng seedlings using RNA sequencing revealed that the expression of ginsenoside biosynthesis genes, including *PgHMGR*, *PgFPS*, *PgSS*, and *PgUGT*, was enhanced in shade conditions but downregulated by red light, indicating that far-red light might play an essential role in ginsenoside production. Further, gene expression analysis in adventitious roots and 2-year-old plants using qRT-PCR showed that the light quality-mediated expression patterns of ginsenoside genes varied with tissue and age. However, unlike the transcriptome, there was no difference in the total ginsenoside content in seedlings among various light conditions. Nevertheless, the amount of major protopanaxadiol-type ginsenosides increased under shade and red light conditions. Unlike seedlings and adventitious roots, there was a decrease in the expression of *PgHMGR*, *PgFPS*, *PgSS*, and *PgDDS* in 2-year-old plants, along with an increase in the ginsenoside content, under far-red light. Taken together, our findings suggest that far-red light is an important environmental factor for ginsenoside biosynthesis and diversification and provide information that can improve the quality of ginseng produced for medicinal purposes.

**Yuseong Chung, Endang Rahmat, Hyeon Hwa Nam, Ayeong Lee, Jun Hong Park, Byeong Cheol Moon, Youngmin Kang. Standardization of *Rehmannia glutinosa* (Gaertn.) DC. steam processing and evaluation of its chemical, anti-oxidant, and anti-inflammatory properties. (2023) Appl. Biol. Chem. 66: 17**

*Rehmannia glutinosa* (Gaertn.) DC., belonging to the family Scrophulariaceae, is an important medicinal herb cultivated in East Asia. Traditionally, *R. glutinosa* is steam processed to increase its efficacy in treating various ailments such as diabetes, hematinic deficiencies and adrenal disorder. However, standardization of processed *R. glutinosa* is highly needed to increase its quality to fulfill global market demand that is safe and possess high level of efficacy. Therefore, this study aimed to optimize the *R. glutinosa* steam processing methods by evaluating some key parameters such as steaming temperature, number of steaming times, steaming duration, and additive supplementation. *R. glutinosa* samples were steam processed at different temperatures (100 °C, 110 °C, and 120 °C), various steaming times (1 to 5 times), several steaming duration (1 to 4 h), and additives supplementation (rice wine, 5% EtOH, 10% EtOH, 20% EtOH, 30% EtOH, and 40% EtOH). As the result, 2 h, 3 replications, and supplementation with 20% EtOH at 120 °C were identified as the optimal conditions for *R*. *glutinosa* steam processing. Optimized processed *R. glutinosa* (SPRR 20%EtOH) resulted in significantly higher content of 5-HMF (7648.60 ± 150.08 µg/g) and iso-verbacoside (203.80 ± 10.72 µg/g) compared with unprocessed *R. glutinosa* (UPR). Compared to those of other samples, SPRR 20% EtOH samples had higher total flavonoid (55.36 ± 1.68 mg/g) and phenolic (69.24 ± 4.56 mg/g) contents and stronger DPPH antioxidant activity (56%). Furthermore, SPRR 20% EtOH had excellent anti-inflammatory activity, as evidenced by the suppression of inducible nitric oxide synthase (iNOS) caused by activation of nuclear factor-κB (NF-κB) through p-p65 pathway in LPS-stimulated RAW 264.7 cells. These findings will provide a basis towards industrialization of *R. glutinosa* processing technology that will be very helpful for oriental medication field.

**Jimin Lee, Nuri Oh, Jae-Young Yun, Hee Soon Choi, Jang-Kyun Seo, Jin-Ho Kang, Choonkyun Jung. Application of CRISPR-Based C-to-G Base editing in rice protoplasts. (2023) Appl. Biol. Chem. 66: 18**

Recently, new types of base editors, C-to-G base editors (CGBEs), that enable cytosine transversions that are unachievable with cytosine base editors (CBEs) and adenosine base editors (ABEs), have been developed in human cells. However, despite their importance in crop genome editing, the efficacy of CGBEs has not yet been extensively evaluated. In our study, based on the previously reported plant-compatible CBE and human CGBE, we demonstrated that our monocot plant-compatible CGBEs (PcCGBEs) enable cytosine transversions (C-to-G) in rice protoplasts. For all targets tested, PcCGBEs (monocot plant-compatible CGBEs) appeared to have substantial levels of C-to-G editing activity. PcCGBE showed a much higher C-to-G base editing activity and C-to-G specificity among C-to-D conversions than the mini-version of PcCGBE. Our demonstration of PcCGBE could provide a platform for the further development of enhanced CGBEs for reliable application as a new crop breeding technology.

**Ngoc Minh Ha, Hoseong Hwang, Seemi Tasnim Alam, Uyen Tran Tu Nguyen, Soon Kwang Lee, Jin-Soo Park, Jin-Chul Kim, Hak Cheol Kwon, Jaeyoung Kwon, Kyungsu Kang. Antimicrobial photodynamic therapy with *Ligularia fischeri* against methicillin-resistant *Staphylococcus aureus* infection in *Caenorhabditis elegans* model. (2023) Appl. Biol. Chem. 66: 19**

The high prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection threatens the effectiveness of current clinical settings. Antimicrobial photodynamic therapy (APDT) is a promising alternative to antibiotics for treating infections due to its low resistance. This study aimed to evaluate the antibacterial properties of APDT with *L. fischeri* extract (LFE) against MRSA and various skin and oral pathogens in vitro and its photopharmaceutical actions in *Caenorhabditis elegans*. The antimicrobial activities of APDT with LFE against pathogens were evaluated using plate counting method. The chemical profile was characterized using high-performance liquid chromatography and spectrophotometry. The growth rate assay, lifespan assay, and bacterial attachment on worms were performed to assess the therapeutics effects in *C. elegans*. The swab method was used for the detection of pathogens on the micropig skin surface. The APDT treatment with *L. fischeri* extract (LFE, 20 µg/mL) and red light (intensity of 120 W/m2) reduced 4.3–4.9 log (colony forming unit/mL) of *Staphylococcus aureus*, MRSA, *Cutibacterium acnes*, *Streptococcus mutans*; and 2.4 log (CFU/mL) of *Candida albicans*. Chemical analysis revealed that LFE enriched three active photosensitizers. APDT reduced bacterial populations on worms, recovered growth retardation, and improved lifespan in MRSA-infected *C. elegans* without causing severe side effects. The surface eradication of MRSA after exposure to LFE with red light was demonstrated on micropig skin. These findings highlight the significance of *L. fischeri* as a natural resource for the safe phototreatment of MRSA infection in the biomedical and cosmeceutical industries.

**Jin-Wook Kim, Young-Kyu Hong, Song-Hee Ryu, Oh-Kyung Kwon, Yong-Bok Lee, Sung Chul Kim. Development of analytical method for veterinary antibiotics and monitoring of residuals in agricultural environment. (2023) Appl. Biol. Chem. 66: 20**

Veterinary antibiotics (VAs) administered to livestock are introduced into the soil through livestock manure and compost. These antibiotics can run off to surface water or leach into groundwater during rainfall, causing problems such as antibiotic contamination or the occurrence of antibiotic-resistant bacteria. In this study, an analytical method for detecting four classes of VAs (penicillin, tetracyclines, macrolides, and sulfonamides) in river water and soil was developed, and the occurrence of residual antibiotics in the agricultural environment was monitored. Soil samples were extracted with the McIlvain buffer solution and pretreatment was conducted using solid-phase extraction, followed by liquid chromatography-tandem mass spectrometry to quantify target VAs. The results of this study showed that the recovery ranged from 62 to 121% in river water and 40.2–149.3% in soil. Among the other VAs, amoxicillin and spiramycin were observed to have low recoveries in all the samples. The method detection limit (MDL) was calculated in the range of 2.1–12.3 ng L−1 in river water and 1.2–13.2 ng kg−1 in soil, and the limit of quantification was 6.6–39.2 ng L−1 and 4.0–42.0 ng kg−1, respectively. This optimal method was then applied to measure the residual concentrations of VAs in river water, sediment, and soil samples around the Muhan watershed in Korea. A total of seven antibiotics were detected, and their concentrations ranged from 0.014 to 0.309 μg L−1in river water, and 1.45–9.04 μg kg−1 in sediment and arable soil. This method can be used to screen VAs in river water and soil and is expected to be used as primary data for examining the occurrence and fate of antibiotics in agricultural environments.

**Eunyoung Park, Jiho Lee, Jeong-Han Kim, Joon-Kwan Moon. X-ray crystal structure, UV–Vis and NMR spectroscopic, and molecular docking studies of pyribencarb isomers. (2023) Appl. Biol. Chem. 66: 21**

The crystal structures of the pyribencarb *E* and *Z* stereoisomers were determined using single-crystal X-ray crystallography. The isomers were confirmed a single data respectively by crystal analysis, LC-UVD mass spectrometry, and NMR spectroscopy. Pyribencarb *E* crystallizes in triclinic *P*− 1 and the *Z* isomer in monoclinic *P*21/*c,* with the crystal structures showing comparable packing motifs. Moreover, molecular docking was carried out with cytochrome *bc*1, revealing binding energies in the ranges of − 24.9 to − 17.6 and − 21.6 to − 14.7 kcal/mol for the *E* and *Z* isomers, respectively. Through a combined experimental and theoretical approach, this study contributes to our understanding of pesticides.

**Hu Shang, Yaling Guo, Liangyu Wu, Jinke Lin. Jasmine tea extract enhances human retinal pigment epithelial cells survival after UVB irradiation. (2023) Appl. Biol. Chem. 66: 22**

To examine the protecting effect of jasmine tea extract (JTE) against ultraviolet B (UVB) induced damage on human retinal pigment epithelial (RPE) cells, the RPE cells were subjected to UVB exposure and sequential JTE administration. The cell viability, intracellular reactive oxygen species (ROS), and apoptosis were determined by MTT, 2ʹ,7ʹ-dichlorodihydrofluorescein diacetate and flow cytometer assays, respectively. Further, the cells treated with UVB irradiation and sequential JTE administration were subjected to RNA-sequencing analysis in order to identify genes and pathways involved in the UVB-induced damage and JTE protecting mechanisms. The results showed that JTE effectively attenuated the UVB-induced cell injury by reducing the excessive intracellular ROS generation, and inhibiting the expression of apoptotic genes such as Bax, Caspase-3/9. This finding may offer a promising candidate for the prevention of UVB exposure related eye diseases.

**Abdul-Raouf Al-Mohammadi, Mohamed Ge Zayda, Mahmoud Ge Zayda, Adel A.-H. Abdel-Rahman, Einas Yousef, Amina Magdy. Some novel peptides containing a modified pyrazolopyrimidine moiety: design, synthesis, and in vitro antibacterial screening. (2023) Appl. Biol. Chem. 66: 23**

Numerous peptide drugs are currently undergoing advanced phases of clinical testing to determine their efficacy in combating antibiotic-resistant bacterial pathogens. Our aim was to prepare some novel peptides containing a modified pyrazolopyrimidine moiety and assess their activity against a set of selected bacteria in comparison to a widely used antibiotic, ciprofloxacin. In this study, eight new peptide compounds incorporating a modified pyrazolopyrimidine moiety were synthesized. Our results revealed that compounds **4** and **5**, which contained only the pyrazolopyrimidine scaffold were less active than the peptide-conjugated pyrazolopyrimidines **10**, **11**, **13**, **14**, **15,** and **17**. The antibacterial activities of the eight novel compounds **4**, **5**, **10**, **11**, **13**, **14**, **15**, and **17** were evaluated against a panel of bacterial strains. All the novel compounds exhibited potent antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* strains compared to the reference antibiotic ciprofloxacin. The tested *Escherichia coli* strain displayed resistance against the newly synthesized compounds. Moreover, *P. aeruginosa* strain displayed resistance against ciprofloxacin and six of the newly synthesized compounds. Compounds **15** and **17** effectively inhibited the growth of the *P. aeruginosa* strain at MIC ≥ 1 μg/mL. Our results are encouraging and urge additional biological and pharmacological screening of the most active compounds against drug-resistant microbial strains.

**Hyemee Kim, Byungyong Ahn. Filbertone, (2E)-5-methyl-2-hepten-4-one, regulates thermogenesis and lipid metabolism in skeletal muscle of a high-fat diet fed mice. (2023) Appl. Biol. Chem. 66: 24**

Filbertone, the principal flavor compound of hazelnuts, is known to have preventive effects against hypothalamic inflammation, obesity and adipocity in vitro and in vivo. However, the effect of filbertone in skeletal muscle remains unknown. In the present study, we determined the effect of filbertone in skeletal muscle of mice fed a high-fat diet (HFD). To identify the underlying molecular and cellular processes of filbertone, we performed whole transcriptome sequencing in skeletal muscle. The muscle transcriptome analysis revealed that the upregulated differentially expressed genes (DEGs) in filbertone-fed mice were substantially associated with several pathways including thermogenesis, fatty acid degradation, oxidative phosphorylation, and branched chain amino acids (BCAAs) degradation. Furthermore, the expression level of thermogenic genes such as uncoupling protein 1 (Ucp1; p < 0.05), cell death-inducing DNA fragmentation factor alpha-like effector A (Cidea; p < 0.05), peroxisome proliferator-activated receptor alpha (Ppara; p < 0.05) and lipid droplet-associated protein genes such as Plin3 (p < 0.05), Plin4 (p < 0.05), and Plin5 (p < 0.05) were significantly upregulated in muscle tissue of HFD with filbertone fed mice compared to HFD only fed mice. Filbertone also elevated the protein level of UCP1 (p < 0.05) and PPARα (p < 0.05). In addition, filbertone reduced the accumulation of intracellular lipids in C2C12 myotubes (p < 0.05). On the basis of these results, we suggest that filbertone has a crucial effect in the regulation of muscle lipid metabolism and energy balance.

**Heejin Nam, Youngkook Moon, Eunjeong Kim, Sooim Shin. Identification of 7-hydroxyindole as an alternative substrate of MauG by in silico and in vitro analysis. (2023) Appl. Biol. Chem. 66: 25**

MauG catalyzes the six-electron oxidation of pre-tryptophan tryptophylquinone (preTTQ) cofactor in methylamine dehydrogenase (MADH) to form mature tryptophan tryptophylquinone (TTQ) via long-range electron transfer. To identify alternative substrates for MauG, docking models for 10 tryptophan-like compounds were constructed using Autodock Vina. These demonstrated spontaneous binding to the preTTQ binding site of MauG, with hydroxyindoles most frequently sharing the natural substrate binding site of MauG. To confirm the result of in silico analysis, 7-hydroxyindole was reacted with *bis*-FeIV of MauG. The spectroscopic change, representing the reactivity of MauG, revealed the highly increased reaction rate (*k3*) toward 7-hydroxyindole, suggesting that *bis*-FeIV MauG extracted an electron from the 7-hydroxyindole and then oxidized to di-ferric MauG.

**Bo Li, Yeni Wang, Xiaoguang Yang. Qianhu (*Peucedanum praeruptorum* Dunn) Improves exercise capacity in mice by regulating Nrf2/HO-1 oxidative stress signaling pathway. (2023) Appl. Biol. Chem. 66: 26**

This study assessed the effect of Qianhu (*Peucedanum praeruptorum* Dunn**)** on the recovery of movement in mice with D-galactose-induced dyskinesia. The evaluation of the ability of mice to exercise revealed that Qianhu increased the running and swimming time to exhaustion in mice with dyskinesia. In addition, measurement of biochemical indices in mice showed that Qianhu altered the serum levels of blood urea nitrogen (BUN), blood lactic acid (BLA), malonaldehyde (MDA), liver glycogen (HG), muscle glycogen (MG), while the levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) remained normal. Additionally, Qianhu regulated the mRNA expression of copper/zinc-superoxide dismutase (Cu/Zn-SOD), manganese-superoxide dismutase (Mn-SOD), catalase (CAT), heme oxygenase 1(HO-1), nuclear factor erythroid2-related factor (Nrf2) and syncytin-1 in mice and also protected mice against D-galactose-induced oxidative stress. The analysis of the chemical composition of Qianhu revealed that it mainly contains isochlorogenic acid B, myricetin, baicalin, luteolin, and kaempferol, which are known excellent antioxidants that protect against tissue damage due to oxidative stress and have anti-aging properties. Thus, these compounds may be the active components in Qianhu that improve the ability of mice to exercise, and may also represent the key compounds for its use as natural medicine or health food.

**Eun Jung Choi, Hee Ho Song, Ka Young Ko, Ki-Bae Hong, Hyung Joo Suh, Yejin Ahn. Fermentation characteristics and radical scavenging capacities of ginseng berry kombucha fermented by *Saccharomyces cerevisiae* and*Gluconobacter oxydans*. (2023) Appl. Biol. Chem. 66: 27**

Kombucha is a healthy carbonated beverage made by fermenting tea extracts such as green tea and black tea through symbiotic culture of bacteria and yeast. In this study, fermentation characteristics and radical scavenging activity of ginseng berry kombucha (GBK) by *Saccharomyces cerevisiae* M-5 and *Gluconobacter oxydans* were measured. As fermentation time increased, pH decreased and titratable acidity increased. Reducing sugars decreased rapidly on day 3. Alcohol content increased dramatically during this period and then decreased. GBK showed increased radical scavenging activity and increased total flavonoid content on day 18 of fermentation compared to before fermentation. In particular, during GBK fermentation, the content of phenolic compounds such as gallic acid (2.09-fold) and chlorogenic acid (2.11-fold) increased, contributing to antioxidant activity. In addition, the major ginsenosides of GBK were identified as Rg2 (10.1 μg/mg) and Re (6.59 μg/mg), and the content of minor ginsenosides, which are easily absorbed forms, increased 2.19-fold by fermentation. GBK also extended survival in a *Drosophila* model treated with 15% hydrogen peroxide. GBK also reduced reactive oxygen species (p < 0.001) through upregulation of gene expression of antioxidant enzymes such as catalase (p < 0.001), superoxide dismutase (p < 0.05), and glutathione peroxidase (p < 0.001). Therefore, GBK can be presented as a functional food that inhibits oxidative stress by increasing radical scavenging activity during fermentation.

**Jingyu Yang, Hailong Si, Bo Dong, Qin Qin. Allicin alleviates coronary atherosclerosis of mice via endothelial nitric oxide synthase(eNOS)/nuclear factor erythroid 2-related factor(Nrf2)/heme oxygenase-1(HO-1) signaling pathway. (2023) Appl. Biol. Chem. 66: 28**

Purpose

Endothelial progenitor cells (EPCs) have been revealed to interventions in atherosclerosis (AS) progressions. Traditional Chinese medicines (TCMs) have been discovered to modulate the functions of EPCs. Herein, effects of allicin on EPCs were explored in coronary atherosclerosis (CAS).

Methods

Allicin (5 or 10 mg/kg/d) was used to treat the ApoE−/− mice fed with high-fat diet (HFD. TC, TG, LDL-C, and HDL-C were examined. HE staining was applied for observation of CAS lesions. In vitro, EPCs were induced by ox-LDL and then treated with allicin and an eNOS inhibitor, L-NAME. Thereafter, the cell viability, apoptosis and migration were examined using CCK-8, flow cytometry and Transwell methods. Western blot was applied for evaluating eNOS, Nrf2 and HO-1 protein expression. NO production, MDA content, and SOD activity were also measured.

Results

Allicin inhibited CAS progression, decreased serum levels of TC, TG, and LDL-C but increased HDL-C. Moreover, counts of circulating EPCs, and the protein levels of eNOS, Nrf2 and HO-1 were increased by allicin treatment in mice fed with HFD. Allicin suppressed MDA contents but enhanced SOD activities. In vitro, allicin reversed the impacts of ox-LDL induction in EPCs, facilitating cell mobility and NO production, and decreasing apoptosis. L-NAME treatment reversed effects of allicin.

Conclusion

Allicin alleviated CAS progressions in mice, modulating the cell apoptosis and migration of EPCs via eNOS/ Nrf2/HO-1 pathway.

**Su Young Shin, Hayeon Kim, Su Gyeong Woo, Jong Chan Hong, Young Hun Song. Analysis of protein binding characteristics among *Arabidopsis* BBX protein family. (2023) Appl. Biol. Chem. 66: 29**

Plants have evolved various mechanisms of adjusting their diurnal and seasonal growth and development in response to variations in day length and light quality. This plasticity is facilitated by intricate regulatory networks that comprise transcription factors, whose expression is modulated by the activity of photoreceptors. In *Arabidopsis*, B-box (BBX) transcription factors, which contain one or two Zn-ligating B-box motifs in their N-termini, serve as key mediators of light signaling for photomorphogenesis, shade avoidance, and photoperiodic flowering. While multiple BBX proteins may function as a single regulatory unit, the binding networks that form among members of the BBX family have not been extensively investigated. Here, we have demonstrated that the homodimerization of two B-box motifs containing CONSTANS protein (BBX1), which regulates light signaling and is the most extensively characterized among all BBX proteins, requires at least three B-box motifs. Therefore, the number of B-box motifs may significantly influence heterodimerization among BBX family members. An interactome analysis of all 32 known B-box family members revealed that the binding affinity between group III and V proteins with only one B-box motif is relatively weaker than that observed among other group members. In fact, the group V proteins BBX26 and BBX27 rarely interact with other BBX members. Taken together, the results of this study emphasize the importance of the B-box motif in network formation among BBX proteins and provide insights into investigating the various signaling pathways mediated by these networks.

**Gi Hyun Lee, Ju Soon Yoo, Ha-Ram Oh, Cheol Woo Min, Jeong Woo Jang, Soumya Mukherjee, Ki-Hong Jung, Yu-Jin Kim, Yiming Wang, Ravi Gupta, Sun Tae Kim. Transcriptome profiling uncovers the involvement of CmXyn1, a glycosyl hydrolase 11, in *Cochliobolus miyabeanus* pathogenicity. (2023) Appl. Biol. Chem. 66: 30**

Necrotrophic pathogen *Cochliobolus miyabeanus* (*C. miyabeanus*) causes rice brown leaf spot disease and drastically affects the yield and quality of rice grains. However, the molecular mechanism of rice-*C. miyabeanus* remains poorly understood due to the limited research conducted on this pathosystem. To elucidate the molecular mechanism of rice-*C. miyabeanus*, a transcriptome analysis was conducted from in vitro and in planta grown *C. miyabeanus.* This analysis led to the identification of a total of 24,060 genes of which 426 in vitro and 57 in planta expressed genes were predicted to encode for secretory proteins. As these 57 genes were specifically expressed in planta and were predicted to be secretory in nature, these were consider as putative effectors, highlighting their possible roles in the fungal pathogenicity. Notably, among these putative effectors, *CmXyn1* which encodes a glycosyl hydrolase 11 displayed the highest expression level under in planta conditions and was thus selected for further functional characterization. Interestingly, the extracellular expression of *CmXyn1* transiently induced cell death in *Nicotiana benthamiana* leaves, while intracellular expression was comparatively lesser effective. In addition, transcriptome analysis on rice leaves during *C. miyabeanus* infection and comparing it to the rice leaf transcriptome data obtained during hemibiotrophic pathogen *Magnaporthe oryzae* infection led to the discovery of 18 receptors/receptor-like kinases that were commonly expressed in response to both pathogens, indicating their key roles in rice defense response. Taken together, our findings provide new insights into rice-*C. miyabeanus* interaction as well as the unique and common defense responses of rice against hemibiotroph and necrotroph model systems.

**Yeong-Ju Park, Sol Sim, Soyeon Jung, Hyunji Seo, Yejin Lee, Jihyun Lee, Chan Lee, Hee-Jae Suh. Simultaneous quantification of ferrous gluconate and calcium gluconate in foods using liquid chromatography–tandem mass spectrometry (LC–MS/MS). (2023) Appl. Biol. Chem. 66: 31**

Ferrous gluconate and calcium gluconate are used as food acidity regulators in South Korea, Japan, the European Union (EU), and other countries. A simultaneous analytical method was developed to quantify ferrous gluconate and calcium gluconate in food using ultra-performance liquid chromatography–tandem mass spectrometry. The limits of detection and quantification of ferrous gluconate were 1.1 and 3.5 mg/kg, respectively, while those of calcium gluconate were 1.4 and 4.8 mg/kg, respectively. The recoveries of ferrous gluconate from processed olives were in the range of 97.7–109.7%, while those of calcium gluconate from beverages were in the range of 94.3–110.8%. The developed simultaneous analytical method was applied to real samples from South Korea, which found ferrous gluconate concentrations of 0.031–0.065 g/kg in processed olives and calcium gluconate concentrations of 3.8–7.8 g/kg in beverages.

**So-Young An, Hyun-Kyu An, Kyoung-Sook Kim, Young-Choon Lee, Seok-Ho Kim.** **Induction of autophagy by oleifolioside A in HCT-116 human colorectal cancer cells. (2023) Appl. Biol. Chem. 66: 32**

In current study, we addressed the anti-cancer effect of oleifolioside A and its mechanism on the regulation of cell death in HCT-116 human colorectal cancer cells. Oleifolioside A inhibited HCT-116 cell proliferation and caused apoptosis associated with sequential activation of caspases 8 and 3, followed by PARP cleavage. Moreover, anti-LC3-positive granules and the increased LC3-II level were observed in HCT-116 cells treated with oleifolioside A, which is the specific characteristics of autophagy. Treatment of autophagy inhibiors, 3-MA and Wort, markedly accelerated the cell death by oleifolioside A and, furthermore, knockdown of Beclin-1 and Atg7 using shRNA increased oleifolioside A-induced apoptosis, suggesting a cytoprotective function of autophagy against oleifolioside A-triggered apoptosis. Treatment of HCT-116 cells with oleifolioside A time-dependently activated extracellular signal-regulated kinase (ERK). Oleifolioside A-induced autophagy was dramatically inhibited by pretreatment with an ERK inhibitor, U0126, which resulted in a marked reduction in cell viability. These findings indicate that oleifolioside A induce autophagy through ERK activation in HCT-116 cells and that autophagy suppression enhances apoptosis induced by oleifolioside A.

**Aisha M. H. Al-Rajhi, Tarek M. Abdel Ghany** **In vitro repress of breast cancer by bio-product of edible *Pleurotus ostreatus* loaded with chitosan nanoparticles. (2023) Appl. Biol. Chem. 66: 33**

Despite advances in early detection and therapy, cancer still is a significant health challenge with the highest priority for investigation. Breast cancer represents the most common cancerous disease among women in the world. The study’s purpose is to estimate the cytotoxic activity of the edible mushroom *Pleurotus ostreatus* extract (PE), chitosan nanoparticles (ChNPs), and PE loaded with ChNPs (PELChNPs), as well as to identify the molecular docking of the cytotoxicity of methyl gallate (MG) as a main component of the PE against breast cancer (MCF-7) cell line. High-performance liquid chromatography (HPLC) analysis of PE exhibited the existence of various phenolic and flavonoid compounds such as MG, gallic acid, chlorogenic acid, hesperetin, naringenin, rutin, and cinnamic acid. The proliferation of the MCF-7 cell line was inhibited at 1, 3.9, and 62.50 µg/mL of PELChNPs, PE, and ChNPs, respectively. PELChNPs were more effective against the MCF-7 cell line than PE, particularly at low concentrations. For instance, at 7.8 µg/mL of PELChNPs and PE, the inhibitory % of MCF-7 proliferation was 20.59±1.75% and 8.57±0.59%, respectively. At 15.6 µg/mL of PELChNPs and PE, the inhibitory % of MCF-7 proliferation was 51.37±1.09% and 25.18±1.64%, respectively. While there is slight difference in the inhibition % of MCF-7 cells (98.64±0.21 and 97.22±0.16%) at high concentration 500 µg/mL of PELChNPs and PE, respectively. IC50 was 15.25 ± 0.54 µg/mL, 46.27 ± 1.94 µg/mL, and 337.38 ± 13.68 µg/mL against MCF-7 cell line of PELChNPs, PE, and ChNPs, respectively. The value of IC50 documented the efficacy of PELChNPs compared with the IC50 (5.91 ± 0.43 µg/mL) of Vinblastine sulfate. Noticeable distortions were observed in the MCF-7 cell line mainly treated with PELChNPs, followed by PE alone. While ChNPs exhibited less effect on the morphology of the MCF-7 cell line. Antioxidant activity of ChNPs, PE, and PELChNPs was evaluated compared with Trolox, which reflected IC50 = 118.33 ± 4.02, 85.63 ± 3.96, 36.80 ± 2.52 and 24.74 ± 0.45 µg/mL. Methyl gallate binding interactions were assessed using molecular docking with the MOE-Dock tool against the target crystal structures of Breast cancer cell line 3HB5. The results shed light on how molecular modeling techniques can inhibit methyl gallate with possible uses in treating breast cancer.

**Ji Soo Kim, Ji Hee Lim, Somi Kim Cho. Effect of antioxidant and anti-inflammatory on bioactive components of carrot (*Daucus carota* L.) leaves from Jeju Island. (2023) Appl. Biol. Chem. 66: 34**

The present study evaluated the potential of carrot (*Daucus carota* L.) leaf, a non-edible part of carrots, which are among the most consumed vegetables worldwide. The antioxidant activities of 70% ethanol extract (EEC) and hot water extract (HEC) of carrot leaves were compared. The results revealed that the total polyphenol content, total flavonoid content, and DPPH and ABTS radical scavenging activities were higher in EEC than in HEC. Both extracts protected the cells against H2O2-induced toxicity and markedly reduced the levels of reactive oxygen species in RAW 264.7 cells. Moreover, pretreatment of RAW 264.7 cells with EEC and HEC prior to H2O2 (500 μM) exposure increased superoxide dismutase and glutathione peroxidase activities in these cells. Notably, EEC and HEC increased intracellular catalase activity by 36.77 and 6.39 times, respectively. Compared to HEC, EEC remarkably inhibited the production of lipopolysaccharide-induced nitric oxide and reduced the gene expression of IL-6, IL-1β, iNOS, COX-2, and TNF-α. Comparative analysis of the composition of the extracts using HPLC–UV suggested notably higher contents of catechin, chlorogenic acid, caffeic acid, rutin, quercetin, and cynaroside in EEC than in HEC. Collectively, these results imply that carrot leaves are a potentially beneficial natural source of antioxidants and anti-inflammatory compounds in functional foods.

**Weiqing Cheng, Zhibin Pan, Hanjing Zheng, Gelian Luo, Zhibin Liu, Suli Xu, Junhan Lin.** **Characterization of phytochemical profile of rhizome of artificial cultured *Polygonatum sibiricum* with multiple rhizome buds. (2023) Appl. Biol. Chem. 66: 35**

Rhizome of *Polygonatum sibiricum* is both a renowned traditional Chinese remedy and a commonly consumed delicacy. Due to the escalating demand and excessive overexploitation, there has been a growing interest in the artificial cultivation of this plant in recent years. To assess the therapeutic benefits of artificially cultivated *P. sibiricum*, it is crucial to identify and classify its phytochemical components, which are the primary bioactive compounds found in its rhizome. In this study, the phytochemical profile of an artificially cultivated *P. sibiricum* rhizomes with multiple rhizome buds (ACM) was characterized by using untargeted UHPLC-Q-Orbitrap-MS based approach. In addition, two-wild-types *P. sibiricum* rhizomes, namely the wild-type with multiple rhizome buds (WTM) and the wild-type with single rhizome bud (WTS), were used for comparison. A total of 183 phytochemicals, including 20 alkaloids, 48 flavonoids, 33 phenolic acids, and 82 terpenoids, were tentatively identified. Generally, the phytochemical profile of ACM was comparable to that of WTM and WTS. In specific, most of the identified alkaloids and phenolic acids, and approximately half of the identified terpenoids, were not significantly different. Notably, several phytochemicals with potent therapeutic properties, such as epiberberine, laetanine, sinapic acid, curcumenol, were present in ACM. Additionally, artificial cultivation increased the abundance of geniposide and naringenin, which have been linked to cardioprotective effects. These findings provide valuable insights for the future utilization of artificially cultivated *P. sibiricum*.

**Weijuan Bai, Fenghong Deng, Xiaojiang Zhang, Yanping Han, Yue’e Xiao, Nan Wang, Xuncai Liu, Qunyan Fan & Baozhong Guo.** **The determination of epidermal growth factor in Edible bird's nest by enzyme-linked immunosorbent assay. (2023) Appl. Biol. Chem. 66: 36**

Edible bird's nest (EBN) is a traditional food which was nourishing and functional. Particularly, there is the epidermal growth factor (EGF) in EBN, which is thought to play an important role in promoting skin repair. However, the type and content of EGF in EBN were not determined yet. In this study, the type of EGF in EBN was identified as bird EGF by enzyme-linked immunosorbent assay and this method was validated to be accurate and precise. Moreover, it was found that the content of EGF in raw-unclean EBN, raw-clean EBN and stewed EBN was 3000 pg/g–4000 pg/g and there were no significant differences, which suggested that the batches, origins, forms, stewing temperatures and stewing times of EBN had no effect on the content of EGF in EBN. However, it was due to that enzyme destroyed the primary structure of EGF, the EGF content of neutral protease and trypsin hydrolysates of EBN was lower than that of flavor enzymes, alkaline protease and pepsin hydrolysates of EGF. This study was the first to determine the type and content of EGF in EBN, and provided a theoretical basis for the selection and processing of EBN and using EBN as a source of EGF.

**Athulya Krishna, Jiseong Lee, Sunil Kumar, Sachithra Thazhathuveedu Sudevan, Prerna Uniyal, Leena K. Pappachen, Hoon Kim, Bijo Mathew.** **Inhibition of monoamine oxidases by benzimidazole chalcone derivatives. (2023) Appl. Biol. Chem. 66: 37**

Ten benzimidazole chalcone derivatives were synthesized, and their monoamine oxidase (MAO) inhibitory activity was evaluated. Most compounds showed higher inhibitory activity against MAO-B than MAO-A. Compound BCH2 exhibited an IC50 value of 0.80 μM, thereby showing the most potent inhibition amongst all. In addition, BCH2 showed the highest MAO-B selectivity index (SI) with an SI value of 44.11 compared to MAO-A. Among the substituents, the halogen group showed the best MAO-B inhibition, and the *ortho*-position of the B ring showed better inhibitory activity than the *para*-site. In comparison with *ortho*-substituents, the inhibitory activity increased in the order, -Cl > -Br > -F > -H. BCH2 was found to be a competitive inhibitor of the enzyme with optimum inhibition kinetics, where Ki was found to be 0.25 ± 0.014 μM. In the reversibility experiment, BCH2 showed a recovery pattern after MAO-B inhibition, similar to that of lazabemide. Thus, BCH2 is a potent, reversible, and selective MAO-B inhibitor and has been suggested as a candidate for the treatment of neurological disorders.

**Tae Ho Lee, Sun Young Park, Ji Young Kim, Jang-Duck Choi, Guiim Moon.** **Establishment of analysis method for the quantification of residues of halquinol and its metabolites in livestock and fishery products using liquid chromatography–tandem mass spectrometry. (2023) Appl. Biol. Chem. 66: 38**

In this study, an analysis method was established for the quantification of residues of halquinol and its metabolites in livestock and fishery products using liquid chromatography–tandem mass spectrometry (LC–MS/MS). We selected beef, pork (muscle and fat), chicken, egg, milk, flat fish, eel, and shrimp as target samples for validation of the method owing to them being typical livestock and fishery products. Validation of the developed analysis method was performed using liquid chromatography–tandem mass spectrometry (LC–MS/MS) at three concentration levels (0.5, 1, and 2 × the maximum residue limits) following the Codex Alimentarius (CODEX) guidelines (CAC/GL 71–2009). For all samples, correlation coefficients (R2) exceeded 0.99, recoveries ranged between 75.59 and 119.36%, and coefficients of variation (CV) ranged between 1.39 and 28.66%, thus satisfying CODEX guidelines. In addition, inter-laboratory validation was conducted, and the resulting recoveries and CVs satisfied the CODEX guidelines; LOQ was established as 10 μg kg–1 for pig muscle and 5 μg kg–1 for the other samples. Therefore, the analysis method developed in this study can accurately and precisely screen for and quantify halquinol and its metabolites in livestock and fishery products.

**Roggers Gang, Motlalepula Matsabisa, Denis Okello, Youngmin Kang.** **Ethnomedicine and ethnopharmacology of medicinal plants used in the treatment of diabetes mellitus in Uganda. (2023) Appl. Biol. Chem. 66: 39**

Diabetes mellitus (DM) is a global health problem owing to its high prevalence and increased morbidity and mortality. The prevalence of DM and impaired glucose tolerance in Uganda is approximately 4.1% and 6.6%, respectively. Medicinal plants are commonly used for the management of DM, especially in developing countries, such as Uganda*.* According to several ethnobotanical surveys conducted in Uganda, various medicinal plants are used in DM management. Meanwhile, ethnopharmacological studies have confirmed the anti-diabetic efficacy of various plants and plant-derived formulations from Uganda. However, these information remain highly fragmented without a single repository for plants used in the management and treatment of DM in Uganda, hindering further investigations. Therefore, this study aimed to comprehensively explore plants used for DM treatment in Uganda and retrieve relevant ethnopharmacological and ethnomedicinal information that can be used for DM therapy development. English peer-reviewed articles and books were searched in scientific databases, especially PubMed, Scopus, Google Scholar, Science Direct, SciFinder, and Medline, to retrieve information on medicinal plants used for DM treatment and management in Uganda. The databases were searched to obtain published literature on the anti-diabetic activities and safety of plants among the identified plants. The family name, plant parts used, anti-diabetic activities, dosage, and mechanisms of action of plant extracts were captured. In total, 46 species belonging to 26 families are used to treat DM in Uganda. Most species belonged to the Fabaceae (20%), Asteraceae (13%), and Solanaceae (7%) families. Anti-diabetic activities of 27 (59%) species have been scientifically investigated, whereas the rest have not been evaluated. This review indicated that various medicinal plants are used in the traditional treatment and management of DM across different regions in Uganda. Scientific investigations have revealed the anti-diabetic potential and safety of several of these plants. However, there is a need to validate the anti-diabetic potential of other unstudied plants. Additionally, isolating and characterizing active principles and elucidating the anti-diabetic mechanism of these plants and performing preclinical and clinical studies in the future could aid in the formulation of an effective and safe treatment for DM.

**Jian Lee, Insun Hwang, Ye-Seul Park, Do Yup Lee.** **Occurrence and health risk assessment of antimony, arsenic, barium, cadmium, chromium, nickel, and lead in fresh fruits consumed in South Korea. (2023) Appl. Biol. Chem. 66: 40**

Although various fruits are consumed as fresh produce in South Korea, information on the concentrations of heavy metals in such fruits remains lacking despite the known toxic effects of the metals. Moreover, the health risks posed by seven potentially toxic metals (As, Ba, Cd, Cr, Ni, Pb, and Sb) ingested through fruit consumption have not been assessed using recent dietary data and occurrence data. Inductively coupled plasma-mass spectrometry was used to quantify these metals in 207 samples of fresh fruits mainly consumed in South Korea. The mean concentrations (mg kg−1 fresh weight) of the metals in all fruit samples were as follows: As < 0.0021, Ba 0.3675, Cd < 0.0022, Cr 0.0307, Ni 0.0815, Pb 0.0236, and Sb < 0.0021. Only Ba showed a significant negative correlation with Pb (ρ =  −0.5385) in the studied fruits at the 95% confidence level. The non-carcinogenic risk of the seven metals in terms of hazard quotients was Pb (0.0149) > As (0.0086) > Ni (0.0081) > Sb (0.0080) > Ba (0.0031) > Cd (0.0027) > Cr (0.0001), and the hazard index, which is the sum of the hazard quotients, was 0.0275 (less than 1). The carcinogenic risks of As and Pb were 4.62E − 07 and 5.05E − 07, respectively (below 1E − 04). The hazard index of seven metals and carcinogenic risks of As and Pb indicated that no health risks were associated with fruit consumption in the Korean population. However, the hazard quotient and carcinogenic risk of Pb in apples were the highest for children aged 1–2 years, indicating that continuous targeted risk monitoring in this age group is required.

**Kyu Jin Sa, So Jung Jang, Sookyeong Lee, Hyun Park, Jungeun Cho, Jungsook Sung, Ju Kyong Lee.** **Characterization of volatile compounds of *Perilla* crop (*Perilla frutescens* L.) in South Korea. (2023) Appl. Biol. Chem. 66: 41**

This study was performed to identify and profile the volatile compounds present in three different types of *Perilla* leaves collected from South Korea. Volatile compounds were analyzed by gas-chromatograph-mass spectrometry. In total, 41 volatile compounds were identified belonging to nine chemical classes (six alcohols, seven aldehydes, two benzodioxoles, two esters, three ethers, four ketones, five monoterpenes, one phenylpropanoid, and eleven sesquiterpenes). In cultivated type of var. *frutescens* (CF), weedy type of var. *frutescens* (WF), and weedy type of var. *crispa* (WC), a total of 34, 39, and 41 volatile compounds, respectively, were identified. The predominant compound in CF and WF was perilla ketone (PK; 87.2% and 64.5%, respectively) and in WC was perilla aldehyde (PA; 26.4%). There were 29 and 27 volatile compounds that showed significant differences of content between WC and CF or WF, respectively. In terms of chemotype based on the volatile compounds, CF and WC were PK type and PA or phenylpropanoid (PP) types, respectively. WF accessions, which were PK and PP types in chemical composition, showed intermediate characteristics in the composition of volatile compounds compared with CF and WC. The results obtained in this study identified successfully the composition and content of volatile compounds in *Perilla* crop in South Korea. These results will provide useful information for industries and research related to *Perilla* crop.

**Ji Hwan Lee, Wonsang Huh, Ji Yun Baek, Jun Yeon Park, So Hyeon Kim, Il-Ho Park, Jaesung Pyo, Chang-Seob Seo, Ki Sung Kang.** **Beneficial effects of WON-21 on the symptoms of a hangover and identification of active compounds: experimental studies on antioxidant, anti-inflammation, and alcohol-metabolizing enzymes. (2023) Appl. Biol. Chem. 66: 42**

Many hangover cure products containing natural ingredients that are also effective against alcohol-related liver damage or improve liver function have recently become available. In addition to curing liver damage, antioxidants, anti-inflammatory agents, and blood ethanol reduction aids are emerging as relief targets that reduce hangover symptoms. We investigated the ameliorating effect of WON-21 herbal medicinal products by studying the mixing ratio of oriental medicine concept with respect to antioxidant potential, anti-inflammation, and aldehyde dehydrogenase (ALDH) and alcohol dehydrogenase (ADH) enzyme activities. WON-21 and its components exerted antioxidant and anti-inflammatory effects. Rutin, taxifolin, and quercetin showed superior antioxidant effects compared to the other components. WON-12 effectively reduced iNOS and COX-2 in LPS-stimulated macrophages. Quercetin and apigenin were 2 compounds effective for the inhibition of iNOS and COX-2. WON-21 and quercetin also significantly increased the activities of ALDH and ADH enzymes in a concentration-dependent manner.

**Soobin Song, Doo-Young Kim, Seon Min Oh, So-Yeun Woo, Il-joo Kim, Mun-Ock Kim, Ji-Yoon Park, Namho Kim, Hae-Young Kim, Juhee Lee, Sang Yoon Kim, Bang Yeon Hwang, Hyung Won Ryu, Sei-Ryang Oh.** **Assessment of iridoid profiles in the growth period of aerial parts of *Pseudolysimachion rotundum* var. *subintegrum* and their antioxidant and MUC5AC inhibitory potential. (2023) Appl. Biol. Chem. 66: 43**

YPL-001 is a drug substance of *Pseudolysimachion rotundum* var. *subintegrum* and has been reported to be a potent COPD inhibitor. For the first time, this study demonstrated a correlation among the iridoid constituents, antioxidants, and MUC5AC inhibition activities in *P. rotundum* during different growth stages (5 to 11 weeks). Single-factor extraction was used to optimize the plant extraction conditions to maximize the major iridoid constituents (70% ethanol, 40 °C, 1 h); isolated metabolites 1–6 were identified using nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS). The contents of each metabolite and antioxidant/MUC5AC inhibition effects were markedly changed according to the growth stages, especially for catalposide (2, 5.97 → 10.99 mg/g, 1.8-fold) and isovanillyl catapol (5, 4.42 → 20.00 mg/g, 4.5-fold), which were the predominant substances in August. Our results indicated that YPL-001 could potentially contribute to enhancing the *P. rotundum* value in accumulated iridoids at the growth stage and the biological effect aspects to develop industrial medicinal crops.

**Jeong Yoon Kim, Ju Yeon Kim, Jae Yeon Park, Jin-Seong Kim, Min-Kyung Seo, Min-Kyoung Shin, Jin-Hyo Kim.** **Synergistic bactericidal effects of carvone and β-lactams against *Xanthomonas campestris* pv. *vesicatoria*. (2023) Appl. Biol. Chem. 66: 44**

*Xanthomonas campestris* pv. *vesicatoria* (Xcv) causes brown spots on the leaves, stems, and fruits of plants, called bacterial leaf scorch (BLS). For the control of pathogens, antibiotics have been used frequently, and they can develop the resistance. In this study, the bactericidal and synergistic effects of caraway oil and its main components against the pathogen (Xcv) were investigated. The tested caraway oil consisted of 58.4% of carvone and 31.1% of limonene. The minimum inhibitory concentration (MIC) of caraway oil and carvone was the same as 125 μg mL−1, and the minimum bactericidal concentration (MBC) was 1000 μg mL−1 for caraway oil and 500 μg mL−1 for carvone, while limonene showed no inhibition below 1000 μg ml−1. In the growth of Xcv, carvone treatment over 31.3 μg mL−1 inhibited dose-dependently, and the bactericidal effect showed after 18 h more than 250 μg mL−1; It was agreed with the release of intracellular components over 250 μg mL−1, especially. Furthermore, carvone damaged the plasmid DNA of Xcv, and it would be the reason for the bactericidal activity. The synergistic effect of carvone was found with β-lactams selectively; the fractional inhibitory concentration (FIC) indexes of carvone with ampicillin or amoxicillin were below 0.5, and the mixture of carvone (125 μg mL−1) and ampicillin (500 μg mL−1) showed the bactericidal activity as well.

**Yun-Gu Kang, Jae-Han Lee, Jun-Yeong Lee, Jun-Ho Kim, Taek-Keun Oh, Jwa-Kyung Sung.** **Effect of pyrolysis conditions on chemical properties of carbonized rice husks for efficient NH4+ adsorption. (2023) Appl. Biol. Chem. 66: 45**

Ammonium ions (NH4+) are commonly found in contaminated water and are a contributing factor to water eutrophication. Carbonized rice husk, derived from various biomass sources, possesses a porous structure, and its characteristics are influenced by the feedstock and pyrolysis conditions. Hence, this study aimed to investigate the applicability of carbonized rice husk as an absorbent for NH4+ removal. The adsorption kinetics were analyzed using the Pseudo-first-order and Pseudo-second-order models, while the adsorption characteristics were assessed using the Langmuir and Freundlich isotherms. The adsorption rate of NH4+ by carbonized rice husk increased until 240 min and then gradually approached equilibrium state. Notably, the highest NH4+ adsorption rate was observed in pH 7.1 carbonized rice husk 36.045 mg/g∙min. Moreover, the NH4+ adsorption capacity exhibited an increase with increasing concentration and quantity of the solution. The pH of the carbonized rice husk was found to influence the NH4+ adsorption process, with higher pH values corresponding to increased NH4+ adsorption rates. The NH4+ sorption rate carbonized rice husk was higher in pH 11.0 at 31.440 mg/g compared to pH 6.1 (7.642 mg/g) and pH 7.1 (10.761 mg/g). These findings highlight the impact of pyrolysis conditions on the adsorption characteristics of carbonized rice husk.

**Jae-Ryeong Sim, Jong-Yeol Lee, Sewon Kim.** **Production of omega-5 gliadin monoclonal antibodies for allergenic evaluation of WDEIA-causing wheat varieties. (2023) Appl. Biol. Chem. 66: 46**

In allergic individuals, ingestion of wheat can lead to wheat-dependent exercise-induced anaphylaxis (WDEIA). Many studies have been conducted to find WDEIA allergen–deficient wheat, including by generating omega-5 gliadin antibodies. However, the reported antibodies have not been specific enough to detect omega-5 gliadins encoded on the 1B chromosome. In this study, we generated monoclonal antibodies against the major allergens causing WDEIA, omega-5 gliadins. Using these antibodies (mono-O5B-1C10), we assessed accumulation of omega-5 gliadins in wild-type and nullisomic-tetrasomic (NT) lines of the wheat (*Triticum aestivum*) varieties Chinese Spring (CS) by one- and two-dimensional gel electrophoresis, followed by Coomassie blue staining or immunoblotting with mono-O5B-1C10. We also tested mono-O5B-1C10 for major omega-5 gliadins in various wheat germplasms. Our results thus demonstrate the specificity of mono-O5B-1C10 for major omega-5 gliadins and potentially useful for identifying of omega-5 gliadin–deficient wheat varieties that should not cause WDEIA.

**Reta Merid Yitbarek, Habtamu Admassu, Fekiya Mohammed Idris, Eskindir Getachew Fentie.** **Optimizing the extraction of essential oil from cinnamon leaf (*Cinnamomum verum*) for use as a potential preservative for minced beef. (2023) Appl. Biol. Chem. 66: 47**

Cinnamon leaf essential oil extraction using steam distillation method is a time-consuming and energy-intensive process. Furthermore, a lower yield and a higher rate of product degradation are this method’s main drawbacks. Thus, the goal of this research is to optimize the extraction process parameters of cinnamon leaf essential oil in response to maximizing the yield while retaining quality by using response surface methodology (RSM). The application of extracted essential oil on minced beef to assess its preservative effect was also the other objective of this research. Extraction time (120–210 min), extraction temperature (105–115 ℃), and feed mass (300–600 g) were the chosen independent variables of the optimization experiment using central composite design (CCD). Furthermore, the extracted essential oil’s antibacterial and microbiological preservative activity on minced beef was evaluated. At extraction time of 175.43 min, extraction temperature of 105 °C, and a feed mass of 600 g, the optimum predicted value of cinnamon leaf essential oil yield and cinnamaldehyde concentration (% area) was 2.9% and 34.6%, respectively. Moreover, the second-order polynomial equation fits the experimental data for 20-run experimental data. The chemical composition of cinnamon leaf essential oil extracted at optimal conditions was dominated by eugenol (60.68%) and cinnamaldehyde (33.94%). Additionally, the optimally extracted cinnamon essential oil inhibited the growth of bacteria, particularly gram-positive bacteria. After twenty-one days of storage at 4 °C, total viable count of minced beef seasoned with cinnamon essential oil at concentration of 1.2% (v/v) was lower than 106 CFU/g. To conclude, optimized cinnamon leaf essential oil extraction process provides better yield while retaining its functional properties.

**Mai K. Ammar, Rasha S. Hanafi, Mouchira A. Choucry, Heba Handoussa** **Structural, functional, nutritional composition and analytical profiling of *Triticum aestivum*L.. (2023) Appl. Biol. Chem. 66: 48**

Wheat is considered as the most important cereal grain globally. It has a vast economic importance as it is used in producing bread, pastries, and household flour and serving as food for livestock among other uses. Different biological activities of wheat were correlated with the presence of polyphenols due to their antioxidant activities and other preventative capabilities. Wheat can also be used as an antidiabetic, anti-inflammatory, anticancer, antimicrobial, and antiaging agent. Omics has established itself during the past 20 years as a crucial tool for comprehending the internal systems of various plant systems including wheat using LC–MS, GC–MS, and UV spectrophotometry as analytical techniques. The current review represents in depth search regarding wheat cultivation, botanical description, economic significance, quantitative phytochemical characterization, and biological importance. Additionally, a critical assessment of the cited omics research on wheat was conducted with an emphasis on the analytical instrument, methods of analysis and results interpretation.

**Shuang Liang, Lijing Zhang, Shanshan Liang. Choucry,** **Targeted delivery of isoliquiritigenin by ultrasonic microbubbles attenuate myocardial injury via suppressing inflammation and oxidative stress and activating AMPK/SIRT1/eNOS signaling pathway in rats. (2023) Appl. Biol. Chem. 66: 49**

To investigate the protective efficacy of ultrasound targeted microbubble destruction (UTMD) combined with Isoliquiritigenin on myocardial injury in rats. The GK rat model of cardiomyopathy was successfully established by the induction of adriamycin. Then these rats with cardiomyopathy were randomly assigned into the model group, isoliquiritigenin microbubbles and ultrasound alone or combination group, using healthy ones as normal control. After 8-week consecutive treatment, the relevance indexes of diabetes, echocardiography as well as the hyperlipidemia, oxidative stress of model animals were examined. In addition, the fibrosis, morphological changes and inflammation response of myocardial tissues were also assessed. After further 4-week intervention, the blood biochemical indexes and the cardiac functions of model rats received the combined treatment were improved (all *P* < 0.05) compare to those received either monotherapy or saline. After chronic treatment, the heart/body weight ratio and serum cardiac index levels in model rats received combined treatment were significantly changed (all *P* < 0.05) compared with others. Furthermore, combination therapy could ameliorate excessive oxidation stress and inflammation response as well as up-regulate the expression levels of AMPK/SIRT1/eNOS signaling pathway. Targeted delivery of isoliquiritigenin by ultrasonic microbubbles can ameliorate the myocardial injury via activating AMPK/SIRT1/eNOS signaling pathways.

**Byeong Jun Jeon, Ji Eun Kang, Jeong Do Kim, Beom Seok Kim. Pentaene macrolides AB023a and takanawaene C produced by *Streptomyces xanthocidicus* strain S3 for controlling pepper anthracnose. (2023) Appl. Biol. Chem. 66: 50**

A natural product library consisting of the culture extracts of 814 actinomycete strains was screened for antifungal compounds that disrupt the cell integrity of plant pathogenic fungi using an adenylate kinase (AK) assay system. The culture extract of *Streptomyces xanthocidicus* strain S3 exhibited high AK activity against various plant pathogens. The active ingredients, AT-1 and AT-2, were isolated from the culture extract using a series of chromatographic procedures. Based on MS, UV, and NMR spectrometric analyses, the structures of AT-1 and AT-2 were determined as the pentaene macrolides, AB023a and takanawaene C. AB023a and takanawaene C displayed broad-spectrum antifungal activity against *Aspergillus oryzae*, *Botrytis cinerea*, *Colletotrichum coccodes*, *C*. *gloeosporioides*, *C*. *orbiculare*, *Cylindrocarpon destructans*, and *Fusarium oxysporum* f. sp. *lycopersici*, showing minimum inhibitory concentrations of 1–32 μg/mL. Treatment of AB023a and takanawaene C successfully inhibited anthracnose development on pepper plants in a concentration-dependent manner without phytotoxicity. The disease control efficacy of both compounds was comparable to that of the commercial fungicide chlorothalonil. Collectively, these results suggest that the polyene macrolides produced by *S. xanthocidicus* strain S3 can be used as natural fungicides for plant disease control.

**Jasung Koo, Gyujin Lee, Donghyun Ka, Changkon Park, Jeong-Yong Suh, Euiyoung Bae. Biochemical characterization of type I-E anti-CRISPR proteins, AcrIE2 and AcrIE4. (2023) Appl. Biol. Chem. 66: 51**

In bacteria and archaea, CRISPRs and Cas proteins constitute an adaptive immune system against invading foreign genetic materials, such as bacteriophages and plasmids. To counteract CRISPR-mediated immunity, bacteriophages encode anti-CRISPR (Acr) proteins that neutralize the host CRISPR–Cas systems. Several Acr proteins that act against type I-E CRISPR–Cas systems have been identified. Here, we describe the biochemical characterization of two type I-E Acr proteins, AcrIE2 and AcrIE4. We determined the crystal structure of AcrIE2 using single-wavelength anomalous diffraction and performed a structural comparison with the previously reported AcrIE2 structures solved by different techniques. Binding assays with type I-E Cas proteins were carried out for the target identification of AcrIE2. We also analyzed the interaction between AcrIE4 and its target Cas component using biochemical methods. Our findings corroborate and expand the knowledge on type I-E Acr proteins, illuminating diverse molecular mechanisms of inhibiting CRISPR-mediated prokaryotic anti-phage defense.

**Ji Hwan Lee, Shihui Jin, Myong Jin Lee, Nguyen Khoi Song Tran, Young-Joo Kim, Sanghyun Lee, Song-Yi Kim, Ki Sung Kang. Mechanism-based biomarkers for the quality control of Dangkwisoo-san: a scoping review. (2023) Appl. Biol. Chem. 66: 52**

Dangkwisoo-san (DS) is a traditional Korean herbal medicine used to treat traumatic diseases, including pulmonary contusions, traumatic pneumothorax, bruising, and ankle sprain. Quality control (QC) biomarkers for DS can help ensure its safety and efficacy. Although chemical quality assessments are performed to ensure consistent efficacy of DS, the identity and quantity of the compounds contained within a given natural product is a frequent complication. We conducted a literature review to identify biological assays that support the chemical QC of DS. The results of our investigation confirmed that in vitro experiments with aqueous and alcoholic extracts of DS exhibited positive effects on many aspects of treatment. With 80% EtOH extraction, a low concentration of DS (1 μg/ml) significantly diminished the expression of inflammatory factors, such as nitric oxide (NO), TNF-α, IL-1β, and IL-6, in the Raw264.7 cell line. MeOH extracts activated NRF2 and antioxidant activities in response to the inflammatory inducer LPS, and water extracts of DS remarkably reduced proinflammatory cytokine levels compared to dexamethasone and cyclosporin treatments. Aqueous extracts of DS at a moderate dose of 125 μg/ml supported bone regeneration, recovered ischemic injury in an eNOS-dependent manner, and prevented metabolic disorders (TRPM7 channel inhibition). Cytokines, NO, and immunoglobulins are potential biological QC biomarkers to assess the anti-inflammation and immune response to DS. Future quality evaluation studies of herbal medicines (herbal prescriptions) should aim to select the mechanism-based in vitro efficacy evaluation methods that can estimate consistent clinical effects.

**Yoo Kyong Han, Le Ba Vinh, Mi-hyun Nam, Ki Yong Lee. Identification of compounds using HPLC-QTOF-MS online antioxidant activity mapping from aerial parts of *Ligularia stenocephala*. (2023) Appl. Biol. Chem. 66: 53**

Inflammation, diabetes, and even malignancies are pharmacological effects connected by antioxidant capacity and free radicals. Many antioxidants scavenge free radicals originating from dietary sources such as fruits, vegetables, and teas. To identify the bioactive components of *Ligularia stenocephala*, an effective method combining HPLC-QTOF-MS and bioactivity evaluation was investigated for the first time. Antioxidant agents were isolated from *L. stenocephala*, a folk medicine used for edema and scrofula in Korea, Japan, and China. The phytochemical investigation of the aerial parts of *L. stenocephala* resulted in the separation and determination of six compounds (**1**–**6**). In particular, the chemical structures were identified as hyperoside (**1**), 3,5-dicaffeoylquinic acid (**2**), 3,5-dicaffeoylquinic acid methyl ester (**3**), trifolin (**4**), rutin (**5**), and 3,4-dicaffeoylquinic acid (**6**). Their structures were identified using 1D and 2D NMR spectroscopy and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) data analysis. The results showed that phenolic components were responsible for the antioxidant inhibitory activity of *L. stenocephala*. Additionally, to understand the mechanisms of the antioxidant inhibitory activity of *L. stenocephala*, a docking simulation study was performed to support the in vitro results. Taken together, this new method is rapid, inexpensive, and can be applied to identify the active components of medicinal herbs without separation.

**Sang Heon Lee, Youngse Oh, Sim-Kyu Bong, Jin Woo Lee, No-June Park, Young-Joo Kim, Hyun Bong Park, Yong Kee Kim, Seung Hyun Kim, Su-Nam Kim. Paedoksan ameliorates allergic disease through inhibition of the phosphorylation of STAT6 in DNCB-induced atopic dermatitis like mice. (2023) Appl. Biol. Chem. 66: 58**

Various allergic diseases such as atopic dermatitis (AD), allergic rhinitis, and asthma are considered incurable conditions that have yet to be fully conquered. Paedoksan (PDS), an herbal preparation consisting of 14 medicines, displays effective anti-inflammatory and anti-allergic properties, yet its underlying molecular mechanism is unknown. This study aims to uncover PDS’s mechanism for treating allergic diseases and suggest its therapeutic potential. Through a network pharmacological prediction, its impact on signal transducer and activator of transcription 6 (STAT6) regulation, a sub-mechanism of interleukin 4 (IL-4), a major inflammatory cytokine involved in degranulation and allergy, was investigated in RBL-2H3 cells and an atopic mouse model. PDS inhibits immunoglobulin E (IgE)-induced degranulation and STAT6 phosphorylation evoked by IL-4 in granulocytes. The downregulation of phospho-STAT6 and thymic stromal lymphopoietin (TSLP) by PDS was confirmed in 2,4-dinitrochlorobenzene (DNCB)-induced mouse skin. The results demonstrate that PDS exhibited remarkable effects on degranulation and STAT6 phosphorylation in RBL-2H3 cells, as well as in an atopic mouse model. Furthermore, the main active components from PDS based on chromatographic analysis showed good accordance with PDS’s effects on RBL-2H3 cells. In summary, these findings collectively suggest that PDS holds the potential to effectively suppress inflammatory and allergic reactions by obstructing the target IL-4 protein and its downstream effects, as elucidated through a network pharmacological analysis.

**Do Manh Cuong, Dae Kyeong Kim, Meran Keshawa Ediriweera, Jong-Eun Park, Jeong Yong Moon, Somi Kim Cho. Effects of the drying method and extraction solvent on antioxidant and anti-inflammatory activity of *Melosira nummuloides* bioproducts. (2023) Appl. Biol. Chem. 66: 59**

*Melosira nummuloides* is a marine diatom with potential use as food, fuel, and a dietary supplement. However, the efficacy of its extraction and drying techniques have not been explored. Here, *M. nummuloides* powders were prepared by two drying methods—hot-air drying (HAD) and freeze-drying (FD)—and extracted with hot water, ethanol, methanol, and chloroform:methanol (CM) at a ratio of 2:1 v/v. The antioxidant and anti-inflammatory activity of each extract was investigated. The CM extract had the greatest 2,2-diphenyl-1-picrylhydrazyl and 2,2ʹ-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity among the solvent extracts, and a slight difference in antioxidant activity was observed across the various drying methods. Compared to other extracts, both the FD-CM and HAD-CM extracts showed stronger anti-inflammatory effects by inhibiting nitric oxide production in lipopolysaccharide-stimulated RAW 264.7 cells. Furthermore, the FD-CM extract contained a wide range of lipophilic compounds. Notably, myristic acid (29.08 ± 0.45 mg/g dry weight powder extract (DW)), oleic acid (25.20 ± 0.92 mg/g DW), palmitoleic acid (10.77 ± 0.41 mg/g DW), eicosapentaenoic acid (12.53 ± 1.00 mg/g DW), neophytadiene (8.42 ± 0.51 mg/g DW), and α-linolenic acid (1.27 ± 0.005 mg/g DW) were among the prominent compounds identified. It is plausible to suggest that the abundance of these lipophilic compounds contributes to the remarkable antioxidative and anti-inflammatory potential exhibited by the FD-CM extract. Our results provide insights into the preferential drying methods and extraction solvents for producing *M. nummuloides*-based products with enhanced antioxidant and anti-inflammatory activity.

**Yeonju Seo, Jongbeom Chae, Ju-Ock Nam. Extract of the bioconverted fine root of ginseng induces apoptosis and cell cycle arrest in mouse colon cancer cells. (2023) Appl. Biol. Chem. 66: 60**

Cancer is the major cause of death worldwide, and the anticancer effect of ginseng and its main root has been studied. However, study of fine root of ginseng (FRG) is still insufficient. The purpose of this study was to discover a new anticancer effect from FRG, which does not show an anticancer effect, through a bioconversion technique. We measured and compared cell viability in FRG- and bioconverted fine root of ginseng (BFRG)-stimulated CT26 cells to investigate differences caused by bioconversion. Cell viability of CT26 was suppressed upon treatment with BFRG, unlike FRG. The effect of BFRG on apoptosis and cell cycle arrest was investigated by flow cytometry. BFRG-stimulated CT26 cells showed an increased apoptotic cells and cell cycle arrest. Additionally, BFRG induced mitochondrial impairment by reducing the expression of anti-apoptosis protein Bcl-2. When confirming the signaling pathway, it was found that the p38 MAPK pathway was activated by BFRG. Collectively, our results reveal anticancer effects against colorectal cancer and represent potential targets for anticancer drug development.

**Jungwon Choi, Hak-Dong Lee, Hyejin Cho, Chang-Dae Lee, Gia Han Tran, Hoon Kim, Sung-Kwon Moon, Sanghyun Lee. Antioxidative phenolic compounds from the aerial parts of *Cyperus exaltatus* var. *iwasakii* and their HPLC analysis. (2023) Appl. Biol. Chem. 66: 61**

The constituents and antioxidant activities of *Cyperus exaltatus* var. *iwasakii* (CE) have not been studied to date. In this study, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6 sulfonic acid) (ABTS) assays were used to evaluate the radical-scavenging activities of the ethanol extract, four fractions, and isolated compounds of CE. In addition, phenolic acids and flavonoids were isolated from the ethanol extract of CE using column chromatography. The compounds identified by spectroscopy were gallic acid, protocatechuic acid, vanillic acid, *p*-coumaric acid, rutin, ferulic acid, isoquercitrin, astragalin, quercetin, luteolin, apigenin, tricin, and kaempferol. Quantitative analysis using high-performance liquid chromatography (HPLC) revealed that the major flavonoids of CE were astragalin and tricin and that the major phenolic acid was *p*-coumaric acid. In addition, comparative analysis of CE from Ganghwa and Hampyeong habitats using HPLC showed that the Hampyeong CE had a higher phytochemical content. Comparative analyses of the isolated compounds were also conducted among five *Cyperus* species. The highest antioxidant activities were found in the ethyl acetate (EtOAc) fraction, and among the compounds isolated from CE, vanillic acid and quercetin showed remarkable antioxidant activity even when compared with ascorbic acid. The results demonstrate the usefulness of CE, which has not been sufficiently studied previously, and will facilitate the evaluation of its potential effectiveness as antioxidant functional plant material.

**Jeong Yeon Kim, Su Kyeong Sin, Jin Hee Park. Electrical signal of pepper during cropping period affected by different amount of fertilizer. (2023) Appl. Biol. Chem. 66: 62**

Precision agriculture requires supply of adequate amount of fertilizer application to increase crop yield and prevent environmental contamination. Objective of the study was to evaluate response of pepper under different fertigation method and amount using plant induced electrical signal (PIES) for precision agriculture. Pepper was fertigated 10 times with recommended additional nitrogen fertilizer and set as a control. Low fertilizer treatment did not receive additional urea and high fertilizer received three times higher amount of nitrogen fertilizer. Conventional treatment was fertigated as basal fertilizer and once with additional fertilizer. The PIES decreased during vegetative stage and remained constant at reproductive stage because of reduced nutrient and water uptake. The PIES showed positive relationship with soil NH4+, NO3−, stem NO3− and leaf N, which resulted in highest PIES value during reproductive stage in high fertilizer treated pepper. Plant growth parameters were also related with the PIES although yield was not affected by different fertilizer treatment.

**Seon Hui Kim, So Yun Park, Ga Eun Kim, Eun Hea Jho. Effect of pH and temperature on the biodegradation of oxytetracycline, streptomycin, and validamycin A in soil. (2023) Appl. Biol. Chem. 66: 63**

Residual antibiotics in agricultural soils can be of concern due to the development of antibiotic resistant microorganisms. Among various antibiotics, oxytetracycline (OTC), streptomycin (ST), and validamycin A (VA) have been used for agricultural purposes in South Korea; however, studies on the biodegradation of these antibiotics in soil are limited. Therefore, this study investigated the effects of pH (5.5, 6.8, and 7.4) and temperature (1.8, 23.0, and 31.2 °C) conditions on the biodegradation of these antibiotics in soil. The biodegradation tests were carried out in the field soil (FS) and rice paddy soil (RS) for 30 d with OTC and ST and 10 d with VA, and the residual antibiotics concentrations were monitored over the degradation period. Under various conditions, the degradation rates of ST was lower (11–69%) than that of OTC (60–90%) and VA (15–96%). The degradation half-lives of OTC and VA tend to decrease with increasing pH value, while the degradation half-life of ST tend to increase with increasing pH value. But, the effect of soil pH on the antibiotics degradation was not statistically significant, except for ST in the FS and RS and VA in the FS. The degradation of three antibiotics was greater at higher temperatures (23.0 °C and 31.2 °C) than at lower temperature (1.8 °C), and the degradation half-lives decreased with increasing temperature. The different degradation characteristics of different antibiotics in soil can be explained by the different characteristics of the antibiotics (e.g., sorption affinity, chemical forms) and soil (e.g., organic matter content). The results suggest that the degradation characteristics of antibiotics need to be considered in order to properly manage the residual antibiotics in soil.

**Minilu Dejene, Hemalatha Palanivel, Heeravathi Senthamarai, Venkatramanan Varadharajan, S. Venkatesa Prabhu, Alazar Yeshitila, Solomon Benor, Shipra Shah. Optimisation of culture conditions for gesho (*Rhamnus prinoides.*L) callus differentiation using Artificial Neural Network-Genetic Algorithm (ANN-GA) Techniques. (2023) Appl. Biol. Chem. 66: 64**

Gesho (*Rhamnus prinoides*) is a medicinal plant with antioxidant and anti-inflammatory activities commonly used in the ethnomedicinal systems of Africa. Using a three-layer neural network, four culture conditions viz., concentration of agar, duration of light exposure, temperature of culture, and relative humidity were used to calculate the callus differentiation rate of gesho. With the ability to quickly identify optimal solutions using high-speed computers, synthetic neural networks have emerged as a rapid, reliable, and accurate fitting technique. They also have the self-directed learning capability that is essential for accurate prediction. The network's final architecture for four selected variables and its performance has been confirmed with high correlation coefficient (R2, 0.9984) between the predicted and actual outputs and the root-mean-square error of 0.0249, were developed after ten-fold cross validation as the training function. In vitro research had been conducted using the genetic algorithm’s suggestions for the optimal culture conditions. The outcomes demonstrated that the actual gesho differentiation rate was 93.87%, which was just 1.86% lesser than the genetic algorithm's predicted value. The projected induced differentiation rate was 87.62%, the actual value was 84.79%, and the predicted value was 2.83% higher than Response Surface Methods optimisation. The environment for the growth of plant tissue can be accurately and efficiently optimised using a genetic algorithm and an artificial neural network. Further biological investigations will presumably utilise this technology.

**HanGyeol Lee, Ji-Yeong Yang, Shin-Hye Kim, So-An Lim, Jae Kwang Kim, Chon-Sik Kang, Kyeong-Hoon Kim, Sik-Won Choi, Woo Duck Seo. Wheat seedling extract and its constituents attenuate RANKL-induced differentiation and fusion of osteoclasts and bone resorption. (2023) Appl. Biol. Chem. 66: 65**

The occurrence of osteoporosis gradually increases within the aging population. As the side effects of therapeutic agents currently used for osteoporosis are increasing, the development of preventive and therapeutic agents derived from natural products without any long-term side effects is important. Here, we investigated the effect of wheat seedling extract (WSE) on the RANKL-mediated differentiation, fusion, and function of osteoclasts. WSE inhibited the differentiation of RANKL-induced bone marrow macrophages and phosphorylation of AKT and ERK. Moreover, the protein and mRNA expression levels of c-Fos and NFATc1 as well as RANKL-induced transcription of *TRAP* and *OSCAR* were suppressed by WSE treatment. DC-STAMP and cathepsin K, which are essential for cell fusion and bone degradation, were also inhibited by WSE. Furthermore, eight components constituting WSE were confirmed to decrease the osteoclast TRAP activity. Taken together, WSE may have potential implications as a useful therapeutic or preventive agent for inhibition of bone loss.

**Arshida Thottile Peedikayil, Jiseong Lee, Mohamed A. Abdelgawad, Mohammed M. Ghoneim, Mohamed E. Shaker, Samy Selim, Sunil Kumar, Sanal Dev, Hoon Kim, Bijo Mathew. Inhibitions of monoamine oxidases by ferulic acid hydrazide derivatives: synthesis, biochemistry, and computational evaluation. (2023) Appl. Biol. Chem. 66: 66**

Monoamine oxidases (MAOs) regulate neurotransmitters, and changes in their regulation lead to neurogenerative diseases (NDs). Therefore, MAO inhibitors are used to treat NDs. Ferulic acid, a phenolic compound found in various plant species, has been demonstrated to have a variety of biological functions, including anti-inflammatory, anticancer, and neuroprotective effects. In this study, ten ferulic acid hydrazide derivatives (**FA1**–**FA10**) were synthesized, and their ability to inhibit monoamine oxidase (MAO) enzymes was tested. Six candidates demonstrated a more pronounced pattern of inhibitory action against MAO-B than against MAO-A. **FA3** had the highest inhibitory efficacy in MAO-B inhibition (IC50 value of 1.88 μM), followed by **FA9** (2.08 μM). **FA3** has a Ki of 1.92 ± 0.73 μM. A reversibility experiment of MAO-B inhibition by **FA3** was conducted using dialysis, and the recovery pattern showed **FA3** was a reversible MAO-B inhibitor with a similar recovery to safinamide, a reversible reference inhibitor. These results indicate that **FA3** is an effective reversible MAO-B inhibitor. In molecular dynamics and docking, **FA3** paired with pi-pi stacking helped stabilize the protein ligand in the active site of MAO-B. According to this study, lead compounds can be used as therapeutic agents to treat neurological conditions, such as Parkinson's disease (PD).

**Heeeun Kim, Inseoung Hwang, Sungbock Ryu, Keedon Han, Yonghoon Kwon. Aryl sulfoxide scaffold useful as herbicide. (2023) Appl. Biol. Chem. 66: 67**

The escalating demand for effective and sustainable weed management strategies, driven by urbanization expansion, is a critical challenge. Herbicides are pivotal tools in modern agriculture, addressing this challenge. Developing novel herbicides with enhanced efficacy and minimal environmental impact is crucial for food security and ecological balance. While numerous herbicides have been developed with varying availability over time and regions, there's a continuous need for innovation. In this study, we explored relatively understudied sulfoxide-containing herbicides and synthesized a smaller yet substantial sulfoxide scaffold for herbicide development. Through screening *Digitaria ciliaris* (Retz.) Koeler, *Amaranthus lividus* L*.*, and *Solanum nigrum* L*.*, we observed promising herbicidal efficacy, especially against Wild Amaranth. Encouraged by preliminary findings, we recognize the potential for refining the core structure. In summary, we fashioned a structurally simple sulfoxide scaffold showcasing discernible herbicidal impact on broadleaf weeds.

**Ji Hwan Lee, Dongyeop Jang, Myong Jin Lee, Myoung-Sook Shin, Chang-Eop Kim, Jun Yeon Park, Ki Sung Kang. Regulation of appetite-related neuropeptides by herbal medicines: research using microarray and network pharmacology. (2023) Appl. Biol. Chem. 66: 68**

Anorexia means loss of appetite and is a state whereby a desire to eat is either reduced or eliminated resulting in reducing or stopping food intake. Sipjeondaebo-tang (SDT) and Hyangsayukgunja-tang (HYT) are prescriptions known to have appetite-improving effects, but studies on their mechanisms and active components are insufficient. The hypothalamus is the center of appetite control, and various appetite control mechanisms are known. We used mouse hypothalamic neuronal GT1-7 cells as appetite control center cells and analyzed the difference in efficacy between SDT and HYT using microarray and network pharmacology. Microarray analysis showed that SDT and HYT affect the regulation of genes related to appetite control in the digestive tract and central nervous system. Using network pharmacology, we analyzed the differential expression of neuropeptide Y receptors, glucagon, corticotropin-releasing hormone receptors 1, and 5-hydroxytryptamine receptor 4 among the 17 anorexia-related genes selected from the comparative toxicogenomics database and also analyzed the active components that affect gene expression. In conclusion, the appetite-related genes contributed to anorexia control, and the difference in the action mechanism of the two complex prescriptions could be explained.

**Minsu Park, Yujin Kweon, Jihyun Eom, Minsun Oh, Chanseok Shin. Development of multi-target dsRNAs targeting *PcNLP* gene family to suppress *Phytophthora capsici* infection in *Nicotiana benthamiana*. (2023) Appl. Biol. Chem. 66: 69**

*Phytophthora capsici*, which causes diseases in solanaceous crops, secretes necrosis and ethylene-inducing peptide 1-like proteins (NLPs) that induce plant defense responses and leaf necrosis. In this study, we used RNA interference (RNAi) technique, a proven strategy for crop protection and gene regulation in plants, to suppress *P*. *capsici* infection through the inhibition of *PcNLPs*. In the RNAi mechanism, Dicer processes double-stranded RNA (dsRNA) into smaller entities known as small interfering RNAs (siRNAs). These siRNAs subsequently integrate into the RNA-induced silencing complex to form sequence-specific base pairing with complementary regions of the target mRNA. This interaction effectively initiates the degradation process of the target mRNA. We designed and synthesized dsRNAs targeting the “AIMY” and “GHRHDWE” conserved motifs of *PcNLP* gene family, which are predicted to be key elements for the expression of NLPs and pathogen infection. After infiltration of dsRNAs targeting the motifs and inoculation with *P*. *capsici*, we confirmed a significant suppression of *P*. *capsici* infection and downregulation of the *PcNLP* gene family. These findings imply that the dsRNA-mediated RNAi technique holds potential for mitigating a wide range of pathogens, while simultaneously suppressing the expression of a particular gene family using dsRNA targeting functional conserved motifs in the gene family.

**Heesung Moon, Junhwa Kwon, Jeongwoo Choi, Dongjin Lee, Dong Cheol Seo. Challenging treatment of food wastes for cleaner production after the African swine fever outbreak in South Korea. (2023) Appl. Biol. Chem. 66: 70**

Food waste is a growing global concern, necessitating effective treatment solutions. South Korea stands out with over 90% of its food waste being recycled, driven by robust resource circulation policies. Across Asia, anaerobic digestion processes are favored for food waste treatment due to their economic and environmental advantages. The South Korean Government aims to expand anaerobic digestion to ensure stable organic waste treatment. However, the 2019 outbreak of African swine fever (ASF) in pig farms led to the cessation of wet feed production, comprising 22% of total feed. This has increased the pressure on alternative recycling methods. The handling of food waste leachate, generating around 1080 t/d during treatment, has become a concern due to the discontinuation of wet feed production. The objective of this study is to develop a food waste policy. It begins by assessing food waste and leachate generation through field surveys of 346 treatment facilities engaged in pretreatment, feeding, composting, and biogasification. To mitigate the impact of ASF outbreaks in the short term, a proposed solution involves diverting food waste leachate to existing sewage treatment plant digesters during non-injection weekends and other off-peak times. This measure aims to completely treat the maximum discharge of approximately 2000 t/d during the peak summer ASF outbreak periods. For the long term, a strategy involving anaerobic digestion is suggested in response to the gradual reduction in wet or dry feed production, along with composting, the conventional treatment method. This transition not only curbs greenhouse gas emissions but also enhances biogas production, a renewable energy source. These efforts align with the Korean Green New Deal’s goal of achieving a 20% share of renewable energy by 2030.

**Eun Hea Jho, Ji Won Yang, Won Jung Ju, Sung-Jong Lee, Md Mehedee Hasan. Effect of co-presence of cadmium or procymidone with microplastic films in soil on lettuce growth. (2023) Appl. Biol. Chem. 66: 72**

Agricultural environment is often contaminated with various chemicals (e.g., pesticides, heavy metals) and microplastics due to the uses of plastic products. The effects of chemical contaminants or microplastics on terrestrial environment have been extensively studied, but the studies on the co-presence of chemical contaminants and microplastics are relatively limited. This study was set to investigate the effect of co-presence of microplastics (i.e., low-density polyethylene (LDPE) and polyvinyl chloride (PVC) microplastic films) and chemical contaminants (i.e., cadmium (Cd) and procymidone (PCM)) in soil on the lettuce growth and Cd and PCM uptake by lettuce using pot tests. The lettuce leaf lengths were not affected by the presence of only Cd or PCM, but the rates of change in the lettuce leaf number were adversely affected by the presence of PCM. The presence of only LDPE or PVC in soil at the concentrations used in this study did not have significant impacts on the lettuce growth. But the co-presence of Cd and LDPE and the co-presence of PCM and PVC resulted in the negligible increases in the lettuce leaf length and leaf number with time, although the lettuce growths were statistically similar in the Cd- or PCM-contaminated soils regardless of the presence of microplastics. The results suggest that the adverse effects of Cd or PCM can be intensified by the co-presence of microplastics, and the effects can be different depending on the types of microplastics. The promoted adverse effects of chemical contaminants in the co-presence of microplastics can be supported by the tendency of the increased absorption of Cd or PCM by lettuce in the co-presence of microplastics. Overall, this study shows the need for management of both chemical contaminants and microplastics that may reside in the agricultural environment.

**Ye Jin Kim, Dae Young Lee, Hye Rim Yang, Kyung-Hoan Im, Sang Un Park, Jae Geun Kim, Jae Kwang Kim. Evaluation of obesity prevention effect of black ginseng on serum, liver, and hypothalamus of mice on a high-fat diet using a metabolomics approach. (2023) Appl. Biol. Chem. 66: 73**

Black ginseng is being studied to prevent obesity caused by a high-fat diet (HFD). The aim of this study was to evaluate the obesity-preventing effect of black ginseng extract (BGE) in the serum, liver, and hypothalamus of mice on an HFD using metabolomic techniques. Mice were divided into four groups which were respectively fed a normal diet (CTL), an HFD, an HFD with a low concentration of BGE (BGEL), and an HFD with a high concentration of BGE (BGEH) for 8 weeks. Metabolite profiling revealed a clear separation between the BGE diet and HFD groups. Lipid metabolism, including saturated fatty acids and cholesterol, was decreased in the BGEH mice. Specifically, neurotransmitters and intermediates of the tricarboxylic acid cycle were increased in the hypothalamus of BGEH mice. The results suggest the obesity prevention effect of black ginseng in that BGEH inhibits body fat accumulation and restores brain function damaged by HFD.

**Chang Jun Lee, Young-Soo Kim, Jinyoung Hur, Guijae Yoo, Sang Yoon Choi. *Asimina triloba* (pawpaw) fruit extract suppresses adipocyte differentiation and lipogenesis-related protein expression in 3T3-L1 cells. (2023) Appl. Biol. Chem. 66: 75**

Obesity is a health condition accompanied by life-threatening comorbidities; hence, there is an increasing need for anti-obesity agents. The anti-cancer effects of the leaves of *Asimina triloba* (pawpaw) has been reported. However, limited research has been conducted on the potential anti-obesity effects of *A. triloba* fruit. Therefore, this study aimed to explore the effects of *A. triloba* fruit extract on murine preadipocytes (3T3-L1). We specifically examined lipid droplet formation in these cells using Oil Red O solution and intracellular pro-adipogenic protein levels were examined using western blot analysis. The results revealed that treatment with *A. triloba* 70% ethanolic fruit extract effectively suppressed lipid droplet formation. Moreover, the expression of crucial proteins involved in adipogenesis, namely sterol regulatory element-binding protein 1, peroxisome proliferator-activated receptor γ, and fatty acid synthase, were significantly inhibited. These findings suggest that *A. triloba* fruit has the potential to prevent obesity by inhibiting fat synthesis and may serve as a natural source for anti-obesity functional agents.

**Paul Toukam Djouonzo, Md Sofequl Islam Mukim, Pamela Nangmo Kemda, Theodora Kopa Kowa, Alembert Tiabou Tchinda, Gabriel Agbor Agbor, Cheol-Ho Pan, Dae-Geun Song. SARS-CoV-2 main protease inhibitors from the stem barks of *Discoglypremna caloneura* (Pax) Prain (Euphorbiaceae) and *Pterocarpus erinaceus* Poir (Fabaceae) and their molecular docking investigation. (2023) Appl. Biol. Chem. 66: 76**

The main viral protease (Mpro) of SARS-CoV-2 provides an excellent target for antivirals, due to its essential and conserved function in the viral replication cycle. We reported in this study, the SARS-CoV-2 main protease inhibitory effect of twelve compounds isolated from *D. caloneura* and *P. erinaceus* together with four derivatives. Among the effectively tested samples, two derivatized compounds displayed significant improvement on the activity from the starting material, friedelin (**1**) through the acetoreduced (**2**) to the acetoxy product (**3**) with respective IC50 values of 42.89, 29.69 and 19.39 µg/mL. The latter displayed the highest activity although lower as compared to that of baicalein, the positive control with IC50 0.41 µg/mL. The molecular docking study showed that an increase in the number of hydrogen bonds between compounds and active site of Mpro resulted in increased inhibition.

**Seyeon Choi, Huiji Kim, Seong-Ah Shin, Moonsu Kim, Sun Young Moon, Minji Kim, Seulah Lee, Jun Hyuck Lee, Hyun Ho Park, Ui Joung Youn, Chang Sup Lee. DB3 from Antarctic lichen inhibits the growth of B16F10 melanoma cells in vitro and in vivo. (2023) Appl. Biol. Chem. 66: 77**

Malignant melanoma is a fatal disease with an increasing global incidence. Despite numerous studies focused on anti-cancer drugs, a variety of side effects of cancer treatment remain challenging. Thus, there is a pressing need to identify novel anti-cancer agents with minimal cytotoxicity and side effects. DB3 (1,3,7,9-tetrahydroxy-2,8-dimethyl-4,6-di[ethanoyl]dibenzofuran) is a member of the dibenzofuran family and is extracted from *Ramalina terebrata* (Antarctic lichen). We investigated if DB3 exerted an antitumor effect on B16F10 melanoma cells. The results revealed that DB3 exerted time- and dose-dependent reduction of cell viability by inducing apoptosis and significantly suppressing cell proliferation through cell cycle arrest in the G0/G1 phase in B16F10 melanoma cells. Additionally, DB3 impeded the migration and invasiveness of B16F10 cells. Subsequently, we observed that DB3 decreased the expression levels of Cdk4/Cyclin D1 and the phosphorylation of p38, JNK, ERK, and AKT. Furthermore, DB3 decreased melanoma tumor growth in a mouse tumor syngraft model. Based on these findings, we propose that DB3 possesses potential for use as an anti-cancer agent for melanoma treatment.

**Gizachew Assefa Kerga, Nurelegne Tefera Shibeshi, Sundramurthy Venkatesa Prabhu, Venkatramanan Varadharajan, Alazar Yeshitla. Biosorption potential of *Purpureocillium lilacinum* biomass for chromium (VI) removal: isolation, characterization, and significance of growth limiting factors. (2023) Appl. Biol. Chem. 66: 78**

Chromium (VI) is known to be harmful element that commonly found industrial waste, mining activities, and wastewater discharges from various industries. When released into the environment, Cr (VI) can contaminate soil, water, and air, posing a serious threat to living organisms. Aiming to Cr decontamination, this work was framed to isolate the fungal species having high Cr tolerance capacity and to exploit as bio-sorbent for the removal of Cr (VI) from aqueous solutions by biosorption. Among the fungal species isolated from the Cr (VI) contaminated soil sample, the filamentous fungus of *Ophiocordycipitaceae* family, *Purpureocillium lilacinum* was identified using molecular sequencing technique, showed maximum tolerance against Cr (VI) with a tolerance index of 1.19 ± 0.23. Further, Plackett Burman Design was applied to investigate for ascertaining the significance of different carbon and nitrogen sources on *P. lilacinus* growth, as well as the influence of environmental factors, such as pH, temperature, and Cr (VI) concentration. The results explicated that glucose was the most preferred carbon source for *P. lilacinu*s, while yeast extract was the most preferred for nitrogen source. The optimum pH value and temperature were found to be 6.0 and 26 °C, respectively. In addition, *P. lilacinus* isolate was identified to survive in high concentrations of Cr (VI), indicating its potential for employing effective bioremediation of chromium-contaminated site.

**Husam Qanash, Aisha M. H. Al-Rajhi, Majed N. Almashjary, Ammar A. Basabrain, Mohannad S. Hazzazi, Tarek M. Abdelghany. Inhibitory potential of rutin and rutin nano-crystals against *Helicobacter pylori*, colon cancer, hemolysis and Butyrylcholinesterase in vitro and in silico. (2023) Appl. Biol. Chem. 66: 79**

Despite the vital activity of many compounds, they lack that effectiveness due to their low solubility in water. Unfortunately, for this reason, rutin often leads to low tissue permeability and insufficient bioavailability, which has greatly limited its pharmacological utility. Therefore, the present investigation is designed to overcome this problem by formulating the rotin to rotin nanocrystals (RNCs) with studying their some pharmacological applications in vitro and in silico. RNCs were created via the ultrasonication approach and showed a spherical shape via Transmission electron microscopy with a mean particle size of 27 nm. RNCs reflected inhibitory action against *Helicobacter pylori* with an inhibition zone (IZ) of 22.67 mm compared to rutin (IZ of 18 mm) and standard control (IZ of 19.5 mm). RNCs exhibited less minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (7.8 µg/mL) than rutin (62.5 µg/mL). The MBC/MIC index of rutin and RNCs indicated their bactericidal properties. RNCs were more acutely **(**92.12%) than rutin (85.43%) for inhibition the *H. pylori* biofilm formation. A promising half maximal inhibitory concentration (IC50) (6.85 µg/mL) was recorded using RNCs for urease inhibition compared to the IC50 value of rutin (97.8 µg/mL). The activity of rutin and RNCs was tested against cancer cells of human colon cancer (HT-29) and normal Vero cells. IC50 values of RNCs were less 168.23 ± 1.15 µg/mL and 297.69 ± 4.23 µg/mL than the IC50 values of rutin 184.96 ± 4.33 µg/mL and 335.31 ± 2.02 µg/mL against HT-29 cells and normal Vero cells, respectively. Different percentages (72.2, 77.3, and 81.9%) of hemolysis inhibition were recorded using RNCs, but 63.6, 68.9, 73.6, and 80.6% were obtained using rutin at 600, 800, and 1000 µg/mL, respectively. Butyrylcholinesterase (BChE) inhibition % was documented at a lower IC50 value for RNCs (12.74 µg/mL) than the IC50 of rutin (18.15 µg/mL). The target molecule underwent molecular docking research against *H. pylori* [Protein Data Bank (PDB) code: 4HI0], HT-29 cells (PDB code: 2HQ6), and BChE (PDB code: 6EMI) in order to enhance the interactions between rutin and the chosen receptors and to estimate its molecular operating environment (MOE) affinity scoring. Rutin has predicted strong binding interactions and potent activity against the examined proteins 4HI0, 2HQ6, and 6EMI with low binding scores of − 7.47778 kcal/mol, − 7.68511 kcal/mol, and − 9.50333 kcal/mol, respectively.

**Thu Thi Hoai Mai, Youngjoo Choi, Hanbyul Park, Jae Lyoung Cheon, Jae-Seok Choi, Donghwan Park, Hekap Kim. Green ultrasound-assisted extraction of fish oil from rainbow trout intestines and purification with adsorbents. (2023) Appl. Biol. Chem. 66: 80**

This study explored the application of green ultrasound-assisted technology for the extraction of oil from the intestines of rainbow trout. Purification methodologies were incorporated using adsorbents in order to enhance the quality of the extracted oil, which was evaluated based on its color, peroxide value (POV), free fatty acids, organic pollutants, and fatty acid composition. The extraction condition for maximum oil recovery was 60 °C for 30 min, with the addition of 1 g of sodium chloride and a water-to-sample ratio of 0:2. The analysis indicated that silica gel exhibited the highest efficiency as an adsorbent for the elimination of peroxides from extracted oil, with optimal results achieved after adsorption for 60 min. Despite undergoing purification, the POV of fish oil still exceeded the quality standard established by the CODEX Alimentarius Commission. In order to optimize the extraction process, the incorporation of antioxidants, including gallic acid, tannic acid, and *Aronia* (black chokeberry) powder, was implemented before the oil refining process. The integration of antioxidants and purification further lowered the POV and mitigated the production of organic pollutants, concurrently enhancing oil quality compared to without antioxidants. Notably, the incorporation of antioxidants during the initial stages of the extraction process resulted in a significant increase in the average concentrations of essential polyunsaturated fatty acids (PUFAs) in the final products. Overall, this study revealed that *Aronia* has the potential to serve as a natural, less-costly antioxidant alternative to pure antioxidants, such as tannic acid and gallic acid. Furthermore, the potential nutritional value of the final refined oil sample derived from rainbow trout intestines can be improved in terms of ω-3 fatty acid content by the developed method.

**Sangyun Kim, Heebak Choi, Taegyu Yi, Dohoon Gwak, Sun-Hwa Ha. Pleiotropic properties of GOLDEN2-LIKE transcription factors for crop improvement. (2023) Appl. Biol. Chem. 66: 81**

Crop improvement can be affected by enhancing the efficiency of photosynthesis-associated bioprocesses such as chlorophyll biosynthesis, chloroplast biogenesis, the functioning of photosystems including light-harvesting complexes, and carbon fixation. To achieve this, the GOLDEN2-LIKE (GLK) transcription factors represent promising targets since they play a positive role for greening traits in diverse plants. To scrutinize the pleiotropic impact of GLKs, we summarized all phenotypic traits reported in functional studies that used transgenic approaches to lose or gain gene functions. Additionally, we also discussed altered plant phenotypes with respect to their physiological–biochemical aspects and environmental stress responses. From these results, we conclude that GLKs consistently increase chlorophyll biosynthesis, enhance chloroplast division, and increase photosynthetic rate. They individually influence other traits including yield, phytochemical accumulation, and biotic and abiotic stress resistance. Collectively, GLKs have potential as key regulators to effect increases in overall agricultural quality across plant species. This suggests that they may be among the most promising target genes for future agro-biotechnology applications.

**Yoon Chae Jeong, Jihyun Park, Yu Jeong Cheon, Ki Seog Lee. Hypothetical protein CuvA (Rv1422) from *Mycobacterium tuberculosis* H37Rv interacts with uridine diphosphate *N*-acetylglucosamine as a key precursor of cell wall. (2023) Appl. Biol. Chem. 66: 82**

*Mycobacterium tuberculosis* CuvA (Rv1422, MtCuvA) has previously been suggested that it may play a critical role in nutrient utilization and cell wall synthesis required for physiological adaptation in a host cell, but its biochemical details remain unclear. Our previous studies showed that MtCuvA can bind to uridine diphosphate (UDP) sugars as a cell wall precursor component. To verify its functional roles, we report here the biochemical properties of MtCuvA for the binding of UDP-*N*-acetylglucosamine (GlcNAc) using site-directed mutagenesis and docking simulation. The *K*D values for UDP-sugars indicate that MtCuvA prefers to bind UDP-GlcNAc as a physiological ligand compared to UDP-glucose. Mutational studies of MtCuvA showed that H12A, T33A, D36A, Q154A, S196, T199A, N226A, and H298A mutants significantly affected the binding to UDP-GlcNAc. We also observed that UDP, but not GlcNAc, could bind to MtCuvA. These results imply that the presence of UDP moiety in the ligand is necessary for interaction with MtCuvA. Moreover, mutational studies of MtCuvA with UDP showed that residues H12, S196, T199, N226, and H298 may be involved in its binding to the UDP moiety, almost consistent with the docking simulation results. Our results provide an insight into the interaction of MtCuvA with UDP-GlcNAc as a key precursor of peptidoglycan.

**Hafiz Muhammad Umer Farooqi, Johoo Lee, Cheong-Ah Lee, Seung-Uk Im, Muhammad Awais Farooqi, Tasbiha Gul, Ghayas Uddin Siddiqui, Dong-Guk Paeng. Acute neuro-biochemical changes induced by nitrogen-tungsten co-doped titanium dioxide nanoparticles. (2023) Appl. Biol. Chem. 66: 83**

Nitrogen-tungsten co-doped titanium dioxide nanoparticles (W-N-doped TiO2 NPs) are employed for the photocatalytic degradation of environmental pollutants. However, the potential impact of these nanoparticles on the central nervous system remains a subject of concern. This study aimed to evaluate the effects of W-N-doped TiO2 NPs on neurophysiological and biochemical parameters of healthy rat brains, including behavioral monitoring, electroencephalogram analysis, and oxidative stress markers quantification. Intraperitoneal administration of W-N-doped TiO2 NPs to rats revealed abnormal brain electrical activity and an altered sense of balance in the treated rats. The ability of W-N-doped TiO2 NPs to cross the blood–brain barrier and accumulate in the brain leads to oxidative stress damage, supported by the elevated levels of reactive oxygen species (ROS), nitrite concentration, and malondialdehyde levels. Additionally, exposure to W-N-doped TiO2 NPs significantly reduced the antioxidant enzyme levels, such as catalase and superoxide dismutase, impacting a significant decrease in dopamine and acetylcholinesterase within the rat neural tissue. Furthermore, the inflammatory biomarker tumor necrosis factor-alpha and 8-hydroxy 2-deoxyguanosine significantly increased in response to W-N-doped TiO2 NPs. The findings revealed the adverse effects of W-N-doped TiO2 NPs on the electrical activity of rat brains and the altered concentration of various neuro-biomarkers, highlighting their potential neurotoxicity.

**Dahye Yoon, Bo-Ram Choi, Woo Cheol Shin, Kwan-Woo Kim, Young-Seob Lee, Dae Young Lee. Metabolomics reveals that *Curcuma longa* and demethoxycurcumin inhibit HCT116 human colon cancer cell growth. (2023) Appl. Biol. Chem. 66: 84**

Studies on the use of natural products to treat cancer are ongoing, and turmeric (*Curcuma longa* L.), a medicinal crop, is known for various effects including anticancer activity. In this study, the inhibitory effect of *C. longa* and demethoxycurcumin on cancer cell growth in a colorectal cancer cell line (HCT116) was investigated by using nuclear magnetic resonance (NMR) spectroscopy-based metabolomics. For this analysis, HCT116 cells were treated with doxorubicin (positive control), *C. longa* extract, or demethoxycurcumin (20, 40, and 60 μM). In the NMR spectra of the HCT116 cell extract, 45 metabolites were identified and quantified. The quantified metabolites were analyzed by biomarker analysis, and significantly changed metabolites were filtered by the area under the curve (AUC) of the receiver operator characteristic (ROC) curve. Multivariate statistical analysis of NMR spectra was conducted to confirm the distribution among groups. Through an S-line plot, it was possible to identify metabolites that contributed to the differences seen in the OPLS-DA score plot. Taken together, the results reveal that *C. longa* extract induces oxidative stress and changes the energy metabolism in HCT116 cells, and that demethoxycurcumin inhibits the energy metabolism strategy for the survival of cancer cells, escape from immune cells, and cancer cell proliferation, thereby enabling the survival of HCT116 cells.

**Sol-ra Oh, Se-keun Park, Pyeongjae Lee, Yong-Min Kim. The ginsenoside Rg2 downregulates MMP-1 expression in keratinocyte (HaCaT)-conditioned medium-treated human fibroblasts (Hs68). (2023) Appl. Biol. Chem. 66: 85**

Keratinocytes exposed to UVB induce the production of cytokines, which activate fibroblasts and increase the expression of matrix metalloproteinases (MMPs). The increased expression of MMPs leads to connective tissue damage and wrinkle formation, resulting in skin aging. In this study, we used human dermal fibroblasts cultured in UVB-irradiated keratinocyte-conditioned medium (UV CM) to investigate the potential anti-aging effects of the ginsenoside Rg2 on skin. The inhibitory effect of Rg2 on the MMP-1 gene and protein was determined by real-time PCR and ELISA. We also examined the expression levels of proteins in the mitogen-activated protein kinase (MAPK) signaling pathway using western blotting, to elucidate the underlying mechanism of the inhibitory effect of Rg2. Rg2 inhibited MMP-1 mRNA and protein expression in a concentration-dependent manner. We found that Rg2 inhibited the phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) but not that of p38. Therefore, our results suggest that Rg2 is a potential material for the prevention and treatment of photoaging.

**N. H. M. Rubel Mozumder, Kyeong Hwan Hwang, Min-Seuk Lee, Eun-Hee Kim, Young-Shick Hong. Metabolic Evidence on Vintage Effect in Tea (*Camellia sinensis* L.) Plants. (2023) Appl. Biol. Chem. 66: 86**

Recent metabolomics studies have reported diverse metabolites of tea depending on tea (*Camellia sinensis*) cultivars, cultivation conditions and geographical location. However, these studies were limited the effects of these conditions on metabolome of tea leaves in a single year. We explored the year-to-year variations in leaf metabolome of two tea (*C. sinensis*) cultivars over a period of five successive years from 2015 to 2019 to determine vintage tea products, such as in grapes or wines, and showed a clear metabolic differentiations of fresh tea leaves. Also, the best conditions of climate were suggested through an association of rainfall and sun-expose time with the metabolism of theanine in taste- or flavor-rich tea cultivar and of catechin compounds in EGCG3″Me-rich tea cultivar, thereby providing the potential vintage tea tailored to the cultivar. Since vintage wine is derived from grapes grown in a year under good climatic conditions, which provides high quality of wine in the best year, the current result highlights important information relevant to tea metabolome associated with climatic conditions in a specific year and the manufacture of vintage tea with unique quality.

**Han-Na Cho, Minji Shin, Ikhyeong Lee, Haeun Ryoo, Bharat Sharma Acharya, Jae-Hyuk Park, Yong Hwa Cheong, Ju-Sik Cho, Se-Won Kang. Impact of biochar and compost amendment on corn yield and greenhouse gas emissions under waterlogged conditions. (2023) Appl. Biol. Chem. 66: 87**

Biochar, widely recognized for its capacity to counteract climate change impacts, has demonstrated substantial benefits in agricultural ecosystems. Nevertheless, empirical studies exploring its efficacy during climatic aberrations such as heavy rainfall are limited. This study investigated the effects of compost and biochar addition on corn growth attributes, yield, and soil CO2 and N2O fluxes under heavy rain (exceeding 5-yr average) and waterlogging conditions. Here, treatments included compost (CP, 7.6 t ha−1); rice husk biochar (RB, 7.6 t ha−1); wood biochar (WB, 7.6 t ha−1); and control (Cn). Under high rainfall and waterlogging, the CP treatment manifested a pronounced enhancement in corn biomass and productivity, exceeding biomass and productivity of Cn treatment by 12.6 and 32.2%, RB treatment by 120 and 195%, and WB treatment by 86.1 and 111%, respectively. Corn yield increased in the order: CP > Cn > WB > RB. Intriguingly, negligible disparity occurred between the RB and WB treatments in straw yield, grain yield, grain index, and corn productivity but both treatments recorded distinctively lower values than CP treatment. Also, the CO2 and N2O fluxes remained largely similar for two biochar treatments but lower than CP treatment. Overall, CP increased corn yield, straw, and grain yield whereas biochars reduced N2O flux during waterlogging. Although derived from a short-term experimental window, these pivotal findings furnish invaluable insights for devising soil amendments for yield and environmental benefits in contexts of extreme climatic perturbations. Our findings offer a robust foundation for refining nutrient management strategies confronted with waterlogging challenges, but long-term studies are necessary for definitive conclusions.

**Yeok Boo Chang, Yejin Ahn, Daebang Seo, Soohyun Bae, Hyung Joo Suh, Yang Hee Hong, Eun Young Jung. *Centella asiatica* lowers body fat accumulation via regulating cholesterol homeostasis- and lipid metabolism-related genes in mice with high-fat, high-sugar diet-induced obesity. (2023) Appl. Biol. Chem. 66: 88**

To understand the mechanisms involved in the anti-obesity effects *Centella asiatica* (CA), we examined body weight, serum levels, white adipose tissue (WAT) weight, histological analysis, and the expression of cholesterol homeostasis- and lipid metabolism-related genes in mice with high-fat, high-sugar diet (HFHSD)-induced obesity that were orally treated with CA for 12 weeks. Eight-week-old, male C57BL/6J mice were assigned to the following four groups (8 mice/group): NOR, normal diet; HFHSD (Control), HFHSD; CA-L, HFHSD + CA 300 mg/kg; CA-H, HFHSD+CA 600 mg/kg. The suspension of powdered CA leaf was fed using oral gavage. CA treatment significantly attenuated HFHSD-induced increase in body weight gain, serum glucose, triacylglycerol, and WAT weight (p < 0.05). Compared to that in HFHSD, adipocyte diameter and macrovesicular area of epididymal WAT significantly decreased with CA treatment (p < 0.05). The mRNA expression levels of peroxisome proliferator-activated receptor gamma (PPARγ), fatty acid synthase (FAS), cluster of differentiation 36 (CD36), 3- hydroxyl-3-methylglutaryl CoA reductase (HMGCR), and stearoyl CoA desaturase 1 (SCD 1) were significantly downregulated in the CA-H compared to the HFHSD (p < 0.05). CA exerts anti-obesity effects by lowering body fat accumulation via regulating gene expression and thus, is a potential lipid-lowering agent.

**Ika Oktavianawati, Mardi Santoso, Mohd Fadzelly Abu Bakar, Yong-Ung Kim, Sri Fatmawati. Recent progress on drugs discovery study for treatment of COVID-19: repurposing existing drugs and current natural bioactive molecules. (2023) Appl. Biol. Chem. 66: 89**

COVID-19 has been a major global health concern for the past three years, and currently we are still experiencing coronavirus patients in the following years. The virus, known as SARS-CoV-2, shares a similar genomic identity with previous viruses such as SARS-CoV and MERS-CoV. To combat the pandemic, modern drugs discovery techniques such as in silico experiments for docking and virtual screening have been employed to design new drugs against COVID-19. However, the release of new drugs for human use requires two safety assessment steps consisting of preclinical and clinical trials. To bypass these steps, scientists are exploring the potential of repurposing existing drugs for COVID-19 treatment. This approach involves evaluating antiviral activity of drugs previously used for treating respiratory diseases against other enveloped viruses such as HPV, HSV, and HIV. The aim of this study is to review repurposing of existing drugs, traditional medicines, and active secondary metabolites from plant-based natural products that target specific protein enzymes related to SARS-CoV-2. The review also analyzes the chemical structure and activity relationship between selected active molecules, particularly flavonol groups, as ligands and proteins or active sites of SARS-CoV-2.

**Seok Joong Kim, Yangji Kim, Thanh Van Duong, Hee Sung Park. Synergistic impact of autocrine motility factor and curcumin on colorectal cancer cell proliferation. (2023) Appl. Biol. Chem. 66: 90**

Colorectal cancer (CRC) presents a formidable challenge, characterized by a steadily increasing incidence. Current approaches to manage CRC, including chemotherapy and targeted therapies, are burdened with significant limitations such as resistance development, adverse events, and high costs. Hence, there is an urgent demand for a more promising alternative. Autocrine motility factor (AMF), known for its role in promoting cancer cell motility, exhibits a unique ability to selectively impede the growth of cancer cells. In our study, we have elucidated the specific inhibitory effect of AMF derived from DU145 prostate cancer cells (D-AMF) on the proliferation of CRC cells. D-AMF effectively downregulated the expression of glucose-6-phosphate dehydrogenase (G6PD) at both the mRNA and protein levels, resulting in a concurrent increase in the generation of reactive oxygen species (ROS). Notably, the combination of D-AMF and curcumin proved highly effective in eliminating curcumin-resistant CRC cells. Therefore, the use of D-AMF in conjunction with curcumin holds promise as an alternative treatment approach for CRC.

**Jong-Hwan Park, Su-Lim Lee, Jae-Hoon Lee, Jun-Suk Rho, Jeong-Min Lee, Seong-Heon Kim, Se-Won Kang, Dong-Cheol Seo. Reduction of ammonia gas by microbial agent treatment in Chinese cabbage cultivation. (2023) Appl. Biol. Chem. 66: 91**

This study aimed to select the optimal microbial agents for ammonia gas reduction in Chinese cabbage cultivation and evaluate their ammonia reduction efficiency. By selecting the optimum microorganism to reduce ammonia emissions, the ammonia emission reduction efficiencies of the nitrification microorganisms, *Alcaligenes faecalis subsp. faecalis* and *Brevibacillus sp.* were 21 and 31%, respectively, which were superior to those of other microorganisms. The best ammonia emission reduction efficiency of the acid-producing microorganisms was 55%. The optimum mixing ratio of microbial agent for removing ammonia gas emitted from NPK-containing soil was: acid-producing microorganism: *Alcaligenes faecalis subsp. faecalis*:*Brevibaillus sp.* = 0.70:0.15:0.15. The optimum treatment amount was 500 L/ha, and the optimum number of microbial agents was basal fertilization (also known as pre-planting fertilization) once and additional fertilization three times, for a total of four times. The reduction efficiency of ammonia emissions from NPK-containing soil under optimum conditions in cabbage cultivation was 27% lower than that of the control (only NPK-containing soil). Therefore, the microbial agent developed in this study can be utilized to effectively reduce the emission of ammonia, a secondary fine particle precursor, while maintaining crop yield in agricultural fields.

**Won Choi, Seo Young Park, Hyun Min Kim, Thanh Dat Mai, Ju Hui Do, Hye Min Jang, Hyeon Bae Hwang, Eun Gyeong Song, Jae Sung Shim, Young Hee Joung. Heterologous expression in *E. coli* and functional characterization of the tomato CPR enzymes. (2023) Appl. Biol. Chem. 66: 92**

NADPH-cytochrome P450 reductase (CPR) is a key enzyme transferring electrons to cytochrome P450. In tomatoes (*Solanum lycopersicum*), two *CPR* genes, *SlCPR1* and *SlCPR2*, were identified. In all the tested tomato tissues, *SlCPR2* showed higher expression levels than *SlCPR1*. *SlCPR2* expression increased significantly with jasmonic acid treatment. No significant changes were observed with salicylic acid or drought stress treatment. The cDNA of *SlCPRs* were expressed in *Escherichia coli* without any amino acid modification. And the heterologously expressed SlCPR enzymes were reacted with several protein and chemical substrates. SlCPR2 was more active than SlCPR1. Both SlCPR1 and SlCPR2 exhibited strong activity across a pH range of 6.0 to 9.0, with peak activity at pH 8.0. The study opens possibilities for CPR control, biocatalyst development, and exploring oxidase enzyme functions.

**Han Na Kim, Jin Hee Park. Monitoring of soil EC for the prediction of soil nutrient regime under different soil water and organic matter contents. (2024) Appl. Biol. Chem. 67: 1**

Smart farms and precision agriculture require automatic monitoring and supply of water and nutrients for crops, but sensors to monitor plant available nutrients in soil are not available. Soil electrical conductivity (EC) is related to nutrients in soil solution, which can be affected by soil organic matter, soil texture, temperature, and water content. Therefore, the objective of this study is to evaluate factors influencing soil EC sensor values by monitoring EC under different soil organic matter and water contents. Ten soil samples with various sand and clay contents, EC, pH, and organic matter contents were selected and saturated with water. Volumetric water content and EC of the soil were monitored while drying the soil. Humic acid and manure were added to soils in order to evaluate the effect of organic matter on soil EC. Soil EC values linearly increased with increasing water content at 10–25% which is favorable water content for plant growth. The EC increased when organic matter was added to soils, which was related to ions released from the organic matter. Soil EC calibration factor for soil water content increased when EC of the soil was high and organic matter was added. The sensor EC values in sandy loam and loam soils was related to the ion contents in pore water, and exchangeable ions in soil, respectively. Sensor EC values were highly correlated with organic matter and K contents in soil and can be used as an indicator for plant available nutrients in soil. Therefore, the sensor EC at optimal soil water content for plant growth can be used to monitor changes in plant available nutrients in soil.

**Jing-Yang Bian, Xiao-Yu Guo, Dong Hun Lee, Xing-Rong Sun, Lin-Shuai Liu, Kai Shao, Kai Liu, Hu-Nan Sun, Taeho Kwon. Non-thermal plasma enhances rice seed germination, seedling development, and root growth under low-temperature stress. (2024) Appl. Biol. Chem. 67: 2**

Recently, non-thermal plasma (NTP) technologies have found widespread application across diverse fields, including plant growth, medical science, and biological and environmental research. Rice (*Oryza sativa L.*) is exceptionally sensitive to temperature changes. Notably, low-temperature stress primarily affects the germination and reproductive stages of rice, often leading to reduced crop yield. This study aimed to identify optimal conditions for enhancing rice seed germination and seedling growth under low temperatures using NTP technology. Our research indicated that NTP treatment at 15.0 kV for 30 s optimally promotes rice seed germination and growth under low-temperature stress. Furthermore, NTP treatment increases the activity and expression of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), under low-temperature conditions. Moreover, it downregulates the expression of β-ketoacyl-[acyl carrier protein] synthase I (KASI) and cis-epoxy carotenoid dioxygenase 3 (NCED3) and upregulates the expression of alternative oxidase (AOX1B), BREVIS RADIX-like homologous gene (BRXL2), WRKY transcription factor 29 (WRKY29), and EREBP transcription factor 2 (EREBP2) in roots after tandem 7 days low-temperature (16 ℃) and 7 days room-temperature (28 ℃) treatments. Transcriptomic analysis revealed the involvement of various key genes in phosphotransferase activity, phosphate-containing compound metabolic processes, and defense responses. These analyses provide comprehensive information on gene expression at the transcriptional level, offering new insights for a deeper understanding of candidate genes required for root growth in rice.

**Kumuduni Niroshika Palansooriya, Piumi Amasha Withana, Yoonah Jeong, Mee Kyung Sang, Yoora Cho, Geonwook Hwang, Scott X. Chang, Yong Sik Ok. Contrasting effects of food waste and its biochar on soil properties and lettuce growth in a microplastic-contaminated soil. (2024) Appl. Biol. Chem. 67: 3**

The incorporation of organic amendments, such as food waste (FW) and biochar, into soil is an established agronomic practice known for enhancing soil fertility and improving overall soil health. However, the individual and combined effects of FW and biochar on soil properties in microplastic (MP)-contaminated soil–plant systems remain poorly understood. To address this knowledge gap, we conducted a field experiment to investigate the individual and combined effects of polystyrene MPs, FW, and FW-derived biochar on soil properties and lettuce growth. Soil chemical properties were unaffected by the addition of MPs. However, the application of FW and biochar increased the soil pH, with the highest pH (8.2) observed in the combined treatment of biochar and MPs. Despite the presence of MPs, FW application resulted in notable increases in soil electrical conductivity (EC; 2.04 dS m−1), available nitrogen (NO3−–N: 325.5 mg kg−1, NH4+–N: 105.2 mg kg−1), available phosphorus (88.4 mg kg−1), and total exchangeable cations (18.6 cmol(+) kg−1). However, these values decreased after lettuce cultivation. In soil cultivated with lettuce, the coexistence of MPs and biochar reduced soil Fluorescein diacetate hydrolase enzyme activity by 46.2% and urease activity by 94.0%. FW addition doubled acid phosphatase activity, whereas FW and its coexistence with MPs decreased alpha diversity. The relative abundance of Actinobacteria decreased with MP application, whereas that of Acidobacteria and Actinobacteria decreased with FW treatment. Gemmatimonadetes and Nitrospirae decreased in soil treated with FW and biochar. The highest relative abundances of Firmicutes and Proteobacteria were observed in the FW-added soils, and Planctomycetes were the highest in the biochar-added soils. FW application negatively affected lettuce growth. Overall, the coexistence of MPs with FW or biochar had limited effects on soil properties and lettuce growth, with FW and biochar serving as the primary factors in modifying soil–plant systems. Future studies should investigate the effects of different MPs and their interactions with organic soil amendments on soil properties and crop growth under different management practices.

**Hye Lin Park, Seong Hee Bhoo, Sang-Won Lee, Man-Ho Cho. Biochemical characterization of a regiospecific flavonoid 3'-*O*-methyltransferase from orange. (2024) Appl. Biol. Chem. 67: 4**

Citrus plants have diverse methoxyflavonoids including, chrysoeriol, isosakuranetin, and nobiletin. In plants, *O*-methyltransferases (OMTs) participate in the methylation of a vast array of secondary metabolites, including flavonoids, phenylpropanoids, and alkaloids. To identify functional OMTs involved in the formation of methoxyflavonoids, orange (*Citrus sinensis*) OMT (CsOMT) genes were retrieved from the Citrus Genome Database. The phylogenetic relationships with functional OMTs suggested that three CsOMTs, CsOMT15, CsOMT16, and CsOMT30, are possible candidates for flavonoid OMTs (FOMTs). These CsOMTs were heterologously expressed in *Escherichia coli*, and their OMT activity was examined with flavonoid substrates. Of the examined CsOMTs, CsOMT16 catalyzed the regiospecific 3'-*O*-methylation of flavonoids to the respective 3'-methoxyflavonoids. A kinetic study demonstrated that CsOMT16 accepts diverse flavonoids as a substrate with a comparable preference. The flavonoids eriodictyol, luteolin, and quercetin were efficiently converted to homoeriodictyol, chrysoeriol, and isorhamnetin by CsOMT16-transformed *E. coli* cells, respectively. These findings suggest that CsOMT16 contributes to the methoxyflavonoid formation in orange and is applicable to the biotechnological production of 3'-methoxyflavonoids.

**Hyosun Park, Suna Kim, Jaecheol Kim, KyeongJin Lee, BoKyung Moon. Bioactive compounds and antioxidant activity in three types of Korean watery kimchi. (2024) Appl. Biol. Chem. 67: 5**

Watery kimchi is a traditional fermented food served with its soup. In this study, we collected 21 samples of *yeolmu mul* kimchi (YMK), *dongchimi* (DC), and *nabak* kimchi (NK), respectively, which are the most popular watery kimchi in Korea. A composite sample of each watery kimchi was prepared for estimation of their bioactive compounds and antioxidant activities. Of the three kimchi types, YMK had the highest total carotenoid content (63.78 ± 4.88 mg/100 g, of which lutein, capsanthin, and β-carotene were the main carotenoids), and DC had the lowest (3.50 ± 0.12 mg/100 g). YMK also had the highest contents of chlorophyll (250.1 ± 3.91 mg/100 g), ascorbic acid (447.16 ± 8.95 mg/100 g), and capsaicinoids (2.51 ± 0.09 mg/100 g) compared to DC and NK. The lactic acid content was highest in NK (582.72 ± 29.10 mg/100 g). Moreover, YMK showed significantly higher antioxidant activity (ABTS and DPPH) than DC and NK (*p* < 0.05). Chlorophyll and antioxidant activity showed a strong positive correlation (*p* < 0.01). The results of this study highlighted watery kimchi as a potentially valuable source of bioactive compounds, and the carotenoids and capsaicinoids were affected by the supporting ingredients used in watery kimchi. Furthermore, watery kimchi provides 4.11% of the recommended daily intake of vitamin A according to the 2020 Korean dietary reference intakes.

**Jiseong Lee, Saranya Kattil Parmbil, Nagendar Kumar Pandit, Sunil Kumar, Asad Syed, Abdallah M. Elgorban, Ling Shing Wong, Ranjana, Hoon Kim, Bijo Mathew. Development of morpholine ring-bearing halogenated α,β-unsaturated ketones as selective monoamine oxidase-B inhibitors. (2024) Appl. Biol. Chem. 67: 6**

Nine morpholine-derived halogenated chalcone derivatives (**MHC1**-**MHC9**) were synthesized, and their inhibitory activity against monoamine oxidase (MAO) was evaluated. **MHC5** showed the highest inhibitory activity against MAO-B with an IC50 value of 0.065 μM, followed by **MHC7** (IC50 = 0.078 μM) and **MHC6** (IC50 = 0.082 μM). The *para*-F substituent **MHC4** was also potent (IC50 = 0.095 μM). The selectivity index values of all the compounds were high for MAO-B over MAO-A, and the values for **MHC5** and **MHC4** were 66.15 and 80.11, respectively. **MHC5** and **MHC4** were competitive MAO-B inhibitors with Ki values of 0.024 ± 0.00062 and 0.041 ± 0.0028 μM, respectively. In reversibility tests, the changes in residual activity before and after the dialysis of **MHC5** and **MHC4** were similar to those of safinamide, a reversible MAO-B reference inhibitor. Additionally, molecular docking and dynamic simulations predicted that the lead molecules **MHC5** and **MHC4** could strongly bind to the MAO-B active site with docking scores of –10.92 ± 0.08 and –10.64 ± 0.14 kcal/mol, respectively. Additionally, **MHC4** and **MHC5** exhibited favorable ADME features, including blood–brain barrier permeability. The experiments confirmed that **MHC5** and **MHC4** are reversible and potent selective inhibitors of MAO-B and are promising candidates for the treatment of neurodegenerative diseases (human health).

**Meran Keshawa Ediriweera, Do Manh Cuong, Somi Kim Cho. Cow milk derived-fat inhibits the proliferation of liver cancer-cells. (2024) Appl. Biol. Chem. 67: 7**

Cow milk is a widely consumed liquid diet, and its fatty acid composition impacts its nutritional and biochemical properties. However, research on the anticancer efficacy of cow milk-derived fat in liver cancer cells is limited. This study investigated the antiproliferative and apoptotic effects of cow milk-derived fat in human hepatoma HepG2 cells. Additionally, the effects of cow milk-derived fat on cell cycle progression and Janus kinase (JAK)-2/signal transducer and activator of transcription (STAT)-3 signaling in HepG2 cells were assessed. Furthermore, the histone deacetylase inhibitory and 2,2-diphenyl-1-picrylhydrazyl radical scavenging potential of cow milk-derived fat were examined. The results demonstrate that cow milk-derived fat can exert antiproliferative and anticlonogenic effects and induce apoptosis in HepG2 cells dose-dependently. Furthermore, cow milk-derived fat induced cell cycle arrest, suppressed the levels of pJAK-2 and pSTAT-3, and inhibited the total histone deacetylase activity in HepG2 cells. The fatty acid profile of cow milk-derived fat revealed that palmitic, oleic, and linoleic acids were abundant as saturated, monounsaturated, and polyunsaturated fatty acids, respectively. Our findings provide a new scientific basis for the development of anticancer strategies that utilize cow milk-derived fat and its derivatives against liver cancer.

**Ziqing Yu, Wen He, Weiwu Shi. Sulforaphane (Sul) reduces renal interstitial fibrosis (RIF) by controlling the inflammation and TGF-β/Smad signaling pathway. (2024) Appl. Biol. Chem. 67: 8**

All chronic renal disorders eventually lead to renal interstitial fibrosis (RIF). Chronic inflammation and pro-fibrotic substances are familiar companions of the fibrotic process. The Sulforaphane (Sul) molecule is particularly useful in protecting the liver from oxidative damage. To investigate the Sul effects on fibrosis markers and inflammatory proteins in the kidney of NRK52E cell line and rats and clarify the mechanism of TGF-β/Smad signaling pathway in a rat model of RIF were developed in the present study. Sul (50, 100, and 200 ng/ml) remarkably reduced the gene expressions of tumor necrosis factor (TNF-α), interleukin-6 (IL-6), interleukin (IL)-1β, collagen 3 (COL3A1), collagen 1 (COL1A1), and α-smooth muscle actin (α-SMA) in fibrotic NRK52E cells compared with those in cells inspired by transforming growth factor-α (TGF-α). Histopathological investigations showed that Sul administration retained renal tissue structure and decreased kidney tissue fibrosis in rats subjected to unilateral ureteral blockage (UUO). The expression level of TNF-α, IL-6, IL-1β, COL3A1, COL1A1, and α-SMA in the rats’ kidneys exposed to UUO was also suppressed by the treatment of Sul. In the present study, western blot analysis showed that Sul upregulated the expressions of fibrotic NRK52E cells Smad7 and rat model UUO groups while simultaneously decreasing the stimulation of Smad2/3 and the expressions of cyclooxygenase-2, NF-κB, Smad4, activator protein-1, and high-mobility group protein B1. Ultimately, Sul’s ability to inhibit the TGF-β/Smad pathway and the development of inflammation factors may mitigate RIF.

**Eman A. Kotb, Riham A. El-Shiekh, Mariam Hassan, Wessam Hamdy Abd-Elsalam, Nebal El Tanbouly, Amira Safwat El Senousy. Potential anti-acne loaded nanogel formulations of *Origanum majorana* L. and *Chrysanthemum morifolium* Ramat. essential oils. (2024) Appl. Biol. Chem. 67: 9**

Acne is a highly prevalent skin disease with a great psychological impact on patients as self-perception, self-confidence, and depression. This work aimed to develop an anti-acne preparation from active anti-bacterial medicinal plants to circumvent the severe side effects and drug resistance commonly reported with topical erythromycin anti-acne preparations. Essential oils: *Salvia officinalis* L. (sage), *Rosmarinus officinalis* L. (rosemary), *Commiphora myrrha* Nees Engl. (myrrh), *Origanum majorana* L. (marjoram), *Pelargonium zonale* L. L’Hér. ex Aiton (geranium) and *Chrysanthemum morifolium* Ramat. (chrysanthemum) were extracted by hydrodistillation and analyzed using gas chromatography/mass spectrometry (GC/MS). The anti-acne activities of the oils against *Cutibacterium* acnes ATCC 6919 were evaluated by microdilution methods to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The most active essential oils were loaded in a film-forming nanogel prepared with chitosan, pluronic F127 and glycerol in the ratio of 3:1:1, prior to investigation in a murine acne in vivo model. Marjoram and chrysanthemum oils showed the highest antimicrobial activity against *C. acnes* (MIC = 0.156% *v/v* and 0.125% *v/v*, respectively). GC/MS of the actives showed that gamma-terpinene (26.46%) and terpinen-4-ol (22.24%) were the predominant constituents in marjoram, whereas chrysanthenone (32.79%) was the main component in chrysanthemum. The formulated essential oil-loaded film-forming nanogels of both oils exhibited significant anti-acne activity in mice via reducing the bacterial loads, activating the antioxidant nuclear factor erythroid 2–related factor 2 (Nrf2) pathway and inhibiting the inflammatory tumor necrosis factor-alpha (TNF-α) pathway. Further studies should be designed to evaluate the clinical evidence for the use of marjoram and chrysanthemum oil products in acne treatment.

**Jisu Lee, Hyun-Joo Lee, Hyunsoo Jang, Jae-Joon Lee, Jung-Heun Ha. High-iron consumption decreases copper accumulation and colon length, and alters serum lipids. (2024) Appl. Biol. Chem. 67: 10**

In this study, we aimed to demonstrate that a significant increase in dietary iron intake disrupts the regulation of copper availability, ultimately leading to systemic copper deficiency. To investigate this, we conducted experiments using five-week-old male weanling Sprague–Dawley rats fed diets based on AIN-93G with some modifications. These diets featured varying iron content, offering choices of adequate iron (~ 120 μg/g, near the upper limit [UL]) or high iron (~ 7544 μg/g), along with low (~ 0.3 μg/g), adequate (~ 6 μg/g), or high (~ 153 μg/g) levels of dietary copper over a 5-week period. Rats consuming the high-iron diets displayed anemia, reduced copper levels in their organs and feces, and shortened colon lengths. Increased dietary iron intake resulted in an overall reduction in copper distribution within the body, likely leading to severe copper deficiency-related disorders in the experimental rats. However, the physiological disturbances caused by a high-iron diet were prevented when additional copper was included in the rodent diet. Furthermore, high iron intake led to copper deprivation, and high iron consumption resulted in elevated serum cholesterol levels. However, increasing dietary copper consumption led to a decrease in overall serum cholesterol levels. Additionally, serum alkaline phosphate and aspartate aminotransferase levels were increased by high-iron feeding, regardless of dietary copper concentration, while alanine aminotransferase levels decreased.

**Sang Gyu Han, Tae Gyu Nam. Simultaneous determination of sulfonylurea herbicides in tomatoes using the QuEChERS method coupled with HPLC. (2024) Appl. Biol. Chem. 67: 12**

The simultaneous determination of trace pesticides in complex matrices containing high concentrations of natural pigments remains challenging. In this study, quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation together with high-performance liquid chromatography with ultraviolet detection (HPLC–UV) was applied for the multi-residue analysis of seven sulfonylurea herbicides (SUHs) in tomatoes. SUH residue was extracted using the QuEChERS procedure, followed by solid-phase extraction (SPE) and dispersive SPE (d-SPE). To reduce the amount of carotenoids in tomato extracts, several d-SPE clean-up procedures were compared, and octadecylsilane (C18) provided the best color removal rate (%) of tomato extracts and recoveries (%) for all the tested SUHs. The validation results indicate good linearity (R2 > 0.9970), accuracy, and precision. Recoveries of 70–120% and relative standard deviations < 20% were achieved for all analytes at three spiked concentrations. The limits of detection and quantification for the 7 SUHs were 0.003 mg kg−1 and 0.008–0.009 mg kg−1, respectively. The developed method was subsequently used to quantify multi-residue SUHs during real sample analysis. None of the tested samples had SUH residue levels higher than the maximum residue limits established by the Korean Ministry of Food and Drug Safety. The results suggest that QuEChERS sample preparation employing a combination of C18 is a high-throughput and rapid clean-up procedure for the multi-residue analysis of SUHs in tomatoes.

**Yu Ra Kim, Sunyoung Park, Ji Young Kim, Jang-Duck Choi, Gui-Im Moon. Simultaneous determination of 31 Sulfonamide residues in various livestock matrices using liquid chromatography-tandem mass spectrometry. (2024) Appl. Biol. Chem. 67: 13**

The widespread use of sulfonamides can result in the residue of sulfonamides in the foods of animal origin that are the major concerns of consumers and regulatory bodies due to their adverse reaction such as the development of antibiotic resistance. A rapid and efficient multi-residue analytical method was developed to screen and confirm 31 sulfonamides in livestock samples in a single run, using ultra-high-performance liquid chromatography combined with comprehensive mass spectrometric approaches. In this study, a novel sample preparation procedure was used, based on a modified QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe). The linearity, sensitivity, accuracy, and precision of the method were validated according to the Codex guidelines. The response of the detector was linear for each target compound over a wide concentration range, with a correlation coefficient (r2) greater than 0.98. The limit of detection (LOD) and limit of quantification (LOQ) ranged from 0.3 to 5 ng *g*−1 and from 1 to 19 ng *g*−1 and the average recoveries (%) for three laboratories ranged from 85 to 109% with a CV (n = 5) below 22%. The applicability of this screening method was verified using real livestock samples. The proposed analytical method achieves identification and quantification of target sulfonamides at trace levels in a short analysis time. None of the samples contained residues that exceeded the maximum residue limit (MRL).

**Jung-Hwan Yoon, Bo-Hyun Kim, Kye-Hoon Kim. Distribution of microplastics in soil by types of land use in metropolitan area of Seoul. (2024) Appl. Biol. Chem. 67: 15**

Plastic pollution is becoming a significant problem in urban areas due to excessive use and careless disposal. While studies on microplastics are increasingly being conducted across various environments, research on microplastics in soil is limited compared to other areas. Microplastics entering the soil through various routes can stay there for a long period of time, threatening soil organisms and eventually humans. Therefore, this study was carried out to investigate the distribution characteristics of microplastics according to types of land use. For this purpose, a total of 54 soil samples were collected from agricultural land, residential areas, roadsides, parks, and forests. The analysis of microplastics in the soil by stereo microscopy showed that the average numbers of microplastics (particles/kg) in agricultural land, residential areas, roadsides, parks, and forests were 5047, 3646, 4987, 2673, and 1097, respectively. Various colors (black, red, green, blue, yellow, white, and transparent) and shapes (fragment, fiber, film, and sphere) of microplastics were found in soils. The combination of black x fragment plastics showed the highest frequency. Microplastics in soil samples from agricultural land, roadside, and residential areas with sizes between 20 µm and 500 µm were determined using Fourier transform infrared spectrometer (FT-IR) and analyzed by MP finder. The number of microplastics detected in the soil with sizes ranging between 20 µm and 500 µm was in the order of roadside > residential areas > agricultural land, which was different from the results by stereomicroscopy. Polyethylene (PE), polypropylene (PP), and polymethyl methacrylate (PMMA) were detected in soils from roadsides. Polyurethane (PU), cellulose acetate (CA), polyethylene terephthalate (PET), PP, and polystyrene (PS) were detected in soils from residential areas, with PU being the most frequently detected.

**Minji Kim, Jangeun An, Seong-Ah Shin, Sun Young Moon, Moonsu Kim, Seyeon Choi, Huiji Kim, Kim-Hoa Phi, Jun Hyuck Lee, Ui Joung Youn, Hyun Ho Park, Chang Sup Lee. Anti-inflammatory effects of TP1 in LPS-induced Raw264.7 macrophages. (2024) Appl. Biol. Chem. 67: 16**

Inflammation is an essential defense mechanism in health; however, excessive inflammation contributes to the pathophysiology of several chronic diseases. Although anti-inflammatory drugs are essential for controlling inflammation, they have several side effects. Recent findings suggest that naturally derived compounds possess physiological activities, including anti-inflammatory, antifungal, antiviral, anticancer, and immunomodulatory activities. Therefore, this study aimed to investigate the anti-inflammatory effects and molecular mechanisms of 2,5,6-trimethoxy-p-terphenyl (TP1), extracted from the Antarctic lichen *Stereocaulon alpinum*, using in vitro models. TP1 treatment decreased the production of nitric oxide (NO) and reactive oxygen species (ROS) in LPS-stimulated Raw264.7 macrophages. Additionally, TP1 treatment significantly decreased the mRNA levels of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) and the mRNA and protein levels of the pro-inflammatory enzymes (inducible nitric oxide synthase and cyclooxygenase-2). Moreover, TP1 suppressed lipopolysaccharide-induced phosphorylation of the NF-κB and MAPK signaling pathways in Raw264.7 macrophages. Conclusively, these results suggest that TP1 ameliorates inflammation by suppressing the expression of pro-inflammatory cytokines, making it a potential anti-inflammatory drug for the treatment of severe inflammatory diseases.

**Tham Thi Mong Doan, Gia Han Tran, Toan Khac Nguyen, Jin Hee Lim, Sanghyun Lee. Antioxidant activity of different cultivars of *Chrysanthemum morifolium* and quantitative analysis of phenolic compounds by HPLC/UV. (2024) Appl. Biol. Chem. 67: 17**

*Chrysanthemum morifolium* is classified within the Asteraceae botanical family and serves as a phytomedicine in many countries. The objective of this study was to quantitatively analyze twelve phenolic compounds through HPLC/UV and to assess the antioxidant abilities using the DPPH and ABTS+ assays in the leaves and flowers of six cultivars of *C. morifolium*: ‘Geumsu’, ‘Ilonka’, ‘Silvia’, ‘Pompadour’, ‘Yes Holic’, and ‘Ford’. The results indicated that the leaves of ‘Geumsu’ and ‘Ford’, as well as the ‘Pompadour’ flowers contained high levels of phenolic compounds and exhibited strong antioxidant abilities. Additionally, a relationship between the phenolic compounds and antioxidant activities was observed. These findings provide foundational knowledge about *C. morifolium* cultivars, which are promising natural sources that can offer health benefits.

**Mangeun Kim, Min Hye Kim, Jinho Kim, Kyungpil Kang, Junsu Lee, Mrinmoy Ghosh, Young-Ok Son. Comprehensive in vitro and in vivo investigations of the therapeutic potential of Jeju lava seawater salt in osteoarthritis. (2024) Appl. Biol. Chem. 67: 18**

Salts play a crucial role in maintaining human health by regulating fluid levels and supporting various physiological processes. However, conventional seawater-derived salts are associated with microplastic pollution and pose potential health risks. Jeju lava seawater (JLS), sourced exclusively from Jeju Island, has emerged as a unique alternative, free of microplastics and enriched with essential minerals such as magnesium, calcium, zinc, and iron. In this study, we investigated the effects of JLS on osteoarthritis (OA) pathogenesis, focusing on chondrocyte metabolism and OA development. We performed surgical destabilization of the medial meniscus to establish a murine model of OA. We examined the expression of catabolic and anabolic factors in JLS-treated chondrocytes. Our cell viability assay revealed that JLS treatment was not cytotoxic to chondrocytes at concentrations ≤ 0.5%. Additionally, JLS treatment resulted in a concentration-dependent increase in the expression of anabolic factors like aggrecan, SOX9, and COL2A1 while decreasing the expression of catabolic factors such as MMP3, MMP13, ADAMTS4, and ADAMTS5 in the chondrocytes stimulated with pro-inflammatory cytokines. Although not statistically significant compared to the control group, JLS intake slightly attenuated the OARSI score, osteophyte score, synovitis score, subchondral bone thickness, and osteophyte size in the mouse model of OA. Conclusively, these results suggest that JLS ameliorates OA by positively influencing chondrocyte metabolism, making it a promising therapeutic candidate for OA management.

**Seung-Gyeom Kim, So-Hyeon Park, Joong-Hyuck Auh. Antioxidant and anti-inflammatory activities of the methanol extract from the bran of the colored wheat, 'Ariheuk'. (2024) Appl. Biol. Chem. 67: 19**

In vitro antioxidant and anti-inflammatory activities were investigated using a 70% acidic methanol extract of the colored wheat bran, 'Ariheuk.' Active metabolites were identified via metabolomic analysis using multivariate statistical comparisons. The 'Ariheuk' bran extract (ABE) contained a higher total anthocyanin content (0.19 mg C3G/g) than the general wheat bran extract (GBE) (0.01 mg C3G/g). ABE exhibited stronger antioxidant and anti-inflammatory activities than GBE. The mechanism underlying the anti-inflammatory effects of ABE was explored by assessing the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) in RAW 264.7 cells stimulated with lipopolysaccharide (LPS). The crude ABE extract was also partially fractionated into three subfractions (ABE-F1, ABE-F2, and ABE-F3) using preparative liquid chromatography (Prep-LC) to identify the active metabolites. The total anthocyanin content was highest in ABE-F3 (1.91 ± 0.06 mg C3G/g). Among the subfractions, ABE-F2 exhibited the highest antioxidant and anti-inflammatory activities. Several distinct metabolites contributing to the activities of ABE-F2 were identified, including various cyanidin and peonidin derivatives and apigenin derivatives, such as corymboside and schaftoside.

**Ju Yeon Moon, Saet Buyl Lee, Yu Jeong Jeong, Gah-Hyun Lim, Gilok Shin, Man-Soo Choi, Jeong Ho Kim, Ki Hun Park, Jiyoung Lee, Jae Cheol Jeong, Cha Young Kim. Soybean flower-specific R2R3-MYB transcription factor gene *GmMYB108* induces anthocyanin production in *Arabidopsis thaliana*. (2024) Appl. Biol. Chem. 67: 20**

R2R3-MYB transcription factors (TFs) are known to play a key role in regulating the expression of structural genes involved in plant flavonoid biosynthesis. However, the regulatory networks and related genes controlling isoflavonoid biosynthesis in soybean are poorly understood. We previously reported that ethephon application increases the production of isoflavonoids in soybean leaves. In this study, we attempted to identify a potential regulatory gene that positively controls isoflavonoid production in response to ethephon treatment in soybean (*Glycine max* L.). RNA sequencing (RNA-seq) revealed that ethephon application led to the upregulation of 22 genes, including the genes for R2R3-MYB TFs, related to isoflavonoid biosynthesis in soybean plants. Ethephon treatment highly induced the expression of *GmMYB108*, and its expression was exclusively enriched in flowers as determined using in silico and real-time quantitative PCR analyses. Furthermore, *GmMYB108* overexpression resulted in an intense accumulation of anthocyanins as well as total flavonoid production in the leaf tissues of transgenic *Arabidopsis* plants. In addition, *GmMYB108* overexpression increased the transcript levels of several genes involved in the biosynthesis of anthocyanins and their regulatory pathways in *Arabidopsis*. These results suggest that *GmMYB108* is a potential positive regulator of the biosynthesis of flavonoids and anthocyanins in soybean flowers.

**So Hee Yang, Suk Weon Kim, Sujin Lee, Yeonjong Koo. Optimized protocols for protoplast isolation, transfection, and regeneration in the Solanum genus for the CRISPR/Cas-mediated transgene-free genome editing. (2024) Appl. Biol. Chem. 67: 21**

The Solanaceae family includes the largest flowering crops such as tomatoes, potatoes, and eggplants. Consumer demand has led to massive development of plants in the Solanum genus, and many different Solanum varieties are now available on the market. The recent advances in Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein 9 (Cas9)-based genome editing have allowed laboratories and smaller crop production companies to utilize the technology in various crops. The traditional transformation method in crops involves the use of Agrobacterium, which is considered the most efficient method for introducing exogenous genetic materials in target plants. The Agrobacterium-mediated transformation method has been also established in the Solanaceae family, enabling CRISPR/Cas-based genome editing in crops like tomatoes, potatoes, and eggplants. However, the Agrobacterium-mediated approach inevitably accompanies the insertion of exogenous DNA into the plant genome and often causes the formation of chimera that require further propagation steps. Alternatively, the CRISPR/Cas components can be introduced into protoplasts in the form of DNA for transient expression or a mixture of protein and RNA to avoid genomic insertion of foreign materials. The protoplast transformation approach involves processes including protoplast preparation, transfection, and regeneration, which require a comprehensive understanding and greater technical mastery of the tissue culture phase. Here we highlight the current research advances in protoplast transformation and discuss how to optimize the procedures of protoplast isolation, transfection, and regeneration for efficient and reproducible CRISPR/Cas-based genome editing in the genus Solanum.

**Young-Ji Jeon, Yun-Gu Kang, Jin-Ah Eun, Taek-Keun Oh. Yield, functional properties and nutritional compositions of leafy vegetables with dehydrated food waste and spent coffee grounds. (2024) Appl. Biol. Chem. 67: 22**

This study determined the fertilizer potentials of the dehydrated food waste powder (DFWP) and spent coffee grounds (SCGs) through assessing their effects on yield, antioxidant activities, mineral and proximate compositions of leaf lettuce and Japanese hogfennel their efficiencies to inorganic fertilizers (N-P2O5-K2O, NPK). In this study, both organic amendments were applied at rates that supplied half, double and recommended nitrogen (N) requirements of the leaf lettuce (15 Mg N/ha) and Japanese hogfennel (10 Mg N/ha) established in Daejeon, South Korea. The recommended treatment of DFWP produced the highest lettuce and Japanese hogfennel yields, respectively. Halving the application rates of the organic amendments generally limited the yielding capacities while doubling them invoked negative yield responses in both crops. The highest antioxidant activities, mineral and proximate contents in both crops were obtained with the recommended dosage of amendments. The SCGs outperformed NPK in all the parameters of the Japanese hogfennel assessed in this study even though its impact on the leaf lettuce was adverse. Therefore, both DFWP and SCGs can effectively supply plant nutrients but their application rates should be regulated so as to avoid NaCl toxicity and elevated phytotoxicity in DFWP and SCGs, respectively.

**Doyoung Kim, Imkyung Oh. Development of fermented beverage with citrus fruit extract using probiotics: impact on antioxidant activity and in vitro digestibility. (2024) Appl. Biol. Chem. 67: 23**

Redhyang *(Citrus hybrid ‘Kanpei’,*CHK) is a subtropical citrus species introduced in Korea due to climate change. To enhance the nutritional value and usability of CHK as a processed food product, CHK extract was fermented with four types of commercial starters (YoFlex Harmony 1.0 (YFH), ABY-3 (ABY), YC-X11 (YXC), and YC-180 (YC)), and their antioxidant activities and changes in chemical properties during fermentation were investigated. The consumer acceptance of probiotic beverages containing fermented CHK extracts and their viability and antioxidant activity through in vitro digestion were also elucidated. The enumeration of lactic acid bacteria (LAB) in all samples after fermentation was above 7.60 log colony-forming units (CFU)/mL, with YC exhibiting the highest number after 24 h. Fermented CHK extracts containing higher levels of organic acids, total polyphenols, and flavonoids tended to exhibit higher antioxidant activities. YFH, ABY, and YC showed maximum antioxidant activity at 24 h, whereas YXC showed differences in the types of LAB at 12 h. After in vitro digestion, YXC showed higher antioxidant activity and LAB viability than the control. This result indicates that CHK extract fermented with YXC can increase antioxidant activity, bioactive ingredients, and sensory preference and positively impact the production of probiotic beverages.

**Se Jeong Kim, San Kim, Sehyeon Jang, Da Hye Gu, Jeong Min Park, Jung A. Ryu, Sung Ran Yoon, Sung Keun Jung. *Curcuma longa* L. extract increased immune responses in RAW 264.7 cells and cyclophosphamide-induced BALB/c mice. (2024) Appl. Biol. Chem. 67: 24**

*Curcuma longa* L. extract (CLE) exerts various biological functions including antioxidant, anti-inflammation, anticancer, and antiallergenic effects. However, its immune-enhancing capacity remains unclear. Therefore, the immune-enhancing effect of CLE was investigated in RAW 264.7 cells and cyclophosphamide (CPP)-induced immunosuppression model. CLE upregulated nitric oxide (NO) and reactive oxygen species production and increased inducible nitric oxide synthase and cyclooxygenase-2 expression without affecting the RAW 264.7 cells viability. The results of quantitative real-time reverse transcription polymerase chain reaction and sandwich enzyme-linked immunosorbent assay showed that CLE increased the gene expression and protein levels of tumor necrosis factor-α, interleukin-6, and interleukin-1β in RAW 264.7 cells. Moreover, CLE upregulated p65, I kappa B kinase α/β, and I kappa B α (IκBα) phosphorylation and downregulated IκBα expression in RAW 264.7 cells. CLE also increased p65 translocation from the cytoplasmic to the nucleus in RAW 264.7 cells. The oral administration of CLE increased organ indexes (including the spleen and thymus) and NO production in peritoneal macrophages and improved natural killer cell activity in CPP-induced immunosuppression BALB/c mice. Overall, CLE could be a useful health functional food material that can improve innate immunity via macrophage activation.

**Chhychhy Chao, Hyong Kyong Nam, Hyun Jin Park, Hyun Woo Kim. Potentials of 3D printing in nutritional and textural customization of personalized food for elderly with dysphagia. (2024) Appl. Biol. Chem. 67: 25**

Elderly individuals commonly experience the risk of dysphagia or difficulties in eating and swallowing food safely. Three-dimensional (3D) food printing is a promising technique widely used in customized food development. This paper reviewed the potential of 3D food printing in nutritional customization and textural modification of personalized food for the elderly with dysphagia. 3D food printing can be used to re-formulate the food ink by combining more than one type of food materials to ensure high calorie and nutrient intake, improve sensory quality, and prevent malnutrition; thus, understanding the functional properties of such macronutrients compounds is essential to design food ink that meets personalized nutrient requirements. Hydrocolloids have been commonly used to modify the desired soft texture and consistent viscoelastic properties of 3D-printed elderly food, as well as improve printability and structural stability. The food standard guidelines have been established and used to categorize texture-modified foods to ensure easy to eat and safe swallowing for the elderly with swallowing difficulties. Finally, the production of personalized food using 3D printing may provide more food options, facilitate safe oral intake, and increase calorie intake to improve the healthy mealtime experience for the elderly.

**Jong Min Oh, Qian Gao, Woong-Hee Shin, Eun-Young Lee, Dawoon Chung, Grace Choi, Sang-Jip Nam, Hoon Kim. Pannorin isolated from marine *Penicillium* sp. SG-W3: a selective monoamine oxidase A inhibitor. (2024) Appl. Biol. Chem. 67: 26**

Six compounds were isolated from *Penicillium* sp. SG-W3, a marine-derived fungus, and their inhibitory activities against target enzymes relating to neurological diseases were evaluated. Compound **1** (pannorin) was a potent and selective monoamine oxidase (MAO)-A inhibitor with a 50% inhibitory concentration (IC50) of 1.734 μM and a selectivity index (SI) of > 23.07 versus MAO-B, and it showed an efficient antioxidant activity. All compounds showed weak inhibitory activities against acetylcholinesterase, butyrylcholinesterase, and β-secretase. The inhibition constant (Ki) of **1** for MAO-A was 1.049 ± 0.030 μM with competitive inhibition. Molecular docking simulation predicted that compound **1** forms hydrogen bonds with MAO-A, and binds more tightly to MAO-A than to MAO-B (− 25.02 and − 24.06 kcal/mol, respectively). These results suggest that compound **1** is a selective, reversible, and competitive MAO-A inhibitor that can be a therapeutic candidate for treating neurological diseases.

**Ping Tang, Sitong Liu, Junshun Zhang, Zhiyi Ai, Yue Hu, Linlin Cui, Hongyang Zou, Xia Li, Yu Wang, Bo Nan, Yuhua Wang. Ginsenosides as dietary supplements with immunomodulatory effects: a review. (2024) Appl. Biol. Chem. 67: 27**

Immune disorders have become one of the public health problems and imposes a serious economic and social burden worldwide. Ginsenosides, the main active constituents of ginseng, are regarded as a novel supplementary strategy for preventing and improving immune disorders and related diseases. This review summarized the recent research progress of ginsenosides in immunomodulation and proposed future directions to promote the development and application of ginsenosides. After critically reviewing the immunomodulatory potential of ginsenosides both in vitro and in vivo and even in clinical data of humans, we provided a perspective that ginsenosides regulated the immune system through activation of immune cells, cytokines, and signaling pathways such as MAPK, PI3K/Akt, STAT, and AMPK, as well as positively affected immune organs, gut flora structure, and systemic inflammatory responses. However, the evidence for the safety and efficacy of ginsenosides is insufficient, and the immune pathways of ginsenosides remain incompletely characterized. We believe that this review will provide a valuable reference for further research on ginsenosides as dietary supplements with immunomodulatory effects.

**Molly E. Murray, Beatriz G. Goncalves, Mary A. Biggs, Sophia A. Frantzeskos, Charlotta G. Lebedenko, Ipsita A. Banerjee. Exploring the binding interactions of NOP receptor with designed natural phytochemical-neuropeptide conjugates: an in silico and SPR study. (2024) Appl. Biol. Chem. 67: 28**

The Nociceptin/orphanin FQ peptide (NOP) receptor is considered a member of the opioid receptor subfamily of G-protein coupled receptors (GPCRs) which has been shown to be present in many parts of the central nervous system (CNS). It plays biologically diverse roles in pain modulation, immune response and in neurodegenerative diseases. In this work, phytochemical conjugates of two known neuropeptides, melanocyte inhibition factor (MiF-1) and mammalian amidated neuropeptide NPFF with pain modulating ability were developed. The binding interactions of those conjugates with NOP receptor was examined as an approach to develop novel natural compounds that can modulate NOP receptor activity. The selected phytochemicals are well-known for their antioxidant abilities and are derived either from natural alkaloids (betanin), polyphenols (gallic acid and sinapic acid) or terpenes (pomolic acid). Each of the phytochemicals selected are antioxidants which may play a role in mitigating diseases. Three conjugates of betanin were designed with each peptide by conjugating each of the three carboxylic acid groups of betanin with the peptides, while all others were mono-conjugates. Our results indicated that the betanin conjugates with both peptides showed strong binding interactions while the pomolate-peptide conjugates showed moderate binding. In general, NPFF and its conjugates showed stronger binding with the receptor. Docking and molecular dynamics studies revealed that binding interactions occurred at the binding pocket encompassing the transmembrane helices TM1, TM3 and TM7 in most cases, with the ligands binding deep within the hydrophobic core. The binding interactions were further confirmed experimentally through SPR analysis, which also showed higher binding with the betanin conjugates. MMGBSA studies indicated that the binding energies of MiF-1 conjugates were higher compared to neat MiF-1. However, in the case of NPFF, while the betanin conjugates showed enhancement, in some cases the binding energies were found to be slightly reduced compared to neat NPFF. Overall our studies reveal that such natural phytochemical derivatives that can bind to the NOP receptor when conjugated to the mammalian amidated neuropeptide NPFF and the short sequence of melanocyte inhibiting factor MiF-1 may be potentially developed for further laboratory studies for potential pharmaceutical applications.

**Amany Ragab, Mohamed A. Taher, Helmy H. El-Rafey, Ahmed Ramadan El-Rokh. Bioactive compounds from *Withania somnifera* dun and their toxicity against some piercing sucking pests. (2024) Appl. Biol. Chem. 67: 29**

Piercing sucking pests are destructive to many strategic crops all over the world. Botanical pesticides can be used to control these pests. A new withanolide derivative **3** named sominone A ((20*R*,22*R*)-1*α*,3*β*,20,27-tetrahydroxywitha-5,24-dienolide) was isolated from the alkaloid fraction of the whole plant of *Withania somnifera*. In addition, there are three known compounds named withasomine **1**, methyl isoferulate **2**, and coagulin Q **4** were also isolated. The structures of isolated compounds were identified using different spectroscopic methods such as 1D, 2D NMR, and HRESIMS spectroscopy. The alkaloid fraction and the four isolated compounds were tested for their pesticidal activity against four piercing sucking pests (*Aphis craccivora* Koch, *Bemisia tabaci* Gennadius, *Nezara viridula* Linnaeus, and *Tetranychus urticae* Koch) that attack many strategic crops under laboratory conditions, along with azadirachtin (Okios 3.2% EC) as a positive control. The results showed that the alkaloid compound (withasomine **1**) was the most toxic to *A. craccivora*, *B. tabaci*, *N. viridula,* and *T. urticae,* with LC50 values of 15.44, 36.61, 85.11, and 128.28 ppm, respectively, compared with the control. Withanolide compounds had moderate effects on all tested pests. Biochemical parameters of six enzymes; *α*-esterase,*β*-esterase, chitinase, acetylcholinesterase, glutathione-*S*-transferase, and peroxidase of *A. craccivora* were estimated at the LC50 value of the most potent compound, withasomine **1** and the values were 38.83, 72.86, 31.45, 506.4, 2.62, and 251.0, respectively. The results demonstrated that all enzymes activity levels were increased compared with the control except a remarkable inhibition in AChE enzyme level was observed compared with control. Therefore, the alkaloid fraction of *W. somnifera* is a promising extract that contains many active compounds that can be used as a natural pesticide against many harmful pests in agriculture crops.

**Jinghui Wang, Qiyou Zheng, Chenxu Wang, Ao Zhou. Classification of soybeans from different habitats based on metabolomic–transcriptomic integration. (2024) Appl. Biol. Chem. 67: 30**

Soybeans are a significant agricultural product in China, with certain geographical locations often yielding higher quality, and thus more expensive, soybean crops. In this study, metabolomics and transcriptomics analyses were conducted on soybean samples from nine regions in Heilongjiang and Liaoning Provinces using untargeted liquid chromatography–mass spectrometry (LC–MS) and Illumina sequencing technologies. The primary objective was to devise an effective and unbiased method for determining the geographical origin of each soybean variety to mitigate potential fraudulent practices. Through multidimensional and unidimensional analyses, successful identification of differentially expressed metabolites (DEMs) and differentially expressed genes (DEGs) was achieved, yielding statistically significant outcomes. Integration of the metabolomics and transcriptomics datasets facilitated the construction of a correlation network model capable of distinguishing soybeans originating from different geographical locations, leading to the identification of significant biomarkers exemplifying noteworthy distinctions. To validate the feasibility of this method in practical applications, partial least squares discriminant analysis was employed to differentiate soybean samples from the nine regions. The results convincingly showcased the applicability and reliability of this approach in accurately pinpointing the geographical origin of soybeans. Distinguishing itself from prior research in soybean traceability, this study incorporates an integrated analysis of metabolomics and transcriptomics data, thereby unveiling biomarkers that offer a more precise differentiation of soybean traits across distinct regions, thereby bridging a critical research gap within the soybean traceability domain. This innovative dual-data integration analysis methodology is poised to enhance the accuracy of soybean traceability tools and lay a new foundation for future agricultural product identification research.

**Won Min Jeong, Seung-Jin Kwag, Jun Young Ha, Seung-Jun Lee, Yeong-In Choe, Dong Yeol Lee, Dong Kyu Jeong, Hwan Hee Bae, Jin-Hee Seo, Young-Sool Hah, Sang Gon Kim. Acetyl genistin modulates myotube differentiation and attenuates dexamethasone-induced muscle atrophy through the FoxO1/3 signaling pathway in C2C12 myotubes. (2024) Appl. Biol. Chem. 67: 31**

Muscle atrophy, a debilitating condition characterized by loss of muscle mass and strength, is a major concern in various clinical settings. Acetyl genistin (AG), a bioactive compound, was evaluated for its role in muscle cell differentiation and its potential protective effects against dexamethasone (dexa)-induced muscle atrophy. Our study demonstrated that AG significantly promoted C2C12 myotube differentiation, as evidenced by enhanced myotube width and increased fusion index. Notably, AG treatment upregulated the expression of myogenic markers, including MHC, MyoD, and MyoG. Moreover, AG displayed protective properties by attenuating dexa-induced muscle atrophy, mainly by suppressing the expression of the atrophy-related genes MAFbx and MuRF1. AG's protective effects are mechanistically attributed to its regulation of the AMPK/FoxO-dependent signaling pathway. Our results highlighted the dual benefits of AG in fostering muscle differentiation and safeguarding against muscle atrophy, positioning it as a promising agent for muscle health and therapeutic applications.

**Sullim Lee, Yunjeong Lee, Yunseo Kim, Hyunji Kim, Haerim Rhyu, Kyoungmi Yoon, Chang-Dae Lee, Sanghyun Lee. Beneficial effects of cannabidiol from *Cannabis*. (2024) Appl. Biol. Chem. 67: 32**

Cannabis, traditionally used for recreation due to psychoactive compounds in its leaves, flowers, and seeds, has not been thoroughly explored for potential therapeutic benefits. Δ9-*trans*-Tetrahydrocannabinol, a key cannabinoid in cannabis, causes hallucinogenic effects and delirium symptoms. In contrast, cannabidiol (CBD) does not induce hallucinations and has shown effectiveness in treating symptoms of various rare, incurable diseases. Cannabis exhibits neuroprotective, anti-inflammatory, anti-thrombotic, anti-bacterial, analgesic, and antiepileptic properties, recently attracting more attention. This review aims to summarize comprehensively the impact of cannabis on human health, focusing on endocannabinoids and their receptors. It also delves into recent CBD research advancements, highlighting the compound’s potential medical applications. Overall, this paper provides valuable insights into the prospective development of medical cannabis, with a particular emphasis on CBD.

**Yun Ji Park, To Quyen Truong, Yeong Bin Choi, Phuong Kim Huynh, Jinyoung Moon, Song Yi Koo, Hyoung Seok Kim, Sang Min Kim. Correlation analysis between artemisinin and its derivative contents and trichome characteristics from different *Artemisia* species.**

**(2024) Appl. Biol. Chem. 67: 33**

*Artemisia* species have significant commercial, medical, and economic value and are widely used in the traditional medicine and pharmaceutical industries. Artemisinin, a powerful antimalarial agent, is an important pharmaceutical metabolite that primarily accumulates within the glandular trichomes (GTs) on the leaf surface of *Artemisia* plants. Trichomes arising from the elongation of epidermal cells can be classified into GTs and non-glandular trichomes (NGTs) based on their morphology. GTs and NGTs are present in *Artemisia* species, and the relationship between GTs and artemisinin has been extensively studied; however, the correlation between NGTs and artemisinin remains relatively unexplored. In this study, we inferred artemisinin derivatives and trichome characteristics based on the type of species, developmental stage, and leaf age and conducted correlation analyses to investigate the factors influencing artemisinin content across different *Artemisia* species. Artemisinin and its derivatives exhibited variations in distribution based on species and leaf age, with a decreasing trend observed across most species as the developmental stage progressed. Noticeable differences among *Artemisia* species were observed in leaf shape, morphology, and trichome distribution. Although the observed data did not evidently differentiate between species, developmental stage, and leaf age groups, principal component analysis revealed that artemisinin was positively associated with the NGTs density, indicating a correlation coefficient of 0.56 (*p* < 0.0001). Therefore, the number of NGTs may affect the artemisinin content in different *Artemisia* species.

**Yun Ji Park, Yeong Bin Choi, Sang-Bin Oh, Jinyoung Moon, To Quyen Truong, Phuong Kim Huynh, Sang Min Kim. Development and application of a high-performance liquid chromatography diode-array detection (HPLC–DAD) method for the simultaneous quantification of phenolic compounds in the aerial part of *Glehnia littoralis*. (2024) Appl. Biol. Chem. 67: 34**

*Glehnia littoralis*, a medicinal herb employed in traditional practices for alleviating fatigue, cough, and a dry throat, is recognized for its beneficial properties due to a diverse array of active compounds found in its extracts. For example, the *G. littoralis* roots (Radix Glehniae) mainly contain coumarins and phenolic acids, serving as the primary focus of this study. Despite the widespread use of the tools in various industries and the development of multiple analytical methods for their examination, the edible aerial parts have industrial potential, and there is currently no analytical method available to identify their key components. In this study, a high-performance liquid chromatography method combined with diode array detection (HPLC–DAD) was developed to simultaneously detect 16 phenolic compounds previously reported to be present in the edible aerial parts of *G. littoralis*. The proposed approach included using gradient elution to change the solvent system from water/acetonitrile to water/methanol. Furthermore, the method validation was conducted, assessing its linearity, limit of detection, limit of quantification, precision, accuracy, and recovery, all of which demonstrated satisfactory results. Subsequently, the developed method was applied to quantify the phenolic compounds in various *G. littoralis* samples obtained from different organs, solvent extraction processes, and processing methods. Moreover, the online HPLC-ABTS (2,2ʹ-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) assay was used to evaluate the antioxidant capacities of individual constituents, identifying four important antioxidants and estimate the overall antioxidant capacity of the *G. littoralis* extract.

**Riham A. El-Shiekh, Rana Elshimy, Asmaa A. Mandour, Hanaa A. H. Kassem, Amal E. Khaleel, Saleh Alseekh, Alisdair R. Fernie, Mohamed A. Salem. *Murraya* *koenigii* (L.) Sprengel seeds and pericarps in relation to their chemical profiles: new approach for multidrug resistant *Acinetobacter* *baumannii* ventilator-associated pneumonia. (2024) Appl. Biol. Chem. 67: 35**

*Acinetobacter* *baumannii* is without a doubt one of the most problematic bacteria causing hospital-acquired nosocomial infections in today's healthcare system. To solve the high prevalence of multi-drug resistant (MDR) in *A.* *baumannii*, we investigated one of the medicinal plants traditionally used as antibacterial agent; namely *Murraya* *koenigii* (L.) Sprengel. The total methanolic extracts of seeds and pericarps were prepared and their anti-bacterial activity was assessed using the agar diffusion method and minimum inhibitory concentration (MIC) was then calculated as compared to tigecycline. Then, an in-vivo murine model was established which confirmed the promising activity of *M.* *koenigii* seeds in demonstrating anti-bacterial and anti-inflammatory actions. The histopathological study of lungs, scoring of pulmonary lesions, counting of bacterial loads after infection by multi-drug resistant *A.* *baumannii* all provided evidence to support these findings. LC–MS/MS profiling coupled to molecular networking and chemometrics detected the presence of carbazole alkaloids, and coumarins as dominate metabolites of the active seed extracts. Positively correlated metabolites to antibacterial potential were 6-(2ʹ,3ʹ-dihydroxy-3-methylbutyl)-8-prenylumbelliferone, scopoline, and 5-methoxymurrayatin. An in-silico study was also performed on the crystal structure of MurF from *A.* *baumannii* (PDB ID: 4QF5), the studied structures of the mentioned extracts revealed good docking interaction at the active site suggestive of competition with the ATP ligand. These collective findings suggest that extracts of *Murraya* *koenigii* (L.) Sprengel seed is a novel prospective for the discovery of drug candidates against infections caused by MDR *A.* *baumannii*.

**Seung-Jun Lee, Kyoung Hwan Cho, Jong Cheol Kim, Ho Jin Choo, Jeong-Yun Hwang, Hyun Chin Cho, Young-Sool Hah. Salem. Comparative analysis of anti-obesity effects of green, fermented, and γ-aminobutyric acid teas in a high-fat diet-induced mouse model. (2024) Appl. Biol. Chem. 67: 36**

Obesity, a prevalent disease associated with numerous chronic conditions, including hyperlipidemia, hyperglycemia, diabetes, and metabolic syndrome, remains a major global health challenge. This study investigated the potential of green tea (GT), fermented tea (FT), and γ-aminobutyric acid (GABA) tea (GBT), which are rich in phytonutrients and polyphenols, for the management of obesity. Using a high-fat diet-induced obese mouse model (C57BL/6N), we explored the effect of these teas on various obesity-related parameters. The mice were categorized into five groups: normal diet with water, high-fat diet with water, and high-fat diet supplemented with GT, FT, or GBT. Over 13 weeks, we monitored body weight, perirenal and liver fat, adipocyte lipid accumulation, and key metabolic indicators, such as serum cholesterol, leptin, insulin, and fasting blood glucose. These teas contain beneficial phytochemicals such as GABA, theanine, and caffeine, and have demonstrated an enhanced antioxidant capacity, which increases the scavenging of free radicals and may reduce oxidative stress. The animal study indicated a decrease in feeding efficiency and significant reductions in body weight liver fat, epididymal fat, and perirenal fat, as well as in adipocyte lipid accumulation. Additionally, notable improvements were observed in metabolic health indicators, including reductions in serum cholesterol, leptin, insulin, and fasting blood glucose levels. Our findings revealed that GT, FT, or GBT significantly counteracted the negative effects of a high-fat diet, suggesting their potential in combating obesity and related metabolic disorders.

**Shruti Sinai Borker, Aman Thakur, Krishna Kanta Pandey, Pallavi Sharma, Vivek Manyapu, Abhishek Khatri, Rakshak Kumar. Nutrient recycling of source-separated human faeces using biochar immobilized indigenous psychrotrophic bacteria for sustaining the agroecosystems of north-western Himalaya. (2024) Appl. Biol. Chem. 67: 37**

The Himalayan composting toilets (CTs) offer a sustainable solution for converting human faeces (HF) into compost, supplementing the low-fertile land of the region. However, CTs face challenges such as delayed composting processes (6–8 months), increased heavy metal content, and foul odour. Therefore, the current study evaluated biochar-amended psychrotrophic bacteria for HF degradation under low-temperature conditions (10 ± 2 °C). Out of 153 psychrotrophic bacteria isolated from HF compost, 17 bacterial strains were selected based on highest and two or more hydrolytic activities. Furthermore, considering the isolation source, bacterial strains were examined for haemolytic activity, biofilm formation, cytotoxicity and seed germination assay. In total, 14 potential strains belonging to *Pseudomonas*, *Microbacterium*, *Arthrobacter*, *Streptomyces*, *Glutamicibacter*, *Rhodococcus*, *Serratia*, *Exiguobacterium*, and *Jeotgalicoccus* genera were considered safe for both human handling and plants. The composting process was conducted in modified plastic drums at 10 ± 2 °C for 90 days through two treatments: Treatment 1 (T1) involving HF, non-immobilized biochar and cocopeat, and Treatment 2 (T2) involving HF, consortium-immobilized biochar and cocopeat. The consortium-immobilized biochar (T2) degraded HF within 90 days with hemicellulose and cellulose degradation ratios of 73.9% and 62.4%, respectively (p ≤ 0.05). The compost maturation indices like C/N ratio (16.5 ± 1.85), total nitrogen (2.66 ± 0.07), total phosphate (0.4 ± 0.005), total potassium (1.8 ± 0.05) also improved in T2 treatment (p ≤ 0.05). Additionally, T2 was more effective in achieving safe levels of faecal coliforms (< 1000 MPN g−1) and reducing heavy metal content compared to T1. 16S rRNA amplicon-based analysis demonstrated an enhancement of bacterial community diversity in T2, with the presence of *Rhodococcus*, *Pseudomonas*, *Arthrobacter*, and *Streptomyces* at the end of the composting period promoting HF degradation. Furthermore, T2-fertilized soil showed a germination index (121 ± 0.4, p ≤ 0.05) and stimulated root, shoot and yield by 110%, 45.2%, and 288%, respectively, in pea (*Pisum sativum* var. AS-10) compared to T1 (49.6%, 19%, and 5.8%, respectively) (p ≤ 0.05). In conclusion, the developed biochar-based formulation proved effective in degrading HF at low temperatures, mitigating foul odours, reducing heavy metals, and enhancing the agronomic value of the final compost. This study presents a promising approach for the sustainable management of HF that can supplement the non-nutritive soil of high-altitude regions.

**Min Ji Kim, Ji Young Kim, Dong Woo Shin, Hyun-Kyung Kim. Investigation of safety and efficacy of febantel and fenbendazole in fish and exposure assessment. (2024) Appl. Biol. Chem. 67: 38**

Fish are susceptible to blood-sucking parasite infections, which cause severe anemia, dyspnea, and ultimately death. However, veterinary drugs available for fish to treat such infectious diseases are lacking; thus, livestock drugs have been repurposed as aquatic animal drugs. Febantel (FBT) and fenbendazole (FBZ) are representative antiparasitic agents for livestock such as cattle, swine, and poultry, and are considered suitable as aquatic animal drugs. Therefore, we investigated the safety and efficacy of FBT and FBZ in fish and performed a risk assessment to determine the maximum residue limit in fish. Most studies indicate that FBT is rapidly converted to FBZ, which is metabolized to oxfendazole and oxfendazole sulfone. FBZ was frequently detectable in the plasma and tissues (e.g., muscle, skin, and the liver) in significant quantities than other metabolites. We regarded the liver as the target organ because reversible hepatocytic changes were observed in fish after administration of 100 mg/kg FBT for 9 days. No toxicological effects, such as increased mortality or decreased appetite, were observed when the fish were administered 50 mg/kg FBT for 3 days. The efficacy of the drugs was verified in various parasites, including *H. heterocerca, H. okamotoi or Z. japonica*, and *M. seriolae*, as causative agents of beko disease through laboratory and field trials. Although toxicity studies on FBZ in fish are limited, its safety has been demonstrated from toxicity studies in a wide range of animal models. The risk from using FBT and FBZ was negligible for human health because the ratio of the estimates of dietary exposure and acceptable daily intake was 78.4%.

**Minyoung Im, Nackhyoung Kim, Ui-Hyun Park, Hyeon Ho Heo, Soo-Jong Um. Piperine reduces hair oiliness by inhibiting adipogenesis of hair stem cells. (2024) Appl. Biol. Chem. 67: 39**

Piperine, an alkaloid compound in black pepper (*Piper nigrum*), has beneficial bioactivities. Specifically, piperine inhibits adipogenesis in 3T3-L1 cells by suppressing the transcriptional activity of PPARγ. Control of hair oiliness, which is related to adipogenic regulation, is important to prevent hair loss. Excessive sebum from the sebaceous gland (SG) can cause acne, folliculitis, or irritated skin by clogging pores. To investigate the in vivo function of piperine in SG, we used mice fed a high-fat diet (HFD). The HFD increased the size and Oil Red O (ORO) staining intensity of SG, which were significantly reduced by piperine. The HFD also upregulated the expression of sebocyte-associated genes, including PPARγ target genes, an effect reversed by piperine. In CD34/CD49f double-positive hair follicle bulge stem cells isolated from mouse vibrissae, piperine inhibited cellular adipogenesis, likely via transcriptional repression of related genes. Furthermore, piperine reduced the thickness of subcutaneous fat. In human dermal papilla cells, piperine inhibited cellular adipogenesis, as shown by the reduction in ORO staining and the downregulation of PPARγ target genes. In conclusion, piperine can be used to reduce hair greasiness by suppressing adipogenesis in hair stem cells.

**YongFei Ming, Yin Li, JianZhi Chu, XiaoShuang Zhou, YuXuan Huang, ShuDe Yang, YueJun Mu, Lin Wang, Rui Zhang, XianHao Cheng. Comparative analysis of metabolites and in vitro hypoglycemic activity of *Taiwanofungus camphoratus* cultured using various methods. (2024) Appl. Biol. Chem. 67: 40**

*Taiwanofungus camphoratus* has attracted much attention because it can abundantly produce various active substances that exhibit blood-sugar lowering, immunity improving, and antioxidant properties. Currently, *T. camphoratus* is cultured using four main methods: cutting wood culture, solid-state fermentation, submerged fermentation, and dish culture. *T. camphoratus* produces different metabolites under different culture methods. In this study, nontargeted metabolomics was used to compare the metabolites of *T. camphoratus* produced under these four culture methods. Principal component analysis and supervised partial least squares-discriminant analysis were used to analyze the differences in the metabolites. Moreover, in vitro hypoglycemic activity of *T. camphoratus* extracts produced under four culture methods was compared by assessing their ability to inhibit the activity of α-glucosidase, α-amylase, and sucrase. A total of 186 metabolites were identified. In total, 127 metabolites were common under the four culture methods. Under solid-state fermentation, submerged fermentation, and cutting wood culture, 12, 1, and 4 metabolites were unique, respectively. The differential metabolites produced by *T. camphoratus* under four culture methods were mainly triterpenoids, phenolic compounds, and fatty acid compounds. α-glucosidase, α-amylase, and sucrase activity inhibition was the best using *T. camphoratus* extract obtained under cutting wood culture; the inhibition rates were 55.97%, 51.96%, and 78.02%, respectively, which were comparable to those exhibited by 0.001, 3, and 12 mg/mL acarbose (positive control). The metabolites produced by *T. camphoratus* and α-glucosidase, α-amylase, and sucrase inhibitory activities were different under the four culture methods. Cutting wood culture exhibited the best enzyme inhibitory activity. This study provided a theoretical basis for further use and development of various culture methods for the rational production of active metabolites of *T. camphoratus*.

**Kyung Hee Hong, Jonghoon Jung, Minji Kim, Min Young Um. Hyperoside ameliorates depression-like behavior in ovariectomized mice. (2024) Appl. Biol. Chem. 67: 41**

The physiological changes caused by the decline in estrogen levels due to menopause are linked to an increased risk of depression. This study investigated the antidepressant effects of hyperoside (HYP), a natural flavonol glycoside, and its associated molecular mechanisms in primary hippocampal neurons and ovariectomized (OVX) mice. HYP treatment increased nitric oxide (NO) production and neuronal nitric oxide synthase (nNOS) expression in primary hippocampal neurons; additionally, it upregulated the expression of brain-derived neurotrophic factor (BDNF) and phosphorylated tropomyosin receptor kinase B (TrkB). In OVX mice, HYP treatment significantly improved depression-like behaviors in an open field test to a level comparable to estrogen treatment. Furthermore, HYP treatment upregulated OVX-induced decreased nNOS expression and BDNF-TrkB signaling in the hippocampus. Therefore, this study suggests that HYP exhibits antidepressant potential by addressing estrogen deficiency-induced alterations, specifically by restoring nNOS expression, promoting NO production, and concurrently enhancing BDNF-TrkB signaling in OVX mice.

**R. Hernández Maqueda, I. Ballesteros, D. Meca, R. Linacero, F. del Moral. Insights into the abundance, expression and diversity of key denitrification genes in an ecologically managed greenhouse agricultural soil. (2024) Appl. Biol. Chem. 67: 43**

Understanding the bacteria associated with nitrification and denitrification is crucial for comprehending the processes that lead to nitrous oxide emissions in agricultural greenhouse soils. Therefore, it is important to determine their abundance and expression to gain insight into these processes.

The aim of this study was to explore the bacterial communities associated with denitrification in a greenhouse agricultural soil amended with crop residues and manure for six years. For this purpose, we proceeded to detect and quantify the genes *nirK* and *nirS* and the gene *nosZ* through clone library construction, sequencing, phylogenetic analysis, and quantitative polymerase chain reaction (qPCR). Sequence analysis based on the clone library revealed that many of the *nirS* or *nirK* genes detected were not closely related to known denitrifier bacteria, but some of the *nosZ* sequences were related to the genera such as *Pseudomonas*, *Halomonas*, and *Marinobacter*. Furthermore, the qPCR revealed a high abundance of DNA copies in *nirK*, 6.08 × 109 ± 1.16 × 109, while *nirS* and *nosZ* showed lower values, 9.05 × 106 ± 1.65 × 106 and 8.71 × 106 ± 1.44 × 106, respectively. However, the highest expression rate was observed for *nirS* (mRNA/DNA ratio = 3.10 × 10− 3), while *nirK* and *nosZ* showed 10-fold lower expression rates (4.4 × 10− 4 and 3.5 × 10− 4, respectively). The results of this work provide a preliminary overview of the diversity, abundance and expression of key genes associated with the denitrification process in this type of soil and are a starting point for further studies to understand how this type of soil management can influence the denitrification process.

**Do-Gyun Park, Hyeon-Cheol Jeong, Eun-Bin Jang, Jong-Mun Lee, Hyoung-Seok Lee, Hye-Ran Park, Sun-Il Lee, Do-Gyun Park, Eun-Bin Jang, Taek-Keun Oh. Effect of rice hull biochar treatment on net ecosystem carbon budget and greenhouse gas emissions in Chinese cabbage cultivation on infertile soil. (2024) Appl. Biol. Chem. 67: 44**

Biochar, with its potential to enhance soil fertility, sequester carbon, boost crop yields and reduce greenhouse gas emissions, offers a solution. Addressing the challenges posed by climate change is crucial for food security and agriculture. However, its widespread adoption in agriculture remains in its infancy. This study assessed the effects of rice hull biochar on cabbage production and greenhouse gas emissions, especially nitrous oxide (N2O). A trial, employing a randomized block design in triplicate was conducted from September 13 to November 23, 2022, where "Cheongomabi" cabbage was cultivated with N-P2O5-K2O fertilization at 32−7.8−19.8 kg 10a−1. Additional fertilizer was applied twice post-sowing. The Biochar application rates were control = 0 ton ha−1, B1 = 1 ton ha−1, B3 = 3 ton ha−1, and B5 = 5 ton ha−1. The aboveground biomass of autumn cabbage harvested 82 days after sowing was 2.40–2.70 kg plant−1 in the control and biochar treatments (B1, B3, and B5), with no significant differences (*p* > 0.05). Cumulative CO2 emissions during cultivation varied across treatment groups, with initial and cumulative emissions of 10.40–17.94 g m−2 day−1 and 3.63–4.43 ton ha−1, respectively. N2O emissions decreased with higher biochar application: reductions of 2.9%, 25.4%, and 41.1% in the B1, B3, and B5 treatments, respectively, compared to the control. The biochar application had no significant impact on yield but curbed soil emissions, Net ecosystem carbon balance during cabbage cultivation ranged from 0.42 to 3.41 ton ha−1 for the B1, B3, and B5 treatments, respectively, compared to control. Overall, the study underscores biochar’s role in mitigating emissions and boosting soil carbon during cabbage cultivation in fall.

**Mohamed Abdel Rida Yaseen, Madiha Hadj Ayed, Jabbar A. A. Al-Saaidi. The potential modulatory impact of garlic-selenium nanoparticles coated with synthetic tocopherol polyethylene glycol-succinate against lead acetate toxicity in male rabbits. (2024) Appl. Biol. Chem. 67: 45**

Toxic heavy metal lead enters in the environment due to industrial and anthropogenic activity threatens ecosystems and public health. Natural garlic extract (GE) exhibits antioxidant properties and various applications against several ailments. Therefore, this study scrutinized the protective effects of tocopherol polyethylene glycol succinate-coated garlic selenium (TPGS-GSNP) against lead acetate (LA) toxicity in rabbits. Sixty-four mature male rabbits were involved and divided into 8 equal groups. They received distilled water (negative control; T1), 30 mg/kg bw of LA (positive control; T2), 800 mg/kg bw of GE (T3), GE + LA (T4), 1 mg/kg bw of TPGS-Selenium (T5), TPGS-S + LA (T6), 1 mg/kg bw of TPGS-GSNP (T7), and TPGS-GSNP + LA (T8). Consequently, treatments were administered three times a week for 12 weeks. Following the treatment period, serum oxidant-antioxidant, protein, and lipid profiles, liver and kidney function, histopathological findings of the adrenal, liver, and kidneys, femur bone marrow chromosomal aberrations, and mitotic activity were collected and analysed. LA exposure showed significant reductions in antioxidant levels, organ weights, and mitotic activity while increasing oxidative stress, corticosteroid levels, and chromosomal aberrations. Importantly, TPGS-GSNP administration significantly improved these markers compared to the LA group. In addition, histological analysis revealed structural improvements of the studied organs in the TPGS-GSNP group compared to the LA group, which displayed high cellular necrotic and degenerative changes. In conclusion, synthetic TPGS-GSNP demonstrated higher protective efficacy against LA-induced toxicity compared to natural GE or selenium alone. However, more future studies could be conducted to explore the potential of TPGS-GSNP as an anticancer or immunomodulatory agent.

**Do Manh Cuong, Sun Hee Yang, Ji Soo Kim, Jeong Yong Moon, Jongkeun Choi, Gyung Min Go, Somi Kim Cho. Evaluation of antioxidant and anti-inflammatory activity and identification of bioactive compound from the marine diatom, *Odontella aurita* extract. (2024) Appl. Biol. Chem. 67: 46**

Increased production of reactive oxygen species (ROS) leads to oxidative stress, with its damaging effect extending to the mitochondria and plasma membrane. Further, prolonged inflammation can result in chronic disease development. The marine microdiatom *Odontella aurita* is recognized for its potential in food and pharmaceutical development. Moreover, it contains antioxidant and anti-inflammatory properties. However, studies regarding the efficacy of their varying extract forms and their underlying mechanisms remain scarce. Therefore, this study aims to explore the antioxidant and anti-inflammatory effects of *Odontella aurita* extracts obtained using various extraction methods (hot water, 70% ethanol, and chloroform:methanol (CM)). Among the three *Odontella aurita* extracts, the CM extract demonstrated superior efficacy in protecting RAW 264.7 cells from H2O2-induced cytotoxicity. It significantly lowered the levels of ROS and enhanced the expression of superoxide dismutase and glutathione peroxidase. Furthermore, the CM extract outperformed other extracts in inhibiting LPS-induced nitric oxide production, reducing mRNA levels in nitric oxide synthase, cyclooxygenase, and the proinflammatory cytokines interleukin IL-1β, IL-6, TNFα. Additionally, CM extract effectively suppressed the activation of NF-κB/IκBα and JAK2-STAT3 in LPS-induced RAW 264.7 cells. HPLC–UV analysis revealed a remarkable 33-fold higher fucoxanthin content in CM compared to the ethanol extract. GC–MS analysis identified elevated levels of cholest-5-en-3-ol, phytol, eicosapentaenoic acid methyl ester, methyl palmitate, palmitoleic acid methyl ester, and neophytadiene in the CM extract. These findings suggest that *Odontella aurita* CM extract is a promising antioxidant candidate for preventing or treating inflammatory diseases, consequently emphasizing its potential for further development.

**Sulaiman A. Alsalamah, Mohammed Ibrahim Alghonaim, Marwah Marwah Bakri, Tarek M. Abdelghany. *Zygnema* sp. as creator of copper oxide nanoparticles and their application in controlling of microbial growth and photo-catalytic degradation of dyes. (2024) Appl. Biol. Chem. 67: 47**

Recently, focus has been placed on renewable sources, as they can be provided in large quantities at the lowest possible cost, in order to create nanoparticles. One of these sources is Zygnema moss which used in the present investigation to create Copper oxide nanoparticles (CuONPs). Several phenols and flavonoids were identified the extract of Zygnema sp. via analysis of High performance liquid chromatography. These constituents served as reducing and stabilizing agents for CuONPs. Characterization of CuONPs was performed via UV-visible spectrum that demonstrated peak at 252 nm, Transmission electron microscopy that showed spherical CuONPs with mean diameter of 30.06 nm, Fourier transform infrared spectroscopy that confirm that presence of several functional groups aided to formation of CuONPs. The crystallographic pattern of CuONPs was recorded via X-ray diffraction analysis. Antimicrobial potential of CuONPs was compared to copper acetate and antibiotic/antifungal drug. CuONPs exhibited more inhibition zones against *S. aureus* (32 ± 0.1 mm), *E. coli* (36 ± 0.1 mm), *S. typhi* (27 ± 0.2 mm), *E. faecalis* (37 ± 0.1 mm), *C. albicans* (34 ± 0.3 mm) than copper acetate and antibiotic/antifungal drug. Promising MIC values of were recorded against *S. aureus, E. coli*, and *S. typhi*. CuONPs at 200 ppm inhibited the growth of *C. lunata*, *F. oxysporium*, *A. flavus*, and *Mucor circinelloid* with inhibtion of 76.92, 73.33, 63.63, and 53.84%, respectively regarded the control 100% growth. The photocatalytic role of CuONPs was recorded for degradation of reactive red (RR195) and reactive blue (RB) dyes with maximum degradation of 84.66% and 90.82%, respectively at 75 min. Moreover, the optimal dyes degradation was 84.66 and 90.82%, respectively at 40 °C.

**Hadush Gebrehiwot, Urgessa Ensermu, Aman Dekebo, Milkyas Endale, Mo Hunsen. Exploring the medicinal potential of Senna siamea roots: an integrated study of antibacterial and antioxidant activities, phytochemical analysis, ADMET profiling, and molecular docking insights. (2024) Appl. Biol. Chem. 67: 48**

Nowadays, infectious diseases pose an alarming global threat to human health. The genus *Senna* is among the most well-known taxonomic categories commonly used in folk medicine to confront these challenges. Motivated by its traditional uses, a comprehensive study was conducted on the roots extract of *Senna siamea*, aiming to address the in vitro antibacterial and antioxidant efficacy of phytochemicals from the dichloromethane: methanol (1:1) roots extract of the plant, along with in silico computational studies. The separation of compounds was achieved using silica gel column chromatography. Whereas, the antibacterial and antioxidant activities were examined using paper disc diffusion and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays, respectively. Silica gel column chromatography of the dichloromethane: methanol (1:1) roots extract afforded lupeol (**1**), *β*-sitosterol (**2a**) and stigmasterol (**2b**), chrysophanol (**3**), betulinic acid (**4**), and glyceryl-1-hexacosanoate (**5**). Although these compounds have been previously reported from the plant, proof of their medicinal applications via in vitro and in silico studies is still lacking. Notably, our findings showed remarkable inhibition zones by the extract (18.00 ± 0.00 mm and 17.17 ± 0.24 mm) against *E. coli* and *S. aureus*, respectively, at 50 mg/mL compared to ciprofloxacin (23.33 ± 0.47 mm and 22.00 ± 0.00 mm, respectively), showcasing its potential antibacterial efficiency. Considerable inhibition zones were also recorded by chrysophanol (**3**) against *E. coli* (16.33 ± 0.24 mm) and *S. pyogenes* (16.00 ± 0.00 mm) at 2 mg/mL, compared to ciprofloxacin which showed 23.33 ± 0.47 mm and 21.67 ± 0.47 mm, respectively, signifying its potent antibacterial activities. In addition, the crude extract and chrysophanol (**3**) exhibited substantial IC50 values (1.24 and 1.71 µg/mL, respectively), suggesting their significant antioxidant potential compared to that of ascorbic acid (IC50: 0.53 µg/mL). Chrysophanol (**3**) fulfilled Lipinski’s rule with no violation and lupeol (**1**), *β*-sitosterol (**2a**), stigmasterol (**2b**), betulinic acid (**4**), and glyceryl-1-hexacosanoate (**5**) displayed one violation each which were in favor of the drug-likeness predictions. All the compounds exhibited no cytotoxicity and except betulinic acid (**4**), all the compounds also showed no carcinogenicity properties which were consistent with the prediction results of ciprofloxacin. The molecular docking computations revealed that all the compound isolates displayed strong and nearly strong binding affinities against all protein targets, ranging from − 6.6 kcal/mol to -9.2 kcal/mol (lupeol (**1**) against *E. coli* DNA gyrase B and topoisomerase II *α*, respectively). Thus, the present findings suggest the roots of *Senna siamea* for potential medicinal applications against multi-drug resistant pathogens hence validating its ethno-medicinal uses.

**Jiaxiang Zheng, Sunyoon Jung, Jung-Heun Ha, Yoonhwa Jeong. *Locusta migratoria* hydrolysates attenuate lipopolysaccharide (LPS)/D-Galactosamine (D-Gal)-induced cytotoxicity and inflammation in Hep G2 cells via NF-κB signaling suppression. (2024) Appl. Biol. Chem. 67: 49**

The prolonged state of hepatic inflammation can lead to liver damage, a critical driving force in the progression of liver-related diseases. *Locusta migratoria* (LM), an edible insect, is recognized for its protein richness and potential to produce a range of bioactive polypeptides, presenting a novel solution for liver disease. This study investigated the hepatoprotective effects of LM hydrolysates in human hepatoma G2 (Hep G2) cells challenged with lipopolysaccharide (LPS)/D-Galactosamine (D-Gal), a model of liver injury. Remarkably, LM hydrolysates significantly ameliorated cell damage, as evidenced by the inhibition of the LPS/D-Gal-induced decrease in cell viability and reduction in lactate dehydrogenase (LDH) release. Furthermore, LM hydrolysates alleviated the release of aspartate aminotransferase (AST) from cells exposed to LPS/D-Gal and lowered the secretion of inflammatory cytokines while suppressing the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a key pathway in inflammation. In particular, LM-N hydrolysate mitigated hepatotoxicity by attenuation of inflammatory responses to reduce interleukin 6 (IL-6) levels, and NF-κB nuclear translocation. These findings suggest that LM hydrolysates could potentially offer hepatoprotective effects by mitigating the inflammatory responses induced by LPS/D-Gal.

**Segun Michael Abegunde, Emmanuel Folorunso Olasehinde & Matthew Ayorinde Adebayo. Exploring the potential of Nauclea latifolia for sustainable synthesis of ZnO nanoparticles: characterization and antibacterial assessment. (2024) Appl. Biol. Chem. 67: 50**

The work presents a report on Zinc oxide nanoparticles (ZnO NPs) synthesized through a green approach using *Nauclea latifolia* fruit extracts, with a view to investigating the prepared nanoparticles for their antimicrobial activities. The ZnO NPs synthesized were characterized using various analytical instruments, including X-ray Diffraction (XRD), Fourier Transform Infrared (FTIR), Ultraviolet-Visible (UV-Vis) spectroscopy, Dynamic Light Scattering (DLS), and Transmission Electron Microscopy (TEM). The instruments provided valuable information on the characteristics of the Zn ONPs. The antibacterial activities of the synthesized ZnO NPs were evaluated with *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E*. *coli)*. The maximum absorption was observed at 379 nm. The average hydrodynamic size and the polydispersity index (PDI) were measured as 81.77 nm and 0.401, respectively. The nanomaterial has a hexagonal wurtzite structure, and the Zn–O bond was detected at 537 cm–1. The nanoparticles were in the nano range with sizes ranging from 10.02 nm to 28.50 nm. The *N. latifolia* fruit extract-mediated ZnO NPs showed excellent performance against the two bacteria at all concentrations of ZnO NPs. The highest inhibition zones for *E. coli* and *S. aureus* at 8 mg/L of ZnO NPs are 21 and 16 mm, respectively. This study provides valuable insights into an efficient, simple, and environmentally friendly route for synthesizing ZnO NPs with a potential application in the biomedical field.

**Eun-Ha Kim, Jung-Won Jung, Oh Suk Yu, So-Young Lee, Myeong-Ji Kim, Sang-Gu Lee, Hyoun-Min Park, Yongmin Jo, Yongsung Joo, Seon-Woo Oh. Natural variation in tocopherols, B vitamins, and isoflavones in seeds of 13 Korean conventional soybean varieties. (2024) Appl. Biol. Chem. 67: 51**

Soybean seeds are excellent sources of tocopherols, B vitamins, and isoflavones, which are well known for their health benefits. This study investigated the influence of environment and genotype on these constituents across 13 Korean soybean varieties cultivated in three locations during the 2017–2019 growing seasons. Statistical analyses, employing both univariate and multivariate methods, revealed significant impacts of genetic and environmental factors on the composition of tocopherols, B vitamins, and isoflavones. Through permutational univariate analysis of variance, the primary contributors to each measured component were identified. Genotype strongly influenced the levels of β- and δ-tocopherols, whereas the interaction between location and year predominantly affected α- and γ-tocopherols. Vitamin B1 content was predominantly determined by genotype, whereas B3 and B6 were influenced by annual variations. Vitamin B2 level was primarily affected by the interplay between environmental and genotypic effects. Genotype had a significant effect on isoflavone components, with the exception of daidzein. Furthermore, early maturing varieties and those with black seed coats exhibited low levels of isoflavone components and total isoflavones, suggesting a relationship between maturity group and seed coat color in isoflavone variation. These findings can be used as reference values for compositional equivalence assessment of genetically modified soybeans.

**Hongjuan Xu, Xiaoyun Bian, Hongxing Wang, Lin Huang, Xiaoxi Chen. Akkermansia muciniphila postbiotic administration mitigates choline-induced plasma Trimethylamine-N-Oxide production in mice. (2024) Appl. Biol. Chem. 67: 52**

Background

Trimethylamine-N-Oxide (TMAO) is believed to be linked to increased likelihood of cardiovascular disease. While probiotics have shown limited effectiveness in reducing TMAO levels, the potential of postbiotics remains underexplored. This study aimed to evaluate the impact of *Akkermansia muciniphila* (*A. muciniphila*) postbiotic administration on choline-induced TMAO production in mice by modifying the gut microbiota.

Methods

Female C57BL/6J mice were divided into six groups, including a control group, high-choline diet group, live *A. muciniphila* probiotic group, pasteurized *A. muciniphila* postbiotic group, sodium butyrate group, and sodium propionate group. Various measurements and analyses were conducted, including TMAO and TMA levels in serum, urine, and cecal contents, as well as the expression of FXR and FMO3 in liver tissues. Additionally, metabolic parameters, body weight, serum lipid profile, hepatic protein expression (FMO3, FXR, CutC, and CutD), and gut microbiota composition were assessed.

Results

Administration of *A. muciniphila* postbiotic significantly reduced choline-induced plasma TMAO levels in mice. Furthermore, improvements in serum lipid profiles and liver enzyme levels suggested potential enhancements in lipid metabolism and liver function. The study also observed modulation of specific proteins related to TMAO production and metabolism, including CutC and CutD.

Conclusion

The findings highlight the potential of *A. muciniphila* postbiotics as a dietary strategy for mitigating cardiovascular disease risk by modulating the gut-TMAO axis. Postbiotics, particularly *A. muciniphila*, offer advantages over probiotics and warrant further investigation for their therapeutic applications in gastrointestinal and metabolic disorders.

**Jae Ho Yeom, Jin-Woo Lee, Seung Myun Hong, Deok Jae Lee, Dong Choon Park, Namhyun Chung. Anticancer activity of peptide W-0803 derived from *Anoplophoa glabripennis*. (2024) Appl. Biol. Chem. 67: 53**

Natural compounds are known as a resource of anti-cancer agents. Anti-cancer capacity toward human epithelial lung cancer cell lines (A549, H460) was examined with and without treatment of trypsin on the extract of *Anoplophora glabripennis*. IC50 values without trypsin treatment were about 21.3 and 25.0 μg/mL for H460 and A549, respectively. When the extract was treated with trypsin, the IC50 values were 16.0 and 15.6 μg/mL for H460 and A549, respectively, indicating that the trypsin treatment increased the anti-cancer capacity. Because trypsin treatment increased the capacity, the extract was treated with trypsin to isolate the peptide W-0803 which has lysin (K) in C-terminal and α-helix structures. With treatment of W-0803, the cell viability decreased dose-dependently for H460 and A549 cells. Apoptosis analysis showed that the cell death with the treatment of peptide W-0803 was mainly by apoptosis. The wound-healing assay also showed that the peptide W-0803 has an inhibitory capability on cell migration of H460 and A549 cells. All these results suggest that the peptide W-0803 is an anti-cancer agent for lung cancer cell treatment.

**Alfan Danny Arbianto, Min Kim, Seon Min Oh, Hyun-Jae Jang, Hyung Won Ryu, Jin-Hyub Paik, Sei-Ryang Oh, Jongmin Ahn. Regional comparison study of *Epimedium koreanum* using UHPLC-QTOF/MS-based metabolomics approach. (2024) Appl. Biol. Chem. 67: 54**

The untargeted metabolomics-based molecular networking approach combined with multivariate analysis, proves to be an effective strategy for distinguishing raw materials in herbal medicine according to specific criteria. It exhibits the correlations between chemical constituents and the geographical habitats of plants, providing a valuable tool for ensuring quality control in mass production within the industry. In this study, we conducted a comprehensive investigation of the chemical compositions of *Epimedium koreanum* Nakai and performed comparative analyses on four extracts collected from distinct regions in South Korea using untargeted metabolomics tools. Through the comprehensive use of UPLC-QTOF/MS analysis and advanced statistical techniques, we elucidated the chemical composition, leading to the identification of key chemical markers. Additionally, the molecular networking analysis revealed distinct clusters of flavonoids and phenolic acids, highlighting the influence of regional factors on the metabolite profiles. These findings offer a promising avenue for enhancing quality control and traceability in the herbal medicine industry, underscoring the important role of geographical variation in the chemical profiles of herbal products.

**Jun Xue, Yu Ping Gao. Novel fabrication of macromolecular multi-functional hydrogel encapsulated with HUCB-derived mesenchymal stem cells to effective regeneration of cardiac repair after acute myocardial infarction. (2024) Appl. Biol. Chem. 67: 55**

Acute myocardial infarction (AMI) has been treated via injectable hydrogels and biomaterial patches invented using tissue engineering advancements over the past decade. Yet the curative potential of injectable hydrogels and stem cells is limited. Here, we propose the development of an injectable and conductive hydrogel composed of oxidised macromolecular hyaluronic acid and chitosan-grafted aniline tetramer polymeric components. In an attempt to enhance the therapeutic potential of AMI therapy, mesenchymal stem cells derived from human umbilical cord blood (HUCB-MSC) have been integrated into the formulation of a conductive hydrogel. For reliable connection to the beating hearts, the hydrogel exhibited suitable adhesive properties. Hydrogel’s potent biocompatibility was determined by in vitro investigations of cell viability and proliferation of NRCMs and H9C2 cardiomyocytes. After myocardial injection, longer HUCB-MSCs survival length, cardiac functioning, and histology in SD rat myocardium were demonstrated, greatly associated by up-regulation and downregulation of cardiac-related relative gene expressions of angiogenic factors and inflammatory factors, respectively. The injectable hydrogel that contained HUCB-MSCs substantially enhanced the therapeutic benefits, indicating a potentially beneficial therapeutic approach to AMI therapy.

**Gyoung-Deuck Kim, Jiho Lee, Joong-Hyuck Auh. Metabolomic screening of anti-inflammatory compounds in *Acanthopanax sessiliflorus* fruit (Ogaza) extract. (2024) Appl. Biol. Chem. 67: 56**

This study investigated the anti-inflammatory compounds in Ogaza, *Acanthopanax sessiliflorus* fruit, and their extracts using metabolomic screening. Ogaza extracts were obtained in various solvents, such as 70% ethanol, 70% methanol, and water. The anti-inflammatory activity was estimated by evaluating nitric oxide production in lipopolysaccharide (LPS)-induced RAW 264.7 cells treated with the extracts. The 70% ethanol extract (EO) showed the most effective anti-inflammatory activity, inhibiting nitric oxide production by approximately 50% and downregulating iNOS expression. The 70% ethanol extract was further fractionated into three partial subfractions by preparative LC to identify the anti-inflammatory compounds. Assessment of the anti-inflammatory activity of each subfraction revealed that the third subfraction (E-F3) showed the highest inhibitory activity against nitric oxide. E-F3 effectively suppressed iNOS expression. Subsequently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) and multivariate statistical analyses were performed to identify the compounds that significantly contributed to the anti-inflammatory activity of the Ogaza extract. Fourteen and 16 compounds in the negative- and positive-ion modes, respectively, were identified as significant constituents of Ogaza. Compounds like quercetin, hyperoside, acanthoside D, oleanolic acid, and scopoletin were identified as potential anti-inflammatory components in Ogaza extract. This study characterized the functional properties of *Acanthopanax sessiliflorus* fruit and indicated the possibility that other compartments of *Acanthopanax sessiliflorus* may also serve as natural sources of nutraceuticals.

**Min Huang, Xiaohong Yin, Jiana Chen, Fangbo Cao. Biochar supplementation altered the expression of antioxidant proteins in rice leaf chloroplasts under high-temperature stress. (2024) Appl. Biol. Chem. 67: 57**

In order to identify the key antioxidant defense systems used to cope with high-temperature stress in rice leaf chloroplasts following biochar supplementation, the present study compared the expression levels of chloroplast proteins related to antioxidant defense in high-temperature stressed rice leaves between without (C0) and with biochar supplementation (C40; 40 g biochar kg–1 soil). A total of sixteen differentially expressed antioxidant chloroplastic proteins were identified. Among them, three antioxidant enzyme proteins and eight thioredoxin proteins were 62–123% and 37–225% higher under the C40 treatment compared to C0, respectively. These results suggest that both antioxidant enzymes and the thioredoxin system are central to the biochar-mediated protection of rice leaves exposed to high-temperature stress.

**Hokyung Song, Tatsuya Unno. A comprehensive database of human and livestock fecal microbiome for community-wide microbial source tracking: a case study in South Korea. (2024) Appl. Biol. Chem. 67: 58**

Fecal waste from livestock farms contains numerous pathogens, and improperly managed waste may flow into water bodies, causing water-borne diseases. Along with the popularization of high-throughput technologies, community-wide microbial source-tracking methods have been actively developed in recent years. This study aimed to construct a comprehensive fecal microbiome database for community-wide microbial source tracking and apply the database to identify contamination sources in the Miho River, South Korea. Total DNA was extracted from the samples, and the 16 S rRNA gene was amplified to characterize the microbial communities. The fecal microbiome database was validated by developing machine-learning models that predict host species based on microbial community structure. All machine learning models developed in this study showed high performance, where the area under the receiver operating characteristic curve was approximately 1. Community-wide microbial source tracking results showed a higher contribution of fecal sources to the contamination of the main streams after heavy rain. In contrast, the contribution of fecal sources remained comparatively stable in tributaries after rainfall. Considering that farms are more concentrated upstream of tributaries compared to the main streams, this result implies that the pathway for manure contaminants to reach the main streams could be groundwater rather than surface runoff. Systematic monitoring of the water quality, which encompasses river water and groundwater, should be conducted in the future. In addition, continuous efforts to identify and plug abandoned wells are necessary to prevent further water contamination.

**Yoonjeong Kim, Jiye Pyeon, Jae-Yeon Lee, Eun-Min Kim, Im-Joung La, Ok-Hwan Lee, Keono Kim, Jeehye Sung, Younghwa Kim. Chemical fingerprint analysis of fermented *Morinda citrifolia* L. (Noni) juice by UHPLC Q-TOF/MS combined with chemometric analysis. (2024) Appl. Biol. Chem. 67: 59**

*Morinda citrifolia* L. (Noni) has been widely used in traditional medicine in tropical zones and has become increasingly popular globally owing to its health benefits. Most noni fruits are consumed as juice, which is traditionally produced by the natural fermentation of noni fruits. In this study, the metabolic profiles of noni fruit juice (NJ1) and fermented noni fruit juices (NJ2 and NJ3) was compared. A total of 74, 83, and 91 compounds including anthraquinones, coumarins, flavonoids, phenolic acids, phenolics, terpenoids, and miscellaneous (acids, carbohydrates, vitamins, fatty acids, etc.) were tentatively identified from NJ1, NJ2, and NJ3 in both positive and negative electrospray ionization modes. The phenolic compound composition differed significantly between noni juice and fermented noni juice. The results of the unsupervised principal component analysis and hierarchical clustering analysis showed that the non-fermented juice group clustered with the fermented juice groups. Asperulosidic acid, isoasperulosidic acid, and rutin levels were higher in the NJ1 group than those in the NJ2 group. Deacetylasperulosidic acid and monotropein contents in NJ2 were higher than those in NJ1. Similarly, NJ1 had higher asperulosidic acid and isoasperulosidic acid than those in NJ3. The findings from this study have the potential to enhance the quality of fermented noni juice.

**Hyo Jung Kim, Hyoun Sook Kim. DUF3055 from *Staphylococcus aureus* adopts unique strategy for structural distinctiveness. (2024) Appl. Biol. Chem. 67: 60**

*Staphylococcus aureus* remains a public health threat with the WHO classifying the pathogen as a high priority in the development of new antimicrobial agents. Whole genome sequencing has revealed a number of conserved genes that may be essential for cell viability and infection. Characterising the structure and function of these proteins will inevitably aid development of new antimicrobials. Therefore, this study elucidated the structure of hypothetical protein DUF3055 from *S. aureus* stain Mu50. The protein possesses an as yet undefined function and a unique fold. The size of DUF3055 made it an ideal candidate for NMR characterisation which in conjunction with circular dichroism revealed the protein to be folded. Crystallisation and structural solution found that the overall dimer fold has a negatively charged surface formed by a β-bulge and tightly crossed α-helices, with a complementary size to a DNA single turn. Our structural observations suggest that hypothetical protein DUF3055 from *S. aureus* has a role in DNA binding and gene regulation.

**Ho Keun Choi, Ga Yeon Kim, Ga Hee Lee, Hee su Jang, Da Hyeon Kang, Jin Pyo Lee, Dong-Ha Lee. Anti-thrombotic effects of arteanoflavone by regulating cyclic nucleotides and aggregation on human platelets. (2024) Appl. Biol. Chem. 67: 61**

Excessive clotting or abnormal platelet accumulation can lead to serious cardiovascular disorders such as atherosclerosis, stroke, and thrombosis. Therefore, it is imperative to identify compounds capable of controlling or impeding platelet aggregation to prevent the onset of cardiovascular diseases. Arteanoflavone, a compound extracted from Artemisia iwayomogi, has not garnered scientific recognition for its potential health benefits, recent studies have substantiated its anti-inflammatory, antioxidant, and anti-allergic properties. However, the precise mechanisms by which arteanoflavone influences platelet aggregation and blood clot formation have not been conclusively established. This research investigates arteanoflavone’s role in these processes, particularly in platelets induced by collagen. The study reveals a significant increase in the production of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) correlating with the administered dosage of arteanoflavone. Concurrently, a noticeable escalation is observed in substrates of cAMP-dependent kinase and cGMP-dependent kinase, specifically VASP and inositol 1,4,5-trisphosphate receptor (IP3R).

Arteanoflavone demonstrates its ability to limit Ca2+ movement in the dense tubular system through IP3R phosphorylation. Moreover, phosphorylated VASP inhibits the binding of fibrinogen to αIIb/β3, thus suppressing platelet activity. Arteanoflavone also stimulates the phosphorylation of PI3K/Akt, a protein linked to platelet granule release, and MAPK (ERK, JNK, and p38) protein, associated with both platelet granule release and TXA2 production.

Lastly, arteanoflavone impedes collagen-induced platelet aggregation and blood clot formation by inhibiting fibrin production in thrombin-induced platelets. Hence, it is suggested that arteanoflavone could be valuable as an agent that effectively deters platelet inhibition and blood clot formation through antiplatelet mechanisms.

**Rania A. Karas, Shaimaa Alexeree, Nora Elzohery, Shams H. Abdel-Hafez, Yasser A. Attia. Antidiabetic potential of Selenium nanoparticles and plasma-rich platelets in diabetic mice. (2024) Appl. Biol. Chem. 67: 62**

Diabetes mellitus is a widespread endocrine disorder, which is categorized as the fourth leading cause of global mortality. Allopathic medicine has yet to provide a satisfactory cure for this condition. Consequently, there is an urgent demand for innovative antidiabetic treatment approaches with enhanced management and minimum side effects and costs. The study investigated the synergistic antidiabetic potential of combining selenium nanoparticles (Se NPs) and plasma-rich platelets (PRP) in diabetic mice. Antidiabetic activity of the proposed combination (Se NPs and PRP) was evaluated from histopathological and biochemical perspectives. The experiment involved alloxan monohydrate induced diabetic mouse model. In the in vivo study, several biochemical parameters for assessing the antidiabetic effect of the novel combination of (Se NPs and PRP) were performed such as blood glucose levels, body weight, lipid profiles, and liver damage markers (AST and ALT). Scavenging antioxidant activity was assessed by evaluation levels of hepatic and renal GSH, MDA, SOD, and CAT activities. Complete histopathological examinations of vital internal organs were carried out. Results revealed that combining Se NPs and PRP presents a novel approach for better diabetes management and reduced complications associated with the disease. These findings have therapeutic implications for managing diabetes mellitus.

**Naseer Maliyakkal, Jong Min Oh, Sunil Kumar, Prashant Gahori, Anandkumar Tengli, Asmy Appadath Beeran, Hoon Kim, Bijo Mathew. Synthesis, biochemistry, and in silico investigations of isatin-based hydrazone derivatives as monoamine oxidase inhibitors. (2024) Appl. Biol. Chem. 67: 63**

Ten isatin-based hydrazone derivatives were synthesized using two subseries, **IA** (isatin + acetophenone) and **IB** (isatin + benzaldehyde), and evaluated for their monoamine oxidases (MAOs) inhibitory activity. All the compounds showed stronger MAO-A inhibition than MAO-B, and the **IB** series showed more effective MAO-A inhibitory activity than **IA** series. Compound **IB4** most potently inhibited MAO-A (half maximal inhibitory concentration IC50 = 0.015 µM), followed by **IB3** (IC50 = 0.019 µM). On the contrary, compound **IB3** showed the highest MAO-B inhibition (IC50 = 0.068 µM), followed by **IB4** (IC50 = 1.87 µM). Compound **IB3** and **IB4** had low selectivity indices of 3.68 and 8.50, respectively. Structurally, the methyl group of **IA** series decreased the inhibition of both MAO-A and MAO-B. Among them, **IB3** and **IB4** (4-Cl and 4-Br in B-ring, respectively) showed higher MAO-A and MAO-B inhibition than the other substitutions. Inhibition constant Ki values of **IB3** and **IB4** for MAO-A were 0.0088 and 0.0063 µM, respectively, and those for MAO-B were 0.048 and 0.060 µM, respectively. **IB3** and **IB4** were competitive, reversible inhibitors of MAO-A and MAO-B. Molecular docking analysis predicted that **IB3** and **IB4** formed stable hydrogen bonds between Asn181 and the NH atom of isatin in the ligand-protein complex. Dynamic analysis revealed that **IB3** and **IB4** are stable with both MAO isoforms. These observations suggest **IB3** and **IB4** are potent and reversible MAO-A and MAO-B inhibitors and both compounds can be used as therapeutic agents for neurological disorders.

**Kyungha Lee, Seong Hee Bhoo, Sang-Won Lee, Man-Ho Cho. Functional identification of three regiospecific flavonoid *O*-methyltransferases in *Rhododendron delavayi* and their applications in the biotechnological production of methoxyflavonoids. (2024) Appl. Biol. Chem. 67: 64**

Rhododendrons produce a variety of methoxyflavonoids, including rarely found 3-methoxyflavonoids and 5-methoxyflavonoids. It was thus suggested that they have a series of regiospecific flavonoid *O*-methyltransferases (FOMTs). The 18 Class II *O*-methyltransferase (OMT) genes were retrieved from the *Rhododendron delavayi* genome, designating them as RdOMTs. A comprehensive biochemical characterization of RdOMTs was performed to identify functional FOMTs. The FOMT activity of recombinant RdOMTs was assayed with flavonoid substrates of different subclasses. Among the examined RdOMTs, RdOMT3, RdOMT10, and RdOMT12 showed FOMT activity for diverse flavonoids. In particular, RdOMT3 consumed only flavonols as a substrate. Structural analyses of the methylated products demonstrated that RdOMT3, RdOMT10, and RdOMT12 catalyze regiospecific methylation of flavonoids at the 3'/5'-, 3-, and 4'-hydroxyl groups, respectively. Their broad substrate spectrum and different regiospecificity suggest that these RdOMTs contribute to the formation of complex methoxyflavonoids in *R. delavayi*. Bioconversion of flavonoids using *E. coli* harboring each RdOMT demonstrated that RdOMT3, RdOMT10, and RdOMT12 are useful tools for the biotechnological production of valuable methoxyflavonoids, including the rarely found 3-methoxyflavonoids.

**Tamanna Kumari, Deepak Phogat, Jatin Phogat, Vineeta Shukla. Biochar & fly ash amendments lower mortality and increase antioxidant activity in chlorpyrifos-exposed earthworms. (2024) Appl. Biol. Chem. 67: 65**

The investigation presented a novel finding regarding mitigating stress induced by chlorpyrifos in *Eisenia fetida* by incorporating biochar derived from rice straw and fly ash as soil amendments. It was observed that phenolic compounds exhibit solubility in methanol, and the methanolic fraction exhibited notable inhibitory effects on lipid peroxidation and displayed antioxidant properties. The defence mechanism of *E. fetida*, comprising catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and various other enzymes, remained effective in neutralizing stressors without disruption when the earthworm was subjected to diverse chemical agents or stressful conditions. These enzymes served as indicative markers of toxicity induced by pesticide exposure, even at sublethal concentrations. The scavenging of free radicals by these enzymes ultimately safeguarded the organism. Fly ash and biochar emerged as two organic alternatives capable of alleviating stress by providing a protective mechanism. In this context, the study examined the impact of biochar and fly ash amendments on earthworm biomarkers. The mortality rate at the median lethal concentration of chlorpyrifos was reduced to less than 50% through 3% and 5% modifications. In contrast to the non-amendment group exposed to sublethal doses, the amendment group exhibited higher levels of oxidative stress and lower protein content. This observation indicated the presence of stress induced by the accumulation of free radicals, which increased in number with higher doses of chlorpyrifos. Moreover, the study highlighted the interconnected nature of total antioxidant capacity and total phenolic capacity values, with a decrease in these parameters signifying a shift in earthworm biomarkers.

**Yeong-Bae Yun, Hae-Yun Kwon, Yurry Um. Changes in growth characteristics and ginsenoside contents of wild-simulated ginseng with different harvest times in South Korea. (2024) Appl. Biol. Chem. 67: 66**

Wild-simulated ginseng (WSG, *Panax ginseng* C.A. Meyer) is grown in mountainous forests, without the chemical treatment or installation of artificial facilities. This study aimed to investigate monthly changes in growth characteristics and ginsenoside contents in WSG to suggest the optimal harvest time. Four-year-old WSG were collected in the same area every month, and their growth characteristics and ginsenoside contents were measured. The growth characteristics of aerial and root parts were measured from May to July and from March to December, respectively. For the aerial part, most growth characteristics of WSG decreased over time, except for stem length. For the root part, rhizome length increased over time except for September, while the root diameter and weight of root part were mostly consistent. The root length increased by September, while the number of rootlets was the highest at May. At July, the total ginsenoside content of WSG was significantly the highest, while the total ginsenoside content at October was the lowest. This result was believed to be due to the F2, Rd, and Rg1 contents of the aerial part, rather than the root part. Also, based on these growths and the ginsenoside contents of WSG, the optimal harvest time for WSG is considered to be late spring–summer (May–July) when the aerial part can be identified.

**Suyoung Kang, So Jin Lee, YongHee Kwon, Doo-Gyung Moon, Jung Hun Sun, Kyu-Won Hwang, Joon-Kwan Moon. Characteristic of phenotype, amino acids and volatile compounds for fresh tea leaves of Korean tea cultivars (Camellia sinensis (L.) O. Kuntze). (2024) Appl. Biol. Chem. 67: 67**

Tea (*Camellia sinensis* (L.) O. Kuntze) is a popular beverage consumed worldwide. To establish fundamental scientific data, we analyzed the amino acids and volatile compounds in seven tea cultivars grown in Korea investigated phenotype also. Phenotypically, the leaf area and greenness index of young shoots and leaf blades were particularly different among the four Korean cultivars. Nine amino acids were detected from each cultivar, with the total amino acid and theanine contents being 9.08–41.42 and 2.81–24.60 mg/g, respectively. Moreover, 107 volatile compounds were identified as common components among tea cultivars using headspace solid-phase microextraction / gas chromatography–mass spectrometry (HS-SPME/GC-MS), and 38 key compounds were identified using partial least squares-discriminant analysis (PLS-DA) and hierarchical cluster analysis (HCA). The (*Z*)-linalool oxide (furanoid) concentrations were significantly high in Korean tea plant cultivars, and linalool concentrations were also high or low, but had high relative contents. Linalool and its various oxides are the major compounds responsible for the tea aroma. In conclusion, Korean tea cultivars have distinct characteristics, and the results of this study will form the basis for identifying Korean tea plant cultivars that can produce high-value tea products.

**Ji Won Kim, Seongmin Hong, Jiyun Go, Jin Seong Park, Gibum Yi. Transcriptomic changes reveal hypoxic stress response in submerged seeds of maize (*Zea mays* L.). (2024) Appl. Biol. Chem. 67: 68**

Maize is highly sensitive to waterlogging stress, and seeds fail to germinate under hypoxic conditions induced by submergence, leading to severe yield losses. We conducted a comparative transcriptome analysis during the initial stages of seed germination, exploring aerobic and hypoxic conditions in two inbred lines, B73 and Okcheon Chal-1. Notably, significant differences emerged between aerobic and hypoxic conditions on the first day of germination, particularly in genes associated with fermentation and phytohormone regulation. However, consistent transcriptomic changes were observed in primary metabolic pathways such as glycolysis, the TCA cycle, and the pentose phosphate pathway. These differences strongly correlate with each other, illustrating the efficacy of the hypoxic response for survival in water. Furthermore, this suggests that germinating seeds serve as a promising model for studying plant hypoxia responses with controlled environmental conditions. Insights from this study contribute to understanding the fundamental mechanisms of hypoxia response and hold promise for developing strategies to cultivate waterlogging-tolerant maize cultivars.

**Sangah Park, Hojin Kim, Miyeon Bang, Byung-Hun Um, Jin Wook Cha. A study on the photoisomerization of phenylpropanoids and the differences in their radical scavenging activity using in-situ NMR spectroscopy and on-line radical scavenging activity analysis. (2024) Appl. Biol. Chem. 67: 69**

Phenylpropanoids are naturally occurring secondary metabolites that exhibit various biological activities such as ultra-violet (UV) light protection and reactive-oxygen species (ROS) scavenging. In this study, we utilized a light-emitting diode (LED) based in-situ UV irradiation nuclear magnetic resonance (NMR) technique to monitor the photoisomerization reactions of these phenylpropanoids under UV irradiation in real-time. Through this approach, we measured the photochemical reaction rates and photostationary state (PSS) ratios of these molecules and observed distinct reaction rate and PSS ratio information depending on the variation of substituent groups in each phenylpropanoid molecule. We also evaluated the radical scavenging activity (RSA) for each photochemical product through diphenyl-1-picrylhydrazyl radical (DPPH) assay and 2,2’-azino-bis(3-ethylbenzenthiazoline-6-sulphonic acid) (ABTS) assay. We found that the photoisomerization product of caffeic acid can increase both DPPH and ABTS radical scavenging activities, and confirmed the enhanced ABTS radical scavenging ability of caffeic acid *cis*-isomer based on the online high-pressure liquid chromatography (HPLC)-ABTS analysis and the PSS ratio information of each isomer.

**Eun-Yeong Bok, Seung-Won Yi, Han Gyu Lee, Jae Kyeom Kim, Kangwook Lee, Seungmin Ha, Bumseok Kim, Young-Hun Jung, Sang-Ik Oh. Comprehensive analysis of a peripheral blood transcriptome signature in piglets infected with *Salmonella* Typhimurium: insight into immune responses. (2024) Appl. Biol. Chem. 67: 70**

*Salmonella* Typhimurium (ST) infection in pigs poses a significant threat to animal health and food safety; the intricate mechanisms underlying host–immune responses and pathogen persistence remain poorly understood. To address this knowledge gap, we comprehensively analyzed the peripheral blood transcriptome in piglets infected with ST. We performed histopathological evaluation, blood parameter analysis, advanced RNA-sequencing techniques, and quantitative reverse transcription PCR (RT-qPCR)-based validation. The increasement in the monocyte counts at 2 days post-infection suggested its potential to serve as a hematological marker for ST infection in piglets. Functional and pathway enrichment analyses of the differentially expressed genes highlighted the pivotal roles of innate and adaptive immune responses, notably in pathways associated with Toll-like receptors, NIK/NF-κB signaling, cytokine signaling, and T cell proliferation. RT-qPCR-based validation using peripheral blood mononuclear cells provided additional insights into the immune system dynamics in response to ST infection, revealing the marked elevation of the interleukin (*IL*)*-15*, *IL-27*, and *CXCL10* levels being significantly elevated in ST-infected piglets. Our comprehensive analysis underscores the multifaceted impact of ST infection on piglets and offers valuable insights into the host–pathogen interactions and the role of host immune system during ST infection.

**Tae Woo Oh, Yeongjun Ban, Youngmin Kang. Anti-neuroinflammatory effects of the KIOM -patented *Polygonum multiflorum* maximized root tuber against LPS-stimulated BV2 Cells. (2024) Appl. Biol. Chem. 67: 71**

The main pathological mechanism of neurodegeneration is neuroinflammation. It is known that the persistent neuroinflammatory response is harmful by causing secondary nerve tissue damage. Meanwhile, *P. multiflorum* is a traditional oriental medicinal herb. It has been used as a hematopoietic agent and is used to treat a variety of diseases and conditions. The aim of the present study was to compare the anti-inflammatory efficacy between the commonly available *P. multiflorum* (C1) and the KIOM-patented in vitro-propagated *P. multiflorum* (K1), which had higher content of active ingredients and biomass, using culture and cultivation conditions of LPS-induced neuroinflammation. After stimulation with LPS and treatment with C1 and K1 in mouse microglial BV-2 cells, nitric oxide (NO) production, pro-inflammatory cytokine secretion, inducible NO synthase (iNOS) expression, MAPK phosphorylation and transcription factor activity were assessed. We examined the antioxidant effect using DPPH and production of nitric oxide (NO). C1 and K1 suppressed the expression of iNOS and COX-2 and the production of pro-inflammatory cytokines. Furthermore, we determined the levels of inflammatory mediators, such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α and mitogen-activated protein kinases and IκBα via Western blotting to understand the regulating mechanisms. Additionally, C1 and K1 also inhibited the activation of p38 and nuclear factor-kappa B (NF-κB) in LPS-stimulated BV2 cells. In all experimental results, excellent anti-neuroinflammatory effects were confirmed at a lower dose in K1 than in C1, which is believed to be due to the increased biomass. Therefore, K1 is expected to be more effective than C1 and can be applied more broadly in the development of prevention and treatment of various inflammatory-mediated neurodegenerative diseases.

**Guilong Cong, Mingyu Li, Sitong Dong, Teng Ai, Xiaopeng Ren, Xianzhen Li, Conggang Wang, Fan Yang. Enhanced extracellular production of maltotetraose amylase from *Pseudomonas saccharophila* in *Bacillus subtilis* through regulatory element optimization. (2024) Appl. Biol. Chem. 67: 72**

Maltotetraose amylase (Mta) catalyzes the hydrolysis of amylaceous polysaccharides into maltotetraose, which is an important functional sugar used in the food industry. However, the lack of efficient expression systems for recombinant Mta has hindered its scale-up production and application. In this study, a codon-optimized *mta* gene from *Pseudomonas saccharophila* was efficiently produced in *Bacillus subtilis* by optimizing the regulatory elements. First, a plasmid library containing 173 different signal peptide sequences placed upstream of *mta* gene was constructed, and transformed into *B. subtilis* strain WB800N(*amyE*Δ1) for high-throughput screening. The signal peptide yhcR was found to significantly enhance the secretion of Mta, reaching an activity of 75.4 U/mL in the culture medium. After optimization of the promoters, the Mta activity was further increased to 100.3 U/mL using a dual-promoter PHpaIIPamyE. Finally, the carbon sources and nitrogen sources for recombinant Mta production were optimized, yielding a highest Mta activity of 288.9 U/mL under the optimal culture conditions. The crude enzyme solution containing recombinant Mta produced a highest maltotetraose yield of 70.3% with 200 g/L of maltodextrin as the substrate. Therefore, the present study have demonstrated a high yield of Mta produced in *B. subtilis*, laying the foundation for large-scale Mta production and application.

**Haydara Ammar Hasan, Jiseong Lee, Sunil Kumar, Saleh Alfarraj, Sulaiman Ali Alharbi, Manu Pant, Hoon Kim, Bijo Mathew. Effect of halogens on 3-[4-(dimethylamino) phenyl]-1-phenylprop-2-en-1-ones: development of a new class of monoamine oxidase-B inhibitors. (2024) Appl. Biol. Chem. 67: 73**

Five dimethylamino-based chalcone derivatives (AC) were synthesized and evaluated for their inhibition degree against monoamine oxidase (MAO) enzymes. All AC compounds showed better inhibitory activity against MAO-B than that against MAO-A. AC4 showed the highest inhibitory ability with an IC50 value of 0.020 µM, similar to that of a reference drug safinamide (IC50 = 0.019 µM) against MAO-B, followed by AC1 (IC50 = 0.068 µM) and AC3 (IC50 = 0.083 µM). Substituent -F in ring A (AC4) increased the MAO-B inhibition, followed by -H (AC1), -Br (AC3), and -Cl (AC2). The selectivity index (SI) value of AC4 was high (SI = 82.00) as well as other compounds (44.41 to 98.15). AC4 was found to be a reversible inhibitor as confirmed through analysis using the dialysis method. Interestingly, AC4 was observed to be a noncompetitive MAO-B inhibitor with a rare case and with Ki values of 0.011 ± 0.0036 µM. These experiments confirmed that AC4 is a reversible and potent selective inhibitor of MAO-B. Molecular docking experiments revealed that AC4 showed the highest inhibitory activity with a docking score (-9.510 kcal/mol). A study using molecular dynamics modeling revealed that the protein–ligand complex was more stable. It was observed that AC4 was non-cytotoxic in the study using L929 cell line. In conclusion, compound AC4 shows promise as a MAO-B inhibitor.

**Mid-Eum Park, Inyoung Kim, Hye Ji Lee, Mi Chung Suh, Kyeong-Ryeol Lee, Hyun Uk Kim. Enhancing seed oil content and fatty acid composition in camelina through overexpression of castor RcWRI1A and RcMYB306. (2024) Appl. Biol. Chem. 67: 74**

Seed triacylglycerol (TAG), a major component of vegetable oil, consists of a glycerol esterified with three fatty acids. Vegetable oil has industrial applications and is widely used as edible oil. The increasing demand for plant oils, owing to population growth, it is crucial to enhance the oil content in seeds. We found castor WRINKLED1A (RcWRI1A) and R2R3-type MYB domain protein 306 (RcMYB306) which have homology with Arabidopsis WRI1 (AtWRI1) and AtMYB96 which regulate genes involved in fatty acid and TAG synthesis, respectively. These castor genes were separately and jointly overexpressed using seed-specific promoters in an oil crop, camelina (*Camelina sativa*). Overexpression of *RcWRI1A*, *RcMYB306*, or *RcWRI1A* + *RcMYB306* increased the total seed oil content in camelina. However, this increase was not significantly different from that observed during the overexpression of *RcWRI1A* or/and *RcMYB306*. *RcWRI1A* overexpression increased the fatty acid content, including 16:0, 18:2, 18:3. Contrastingly, *RcMYB306* overexpression increased the 18:1, 18:2, 18:3, 20:0 and 20:1 fatty acid. In the *RcWRI1A + RcMYB306* lines, changes in fatty acid composition demonstrated the combined effects of these transcription factors. These results suggest that RcWRI1A and RcMYB306 can be used to improve the productivity of oil crops.

**Aisha M. H. Al-Rajhi, Tarek M. Abdelghany, Mohammed S. Almuhayawi, Mohammed H. Alruhaili, Soad K. Al Jaouni, Samy Selim. The green approach of chitosan/Fe2O3/ZnO-nanocomposite synthesis with an evaluation of its biological activities. (2024) Appl. Biol. Chem. 67: 75**

Biopolymers embedded with nanoparticles of metal oxides (MOs) demonstrate a wide range of bio-functions. Chitosan-incorporated MOs are an interesting class of support matrices for enhancing the biological function, compared to other support matrices. Therefore, the importance of this study lies in exploiting chitosan as a carrier not of one metal as in previous studies, but of two metals in the form of a nanocomposite to carry out several biological functions. The coprecipitation approach was employed to synthesize chitosan/Fe2O3/ZnO-nanocomposite in the present research. The characterization of chitosan/Fe2O3/ZnO-nanocomposite was performed to find out the morphology and dispersion properties of chitosan/Fe2O3/ZnO-nanocomposite. The X-ray diffraction (XRD) investigation revealed that these were crystalline. Fourier transforms infrared (FTIR) spectrum bands were viewed at 400/cm and 900/cm, due to the stretching vibration of Fe and Zn oxygen bond. TEM showed that chitosan/Fe2O3/ZnO-nanocomposite was of 20–95 nm in size. chitosan/Fe2O3/ZnO-nanocomposite exhibited inhibitory potential against *Staphylococcus aureus*,*Bacillus subtilis, Escherichia coli*, and *Candida albicans* with inhibition zones of 25 ± 0.1, 28 ± 0.2, 27 ± 0.1, and 27 ± 0.2 mm, respectively while didn’t inhibited *Aspergillus niger*. MIC value of nanocomposite was 15.62 ± 0.33 µg/mL for*C. albicans, B. subtilis* and *E. coli*, while it was 62.50 ± 0.66 µg/mL for *Pseudomonas aeruginosa*. Ranged values of nanocomposite MBC (15.62 ± 0.33 to 125 ± 1 µg/mL) were attributed to all tested bacteria. Different concentrations of chitosan/Fe2O3/ZnO-nanocomposite MBC (25, 50, and 75%) reflected anti-biofilm activity against*E. coli* (85.0, 93.2, and 96.0%),*B. subtilis* (84.88, 92.21, and 96.99%), *S. aureus* 81.64, 90.52, and 94.64%) and *P. aurogenosa* (90.11, 94.43, and 98.24%), respectively. The differences in the levels of antimicrobial activities may depend on the type of examined microbes. Antioxidant activity of chitosan/Fe2O3/ZnO-nanocomposite was recorded with excellent IC50 values of 16.06 and 32.6 µg/mL using DPPH and ABTS scavenging, respectively. Wound heal by chitosan/Fe2O3/ZnO-nanocomposite was achieved with 100% compared to the untreated cells (76.75% of wound closer). The cytotoxicity outcomes showed that the IC50 of the chitosan/Fe2O3/ZnO-nanocomposite was 564.32 ± 1.46 µg/mL normal WI-38 cells. Based on the achieved findings, the chitosan/Fe2O3/ZnO-nanocomposite is a very promising agent for perform pharmacological activities.

**Ji-Yeon Yang, Jeong-Hyun Lim, Soo-Jin Park, Youmi Jo, Si Young Yang, Min-Kyoung Paik, So-Hye Hong. Potential endocrine-disrupting effects of iprodione via estrogen and androgen receptors: evaluation using in vitro assay and an in silico model. (2024) Appl. Biol. Chem. 67: 76**

This study was conducted to provide evidence, using in vitro and in silico testing methods, regarding the adverse effects of iprodione, a representative dichlorophenyl dicarboxamide fungicide, on the endocrine system. In the present study, we used the HeLa9903 stably transfected transactivation assay (OECD TG 455), 22Rv1/MMTV\_GR‒KO androgen receptor transcriptional activation assay (OECD TG 458), and toxicity prediction using VEGA QSAR. Our results showed that iprodione had no estrogen receptor antagonistic or androgen receptor agonistic effects; however, iprodione was determined to be an estrogen receptor agonist (log PC10 value is less than − 9) and androgen receptor antagonist (log IC30 value is − 4.58) without intrinsic toxicity against the human cell lines used in this study. VEGA QSAR was used to evaluate five substances with structures similar to that of iprodione. Among them, four chemicals were found to have positive androgen receptor and aromatase activities and have been observed to be developmental toxicants. These results suggest that iprodione regulates steroid hormone receptor interactions and is a potential reproductive toxicant.

**Bihon Abera, Yadessa Melaku, Kebede Shenkute, Aman Dekebo, Negera Abdissa, Milkyas Endale, Temesgen Negassa, Messay Woldemariam, Mo Hunsen. In vitro antibacterial, antioxidant, *in silico* molecular docking and ADEMT analysis of chemical constituents from the roots of *Acokanthera schimperi* and *Rhus glutinosa*. (2024) Appl. Biol. Chem. 67: 77**

*Acokanthera schimperi* is a medicinal plant traditionally used for the treatment of wounds, scabies, and malaria. *Rhus glutinosa* has been also utilized for the management of ectoparasites and hemorrhoids. Silica gel column chromatography separation of CH2Cl2/MeOH (1:1) extract root of *A. schimperi* afforded oleic acid (**1**), lupeol (**2**), dihydroferulic acid (**3**), acovenosigenin A- 3-O-α-L-rhamnopyranoside (**4**) and sucrose (**5**) whereas CH2Cl2/ MeOH (1:1) and MeOH roots extracts of *R. glutinosa* afforded β-sitosterol (**6**), (E)-5-(heptadec-14-en-1-yl)-4,5-dihydroxycyclohex-2-enone (**7**), methyl gallate (**8**), and gallic acid (**9**). The structures of the compounds were established using spectroscopic (1D and 2D NMR) and FT-IR techniques. Disc diffusin and DPPH assay were used, respectively, to evaluate the antibacterial and antioxidant potential of the extracts and isolated compounds. MeOH extract root of *A. schimperi* showed a modest antibacterial effect against *E.coli* with an inhibition zone (ZI) of 16 ± 0.0 mm compared to ciprofloxacin (ZI of 27.0 ± 0.0 mm). CH2Cl2/MeOH (1:1) and MeOH root extracts of *R. glutinosa* showed maximum activity against *S. aureus* with ZI of 17.3 ± 0.04 and 18.0 ± 0.0 mm, respectively. At 5 mg/mL, the highest activity was noted against *S. aureus* by **8** with ZI of 18.6 ± 0.08 mm. Dihydroferulic acid (**3**), methyl gallate (**8**), and gallic acid (**9**) displayed potent scavenging of DPPH radical with respective IC50 of 10.66, 7.48, and 6.08 µg/mL, compared with ascorbic acid (IC50 of 5.83 µg/mL). Molecular docking results showed that lupeol (**2**) exhibited strong binding energy of -7.7 and − 10 kcal/mol towards PDB ID: 4F86 and PDB ID: 3T07, respectively, compared to ciprofloxacin (-6.5 and − 7.2 kcal/mole). Towards PDB ID: 1DNU receptor, compounds **3**, **8**, and **9** showed minimum binding energy of -5.1, -4.8, and − 4.9 kcal/mol, respectively, compared to ascorbic acid (-5.7 kcal/mol). The Swiss ADME prediction results indicated that compounds **2**, **3**, **8**, and **9** obeyed the Lipinksi rule of five and Veber rule with 0 violations. The in vitro antibacterial and antioxidant results supported by *in silico* analysis indicated that compounds **2**, **3**, **8**, and **9** can potentially be lead candidates for the treatment of pathogenic and free radical-induced disorders.

**Taehoon Oh, Sunin Jung, Seon Min Oh, Mi Hyeon Park, Hyoung-Geun Kim, Su-Yeon Lee, Sung-Kyun Ko, Hyung Won Ryu. Inhibitory effect of human indoleamine 2,3-dioxygenase 1 (hIDO1) by kazinols of 1,3-diphenylpropane derivatives. (2024) Appl. Biol. Chem. 67: 78**

This study focused on identifying and characterizing 1,3-diphenylpropane derivatives from flavonoids that inhibit human indoleamine 2,3-dioxygenase 1 (hIDO1) enzymes, which play a role in immune regulation and are associated with various diseases. A series of isolated metabolites (1–7) demonstrated modest to high inhibition of hIDO1, with binding degree values ranging from 26.31 to 72.17%. In particular, during a target-based screening of natural products using hIDO1, kazinol J (6, a 1,3-diphenylpropane derivative) was found to potently inhibit hIDO1, with a binding degree of 72.17% at 1 ppm. Kazinol J (6) showed concentration-dependent and mixed inhibition kinetics and achieved slow and time-dependent inhibition of hIDO1. Additionally, docking simulations were performed to evaluate the inhibitory potential and binding interactions of the compounds with hIDO1. These findings suggest that these 1,3-diphenylpropane derivatives can serve as therapeutic agents for conditions involving hIDO1 dysregulation, such as cancer, autoimmune disorders, and infectious diseases.

**Ki Mo Kim, A.-Rang Im, Ki-Shuk Shim, Chang-Seob Seo, Yongnam Lee, Jonghun Lee, Ji Seok Yoo, Sunga Choi, Sungwook Chae. Chikusetsusaponin IVa from *Dolichos lablab* Linne attenuates UVB-induced skin photoaging in mice by suppressing MAPK/AP-1 signaling. (2024) Appl. Biol. Chem. 67: 79**

Ultraviolet-B (UVB) radiation-induced photoaging of the skin is characterized by amplified expression of matrix metalloproteinase-1 (MMP-1) and reduced collagen fibers, both of which contribute to skin wrinkle formation. Edible natural products can protect against skin photoaging. Here, we investigate the protective effect of *Dolichos lablab* Linne (DLL) water extract against UVB radiation-prompted skin damage and attempt to uncover its fundamental mechanisms in human keratinocytes (HaCaT) and HR-1 hairless mouse. We found DLL extract rescued the reduction in cell viability associated with UVB exposure without any associated cytotoxic effects. It also protected against skin photoaging by inhibiting mitogen-activating protein kinase (MAPK) signaling, thereby preventing the UVB-associated increase in MMP-1 and -9 expression. DLL extract also increased the expression of both superoxide dismutase 1 (SOD1) and catalase (CAT). We identified chikusetsusaponin IVa, soyasaponin Bb, and sandosaponin A as bioactive components of DLL. Although we have not yet identified the mechanisms by which these compounds reduce the effects of photoaging, we have demonstrated that chikusetsusaponin IVa, soyasaponin Bb, and sandosaponin A reduce MMP-1, MMP-9, p–c-Fos, and p–c-Jun expression, while also avoiding any cytotoxicity. We found oral administration of DLL extract effectively alleviated dorsal epidermal thickening and skin dehydration in HR-1 hairless mouse visible to UVB. DLL extract also prevents UVB-induced activation of the MAPK/AP-1 signaling pathway, thereby reducing the expression of MMPs in dorsal mouse skin. Our results indicate that chikusetsusaponin IVa, soyasaponin Bb, and sandosaponin A are bioavailable components of DLL extract that can reduce UVB-induced skin damage via MMPs by deactivating the MAPK/AP-1 signaling pathway. These findings suggest DLL extract can be used as a skin anti-photoaging agent.

**Haeng Lim Lee, Selim Ashoor, Zhuang Yao, Yu-Sin Jang. Characterization of acidogenic phase metabolism in *Clostridium acetobutylicum* ATCC 824 (pCD07239) under different culture conditions. (2024) Appl. Biol. Chem. 67: 80**

In this study, we investigated the metabolic behavior of the engineered *Clostridium acetobutylicum* ATCC 824 (pCD07239) strain during the acidogenic phase under varying glucose concentrations and pH conditions. Unlike the wild-type *C. acetobutylicum* ATCC 824, the engineered strain exhibited negligible butyrate production and simultaneous butanol production during the acidogenic phase under limited glucose condition of 25 g/L. Specifically, batch fermentations of the engineered strain with 25 g/L glucose at a pH of around 5.0 (initially uncontrolled) demonstrated butanol production of 2.99 g/L, while butyrate remained below 0.30 g/L. Separately, in batch fermentations at pH 6.0 with 90 g/L glucose, acetate production nearly doubled compared to fermentations at pH 5.0 with the same glucose concentrations, reaching a maximum concentration of 11.43 g/L, while butyrate production remained relatively low at 4.04 g/L. Under these pH 6.0 and 90 g/L glucose conditions, butanol production reached 9.86 g/L. These findings indicate that *C. acetobutylicum* ATCC 824 (pCD07239) maintained low butyrate production, even under conditions favoring acidogenesis, and consistently produced butanol. Additionally, the negligible production of acetone at pH 6.0 further indicates that the traditional phase transition was not prominent, suggesting altered regulation mechanisms in the engineered strain. These findings highlight *C. acetobutylicum* ATCC 824 (pCD07239) strain’s unique metabolic profile and its potential for efficient biobutanol production under diverse conditions.

**Hong Wang, Xiang Liao, Chunyao Lin, Weidong Bai, Gengsheng Xiao, Xingyuan Huang, Gongliang Liu. Optimization of fermentation conditions, physicochemical profile and sensory quality analysis of seedless wampee wine. (2024) Appl. Biol. Chem. 67: 81**

The aims of the present stud were to optimize fermentation parameters of seedless wampee wine using response surface methodology (RSM) and evaluate the changes in flavor metabolites during fermentation. Seedless wampee wine of optimal sensory quality was produced using an inoculum concentration of 0.6%, initial sugar levels of 200 g/L, a fermentation temperature of 22 °C, and a fermentation period of 9 days. Then the flavor compound profiles (amino acids, organic acids and volatile aroma compounds) of seedless wampee wine during the fermentation under optimal conditions were analyzed using high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometr (GC-MS). The main fermented phase of fermentation resulted in fluctuations in both total amino acids and organic acids, with stabilization occurring later on. A total of 54 volatile components, including esters, alcohols, terpenes, and acids, were putatively identified. Terpenes were the primary drivers of the flavor characteristics of seedless wampee. The rise of esters and decline of terpenes have the potential to significantly alter the flavor of wine during fermentation. These results would contribute to the further development of seedless wampee wine.

**Yoora Cho, Juin Yau Lim, Avanthi Deshani Igalavithana, Geonwook Hwang, Mee Kyung Sang, Ondřej Mašek, Yong Sik Ok. AI-guided investigation of biochar’s efficacy in Pb immobilization for remediation of Pb contaminated agricultural land. (2024) Appl. Biol. Chem. 67: 82**

This study evaluated the lead (Pb) immobilization efficiency of biochar in contaminated agricultural soil. The biochar was produced from a range of major biomass residues and pyrolyzed under well-controlled conditions. Ten different types of standard biochar samples were derived from five different feedstocks (i.e., softwood, miscanthus straw, rice husk, oilseed rape straw, wheat straw) and pyrolyzed at 550 ℃ and 700 ℃. Pb-contaminated soil near an abandoned mine was incubated with 2.5% (w w− 1) of biochar. Incubation was conducted for various durations at room temperature under both short-term (21 days) and long-term (214 days) conditions. This variation explicitly accounted for the simulated microplastic contamination during the long-term incubation period. A novel framework has been developed to predict the long-term immobilization effect of various biochar types using a machine-learning approach, following the successful identification of optimal biochar implementations. This prediction method utilizes a small on-field dataset by employing a data augmentation approach, showcasing an innovative approach to forecasting the effects of different biochar types over time. After the incubation period, soil samples were analyzed for their chemical properties. As a result, oil seed rape biochar was the highest in pH, EC, exchangeable Ca2+, Mg2+, and K+, total nitrogen content, soil organic matter content, and available phosphate. In return, OSR 700 treated soils showed the highest content of exchangeable cations and the lowest content of available Pb after the incubation period. The most efficient biochar for immobilizing lead (Pb) in soil appears to be OSR 700, based on the available evidence.

**Yu-Wei Chen, Gao Feng, Xia Hong, Meng Wang, Quan Zhang, Zhao-Yong Sun, Ya-Ting Chen, Yue-Qin Tang. Effects of high solid content and straw proportion on volatile fatty acids production from straw, sludge and food wastes: performance and microbial community characteristics. (2024) Appl. Biol. Chem. 67: 83**

Anaerobic digestion (AD) is an efficient technology for treating organic solid wastes, and the volatile fatty acids (VFAs) produced during AD have significant value due to their wide range of applications and higher added value compared to methane. This study investigated the long-term effects of high solid content and straw proportion in mixed substrates (straw, sludge, and food wastes) on VFAs production through semi-continuous reactors under thermophilic and mesophilic conditions. Results showed that both reactors achieved a maximum VFAs concentration of ~ 22 g/L as the straw proportion increased to 50%. Acetate (48.3 – 64.5%) was the main component of produced VFAs in both reactors, while butyrate and propionate production in thermophilic temperature were superior compared to mesophilic conditions. Microbial community analysis revealed that *Defluviitoga* plays a pivotal role in acidogenesis within both reactors; besides, unclassified Hungateiclostridiaceae and *Caproiciproducen* were found to be dominant in thermophilic reactor, while Lachnospiraceae\_NK3A20\_group and Rikenellaceae\_RC9\_gut\_group were essential for VFAs production under mesophilic conditions. These findings provide valuable insights for the biotechnological exploration of acidogenic fermentation for large-scale mechanized production of VFAs from agricultural wastes.

**Hyemi Kim, Ga Yeong Cheon, Jae Hee Kim, Ra-Yeong Choi, In-Woo Kim, Hyung Joo Suh, Ki-Bae Hong, Sung Hee Han. Preparation of chitosan oligosaccharides from chitosan of *tenebrio molitor* and its prebiotic activity. (2024) Appl. Biol. Chem. 67: 84**

This study aimed to establish the optimal production conditions for mealworm chitosan oligosaccharides (MCOS) using the response surface methodology and measure the prebiotic effect of MCOS prepared on cecal microbiota through in vitro anaerobic fermentation. The optimal conditions for MCOS production using chitosanase were 2.5% substrate, 30 mg/g enzyme, and 6 h reaction time. Matrix-assisted laser desorption ionization-time of flight mass spectrometry, Fourier transform infrared spectroscopy, and in vitro assays to confirm that the chemical structure and physicochemical properties of MCOS are similar to those of commercially available chitosan oligosaccharides. The growth of *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, and *Bifidobacterium bifidum* was increased by MOCS and confirmed that the prebiotic effect of MCOS was significant in a concentration-dependent manner. The addition of 1% and 2% MCOS to in vitro anaerobic fermentation resulted in changes in the content of short-chain fatty acids (SCFAs) and an increase in *Verrucomicrobiota* abundance compared with the control. In the case of *Romboutsia*,*Turicibacter*, and *Akkermansia*, a significant increase was confirmed in the MCOS-containing groups compared to that in the control group. Compared to 2% MCOS, 1% MCOS more significantly affected *Lactobacillus* levels. MCOS produced by chitosanase under optimal conditions contains oligosaccharides with 2–6 degree of polymerization and exerts a prebiotic effect that affects changes in the SCFA content and microbiota composition in the cecum.

**Mariam S. El-Alfy, Mohamed E. Mostafa, Abelaziz M. Dawidar, Mamdouh Abdel-Mogib. Phytochemical composition and green insecticides from *Citrus aurantifolia* fruit peels against whitefly, *Bemisia tabaci*. (2024) Appl. Biol. Chem. 67: 85**

Insecticidal potential of extracts of *Citrus aurantifolia*, family Rutaceae, was evaluated to control whiteflies, *Bemisia tabaci*. Biocidal activity directed chromatographic separation of chloroform and butanol fractions, with spectral identification (1D-NMR, 2D-NMR, ESIMS) of the active fractions have been resulted in separation and structural elucidation of for previously described coumarins (bergapten **1**, limettin **2**, isopimpinellin **3**, oxypeucedanin hydrate **4**) in addition to a new dimeric coumarin (12R, 12’R)-aurantifolin **5**, two known limonoids; 21,23-dihydro-23-methoxy-21-oxolimonin **6**, 21,23-dihydro-23-methoxy-21-oxonomilin **7**, and two known flavonoid glycosides; scoparin **8**, and narcissin **9**. Amongst these compounds, narcissin **9** was the most effective after 24 h. of treatment while, (12R, 12’R)-aurantifolin **5** was the most potent against *B. tabaci*, 3rd instar nymphs after 72 h. of treatment and under laboratory conditions, with LC50 values of 33.31and 15.92 ppm, respectively comparing with the positive control azadirachtin.

**Samina Hanif, Zainab Shahzadi, Irfan Anjum, Zubaida Yousaf, Arusa Aftab, Sana Javed, Zainab Maqboo, Riaz Ullah, Zafar Iqbal, Muhammad Ahmer Raza. Colchicine, serotobenine, and kinobeon A: novel therapeutic compounds in *Carthamus tinctorius L.* for the management of diabetes. (2024) Appl. Biol. Chem. 67: 86**

Diabetes, a global health concern, poses increasing mortality risks. The pathogenesis of diabetes involves multiple mechanisms, with oxidative stress being one of the key contributors. As synthetic drugs have various side effects, which can be minimized by using herbal plants. This study focuses on the In vitro antioxidant potential, α-amylase inhibition potential, identification of bioactive compounds, and hub genes in diabetes treatment mechanism by using *C. tinctorius* Extraction of *C. tinctorious* lead and flower was performed using different solvents (Distilled water, methanol, chloroform, and Dimethyl ether). After extraction different concentrations range from 25–200 mg/mL) was made and checked against activities. The antioxidant potential was assessed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), total phenolic contents (TPC), and total antioxidant capacity (TAC) assays, while antidiabetic activity was evaluated through α-amylase inhibition assay. Phytochemicals was identified by GC–MS analysis, followed by ADMET screening and network pharmacology analysis using Swiss Target Prediction, Gene Card, DesGeNet, DAVID, STRING, Cytoscape, and drug revitalization databases. Results revealed positive correlations with DPPH, TAC, and TPC. Methanol extract exhibited the highest inhibitory concentration. Screening of 46 compounds was performed by studying their pharmacokinetic properties which revealed 9 compounds effective against 204 diabetes targets. Moreover, their network analysis identified four hub genes, including AKT1, JUN, EGFR, and MMP9. These genes found highly associated with drugs like Colchicine and Serotobenine. Revitalization analysis also highlighted four genes (EGFR, PTGS2, AKT1, and MMP9) strongly correlated with FDA-approved drugs. The study suggests *C. tinctorius* methanol extract is a potential source for novel drugs.

**Ji-Hyun Kim, Weon-Young Choi, Seung-Jun Jeong, Ka Hyon Park, Gyuseok Lee, Mangeun Kim, Soo-Chang Joo, Seongjun Kim, Beom-Jin Cho, Young-Ok Son, Je-Hwang Ryu. Assessing the therapeutic potential of *Ganoderma lucidum* spore oil in alleviating periodontal tissue damage in murine periodontitis model. (2024) Appl. Biol. Chem. 67: 87**

Periodontal disease presents a significant challenge in oral health due to its chronic inflammatory nature and subsequent degradation of tooth-supporting structures. Natural compounds have attracted attention for their potential therapeutic effects in alleviating symptoms of periodontitis (PD). In this study, we investigated the impact of *Ganoderma lucidum* spore oil (GLSO), a lipid component extracted from broken-walled GLS using the supercritical CO2 extraction method, on PD pathogenesis in vitro and in vivo. Treatment of human gingival fibroblasts with GLSO resulted in a significant reduction in the expression of inflammatory factors, including matrix metalloproteinase (MMP)-1 and interleukin (IL)-8, upregulated by lipopolysaccharide or IL-1β. Molecular mechanism studies revealed that the observed decrease in inflammatory factor expression may be attributed to the inhibition of phosphorylated c-Jun N-terminal kinase activity by GLSO. Furthermore, intraperitoneal injection of GLSO in a ligature-induced PD mouse model led to a notable reduction in periodontal inflammation and alveolar bone loss, accompanied by decreased levels of MMP-1 and IL-8. These in vivo results support the potential therapeutic efficacy of GLSO in alleviating PD symptoms. Overall, our study provides novel insights into the beneficial effects of GLSO in PD management. Further research is warranted to elucidate the underlying molecular mechanisms and explore the clinical applicability of GLSO as a promising therapeutic agent for PD treatment.

**Ahyoung Yoo, Hyunjung Lee, Jung-In Kim, Jeong-Hoon Hahm, Chang Hwa Jung, Jiyun Ahn. Effect of the combination of *Lithospermum erythrorhizon* and *Lonicera japonica* on dexamethasone-induced muscle atrophy in mice. (2024) Appl. Biol. Chem. 67: 88**

Skeletal muscle atrophy occurs in several pathological conditions. Among other reasons, high-dose or long-term administration of glucocorticoids increases circulating glucocorticoid levels and causes muscle atrophy. The purpose of this study was to investigate whether *Lithospermum erythrorhizon* and *Lonicera japonica* complex extract (LELJ) has a beneficial effect on dexamethasone (Dexa)-induced muscle atrophy. In Dexa-induced myotube atrophy, treatment with LELJ increased myotube diameter, decreased the expression of muscle atrophy markers, and increased the expression of myosin heavy chain (MHC) isoforms. Supplementation with LELJ improved muscle function and performance in mice with Dexa-induced muscle atrophy as demonstrated by grip strength and running tests. Additionally, it increased skeletal muscle mass, size, and expression of MHC isoforms and protein synthesis-related markers. Furthermore, it reduced the upregulated protein levels of skeletal muscle atrophy markers in Dexa-treated mice. Supplementation with LELJ reversed Dexa-induced translocation of the glucocorticoid receptor and forkhead box O3 from the cytosol to the nucleus in skeletal muscles. LELJ also ameliorated age-related muscle loss by extending lifespan and increasing locomotor capacity in *Caenorhabditis elegans*. We identified loganin and lithospermic acid as bioactive compounds of LELJ and found that treatment with these agents increased myotube diameter, MHC isoform, and puromycin protein levels, and decreased atrophy markers in Dexa-treated myotubes. The current findings underscore how LELJ can prevent Dexa-induced skeletal muscle atrophy, attributing the effects to loganin and lithospermic acid.

**Hyegyeong Lee, Kiyun Kim, Junhyeong Park, Joon-Goo Lee. Contamination of trichlorobenzene isomers in food: toxicity, analytical methods, occurrence in food, and risk assessments. (2024) Appl. Biol. Chem. 67: 89**

Trichlorobenzenes (TCBs), comprising the isomers 1,2,3-, 1,2,4-, and 1,3,5-TCB, disrupt metabolic processes by inducing liver enzymes involved in xenobiotic metabolism, suggesting a broad toxicological impact. Specifically, exposure to TCBs is associated with significant organ-specific toxicities, such as increased liver and kidney weights in rodents and cytotoxic effects in mammalian cells, which include DNA damage without metabolic activation. Used extensively in industrial and agricultural sectors, TCBs are prevalent pollutants in various ecosystems, including air, food, surface water, groundwater, sediment, soil, and sewage. This is a concern because of their tendency to accumulate in lipid-containing tissues of animals and humans and potentially serious risks to human health and ecosystems. Information showing the presence of TCBs in food, drinking water, and even human breast milk underscores the need for ongoing assessment of the extent of these contaminants in food to measure the potential exposure to these chemicals. TCBs are extracted from various food sample matrices, and then instrumental analysis is performed, typically gas chromatography (GC) coupled with a variety of detectors. This review discusses the occurrence and risk assessment of TCBs in foods, as well as the toxicology and analytical methods related to TCBs.

**Hosam Ki, Sung Geon Yoon, Jeung Hi Han, Byeongmin Shin, Young Soo Kim, Yang Gyu Choi, Kwang Yeon Hwang. Development of *Bacillus stratosphericus* Lysate Concentrate to Control Sebum Secretion through In vitro Studies and Clinical Trial. (2024) Appl. Biol. Chem. 67: 90**

The sebum on human skin is generated for various causes. The composition of the formed sebum increases the proliferation of *Cutibacterium acnes* (*C. acnes*) residing on the skin. As *C. acnes* proliferates, it produces skin irritants that stimulate the sebaceous glands, increasing sebum production. Skin troubles such as acne may occur. The lysate concentrates of *Bacillus stratosphericus* (*B. stratosphericus*), first discovered in the stratosphere, confirmed a 66.35% inhibition of Nitric Oxide (NO) production at 0.50 mg/ml concentration in vitro. Additionally, the growth inhibition efficacy of *B. stratosphericus* lysate concentrate (BSLC) against *C. acnes* was confirmed, showing a 95.1% inhibition of growth proliferation at a consistency of 0.50 mg/ml. Based on the in vitro results, the efficacy of BSLC in degrading and reducing sebum was confirmed by reacting it with artificial sebum to various concentrations. The results showed a concentration-dependent decrease in artificial sebum ccording to the efficacy results confirmed in vitro, a clinical trial was conducted to evaluate the daily sebum reduction efficacy of a serum formulation containing 50 mg/ml of BSLC. After a 4-week application, the test group containing BSLC determined a significant 28.68% reduction in sebum levels, demonstrating the practical implications of the research. In conclusion, BSLC is considered to have sufficient industrial value as a valuable ingredient for the cosmetics industry aimed at sebum improvement.

**RunCheng Zhou, QiLin Liang, Han Lei, Tianci Liang, Simin Chen, Xin Chen. Preparation of *α*-zein loaded with baicalincomposites: A study on their in vitro simulated digestive behavior and molecular dynamics simulation. (2024) Appl. Biol. Chem. 67: 92**

In order to improve the bioavailability of baicalin, this article prepared for *α-*zein loaded with baicalin composites (*α-*zein@BA) by pH driven method and they were characterized using scanning electron microscopy, infrared spectroscopy, and measurement of particle size distribution in water solution phase techniques. The digestive behavior and antioxidant activity of composites before and after simulating gastrointestinal fluid in vitro were studied as well. At the same time, molecular dynamics simulation techniques were used to reveal the molecular mechanism behind the formation of the composite between the two. The results indicated that the composites of *α*-zein@BA were observed to be approximately spherical under a scanning electron microscope, and their particle size was mainly distributed in the range of 94.55-145.10 μm in aqueous solution, whose encapsulation efficiency of baicalin was (86.61 ± 0.71) %. Infrared spectroscopy analysis indicated that *α*-zein and baicalin mainly formed complexes through hydrogen bonding, electrostatic and hydrophobic interactions. The measurement results of baicalin residue in simulated digestion of gastric and intestinal fluids in vitro are as follows: *α-*zein@BA > Baicalin, while both significantly increased in the gastric digestion stage (*P* < 0.05) and significantly decreased in the intestinal digestion stage (*P* < 0.05). Molecular dynamics simulation studies have shown that baicalin has a promoting effect on protein structural stability, and protein 158SER and GLN196 were mainly formed hydrogen bonds with it, while hydrophobic interactions were mainly manifested between non-polar amino acids such as PHE201 and PRO200. This study indicates that *α*-zein and baicalin can form stable composites, improving the bioavailability of baicalin.

**Ji Yeon Song, Hyunsook An, Soojeong Kim. Preparation of *α*-zein loaded with baicalincomposites: In vitro effectiveness of CB469, a MET tyrosine kinase inhibitor in MET-activated cancer cells. (2024) Appl. Biol. Chem. 67: 94**

Gene alterations in receptor tyrosine kinases can result in oncogenic driver mutation in non-small cell lung cancer (NSCLC) including in genes like EGFR, ALK and MET. MET amplifications and MET exon14 skipping are the primary genetic changes in MET-altered cancers. Acquired MET mutations mediate resistance to clinical MET-targeted therapy in NSCLC. MET kinase domain secondary mutations (D1228X, Y1230X) confer resistance to type I MET tyrosine kinase inhibitors (TKIs) in METexon14-altered or MET amplified NSCLC. Here, we investigated the preclinical activity of a novel MET inhibitor, CB469, with cell growth, signaling pathway and colony formation. We confirmed that CB469 inhibited the activity of MET wild and secondary mutant kinases, D1228N and Y1230H, as a type II inhibitor. CB469 also inhibited cell growth and cell signaling proteins in MET-activated or MET exon14 skipping-mutated cancer cell lines and NIH/3T3 cells expressing an engineered MET mutant. CB469 exhibited the inhibitory efficacy comparable with that of capmatinib in migration of EBC-1(*METwt*) and Hs746T(*METΔex14*) cells. Finally, CB469 showed selective and potent inhibition in MET-activated cancer cells among MET TKIs leading to enhanced selectivity for MET-mutant versus wild type MET with inhibition of cell growth in NIH/3T3 cells expressing an engineered MET mutant variant.

**Si-Young Ahn, Chang-Dae Lee, Ja Jung Ku, Sanghyun Lee, Sullim Lee. Anti-aging potential of Cephalotaxus harringtonia extracts: the role of harringtonine and homoharringtonine in skin protection. (2024) Appl. Biol. Chem. 67: 96**

Photoaging damages the skin layers. The tumor necrosis factor-alpha (TNF-α) plays a crucial role in the central mechanism of photoaging. TNF-α production leads to direct damage to skin cells and facilitates the degradation of vital extracellular matrix (ECM) proteins. TNF-α stimulates matrix metalloproteinase-1 (MMP-1) activation This accelerates the loss of skin elasticity and wrinkle formation. Thus, preventing photoaging and delaying the skin aging process are important research objectives, and the development of new anti-aging substances that target the TNF-α and MMP-1 pathways is promising. In this context, the efficacies of four extracts derived from two types of *Cephalotaxus harringtonia* (CH) buds (CH-10Y-buds, CH-200Y-buds) and leaves (CH-10Y-leaves, CH-200Y-leaves) were investigated, exhibiting a significant reduction in reactive oxygen species (ROS). Among the four extracts, CH-10Y-buds was the most effective in reducing ROS and exhibited the highest amounts of harringtonine and homoharringtonine. The activities of harringtonine, homoharringtonine, and ginkgetin were evaluated; harringtonine exhibited a high efficacy in inhibiting TNF-α-induced inflammatory responses and MMP-1 activation, thereby reducing collagen degradation. These findings suggest that CH-10Y-buds and their components herringtonin are promising candidates for preventing damage caused by photoaging. Our results can facilitate the development of new methods for maintaining skin health and inhibiting the skin aging process. Further research is necessary to comprehensively evaluate the potential efficacy of these candidate substances and investigate their applicability to actual skin. Such studies will aid in the development of more effective anti-aging strategies in the future.

**Hyeonmin Lee, Minsu Park, Yujin Kweon, Dowhan Lee, Chanseok Shin. Targeted dsRNA-mediated suppression of Phytophthora infestans infection via Avr3a. (2024) Appl. Biol. Chem. 67: 97**

*Phytophthora infestans* (*P*. *infestans*) is a highly destructive oomycete that causes the late blight in Solanaceous crops, such as potatoes and tomatoes, reducing crop yield. Although many pesticides are used to control *P*. *infestans*, the pathogen has evolved resistance to these chemical pesticides over time. In this study, we employed RNAi technology as an alternative strategy to suppress *P*. *infestans* infection. We designed and synthesized two dsRNAs targeting 5' and 3' regions of the *Avirulence Protein 3a* (*Avr3a*) gene, a key effector essential for the virulence of *P*. *infestans*. Interestingly, the dsRNA targeting the 5' region which contains the conserved RxLR-EER motif of Avr3a exhibited more substantial suppression of *P*. *infestans* infection and *Avr3a* expression level compared to the 3' region targeting dsRNA. Additionally, we identified changes in the expression of genes related to pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) in plants treated with these dsRNAs. In leaves treated with dsRNAs targeting *Avr3a*, the expression of PTI-related genes was restored, while ETI-related genes showed lower expression levels compared to the mock-treated leaves. These results suggest that dsRNAs targeting *Avr3a* effectively suppress *P*. *infestans* infection, enabling plants to achieve balanced immunity and enhanced defense.

**Mi-Rae Shin, Minju Kim, Hui Yeon An, Hwang-Yong Choi, Youngseok Ham, Hakjoo Choi, Seong-Soo Roh. Effects of oral hyaluronic acid on monosodium iodoacetate-induced osteoarthritis in rats: mechanistic insights and therapeutic implications. (2024) Appl. Biol. Chem. 67: 98**

This study aimed to meticulously assess the effectiveness of hyaluronic acid (HA) in mitigating symptoms associated with monosodium iodoacetate (MIA)-induced osteoarthritis (OA) symptoms in rodent models and to investigate the underlying mechanistic pathways. Eight-week-old rats were randomly allocated to a normal control group and three experimental groups (*n* = 10 per group). The normal group did not undergo any treatment. The experimental groups were administered MIA for 1 week to induce osteoarthritis, and orally administered distilled water (control group), 2 mg/kg indomethacin (INDO group), or 20 mg/kg HA (HA20 group) daily for 4 weeks. The HA20 group showed a significant improvement in hind-paw weight-bearing distribution after 4 weeks compared to the control group. HA suppressed inflammatory responses by reducing the overproduction of prostaglandin E2 and leukotriene B4 and protected the vital components of the articular ECM, including glycosaminoglycans and aggrecan. HA treatment effectively reduced inflammation, protected cartilage by inhibiting MMP expression, and suppressed inflammatory mediator production. This study demonstrates that HA has potential to alleviate OA symptoms in a rodent model stimulated with MIA, rendering it a promising therapeutic agent for OA.

**Shin-Won Lee, Garok Lee, Ji-Hyeon Jo, Youri Yang, Joong-Hoon Ahn. Biosynthesis of phloretin and its *C*-glycosides through stepwise culture of *Escherichia coli*. (2024) Appl. Biol. Chem. 67: 99**

Phloretin (PT) belongs to the dihydrochalcones (DHCs) family and is found in apple and rooibos tea. Its glycosides, including phlorizin (PT 2′-*O*-glucoside), trilobatin (PT 4′-*O*-glucoside), and nothofagin (NF, PT 3′-*C*-glucoside), are present in various plants. Phloretin and its related glycosides possess health benefits, including antioxidant, anti-inflammatory, and antibacterial activities. To biosynthesize PT and its glycosides, the relevant pathways in plants were studied and introduced into *Escherichia coli*. We reconstructed the biosynthetic pathways pertaining to PT and three PT *C*-glycosides (NF, PT 3′, 5′-di-*C*-glucoside [PDG], and PT 3′-*C*-arabinoside [PARA]) in *E. coli.* To prevent the undesirable synthesis of flavonoids instead of PT, we strategically divided the entire pathway into two parts: the first involved the synthesis of tyrosine to phloretic acid (PA), while the second involved the synthesis of PA to PT and its glycosides. The gene set pertaining to each part was incorporated into a different engineered microbe. We optimized phloretin microbial biosynthesis by improving enzyme affinity, identifying the gene that increased the output, refining the production design to a stepwise culture approach, and analyzing the culture conditions (substrate and yeast extract concentrations and pH) conducive to maximum output and the prevention of product degradation. Using the stepwise culture approach, 12.8 mg/L of PT, 26.1 mg/L of NF, 30.0 mg/L of PDG, and 18.1 mg/L of PARA were synthesized. This study provides valuable information for future approaches in the microbe-based synthesis of PT derivatives.

**You Rim Min, Jun-Bae Hong, Sam Han, Min-Ji Choi, Seong Bo Shim, Hae-Won Jang, Jung-Bin Lee. Quantitative analysis of seven commonly used synthetic food color additives by HPLC-PDA. (2024) Appl. Biol. Chem. 67: 100**

Sixteen color additives (tar colors) were detected in 128 food samples (macarons, meringue cookies, and coque macarons) using HPLC with a photodiode array detector at 420 nm, 520 nm, and 620 nm for the yellow, red, and blue and green color types, respectively. The tar color recovery rates ranged from 81.3 to 95.6%, and their limits of detection (LOD) and quantification (LOQ) were 0.001–0.049 mg/kg and 0.004–0.147 mg/kg, respectively. Seven tar colors (Y4, Y5, R3, R40, R102, B1, and B2) were detected in 129 samples. All the samples did not contain nine tar colors (R2, G3, Azo, R106, QY, ORII, BBN, PBV, and GS). The quantity of tar colors (Y4, Y5, R40, and B1) in 15 samples exceeded the Korean Ministry of Food and Drug Safety (MFDS) standard. Ninety samples (70%) used a mixture of two or more tar colors, and the amount used was 11.0–1643.3 mg/kg. The quantity of combined tar colors in 15 samples exceeded 300 mg/kg. Through these findings, this study aims to contribute to the development of safer and more reliable desserts containing tar colors, by enhancing safety measures and ensuring improved quality control for consumer protection.

**Hyunchae Joung, Jaeryang Chu, Yoo Jin Kwon, Kyung Hwan Kim, Chang Hun Shin, Jung-Heun Ha. Assessment of the safety and hepatic lipid-lowering effects of *Lactobacillus delbrueckii* subsp. *lactis* CKDB001. (2024) Appl. Biol. Chem. 67: 101**

Probiotics have been shown to provide health benefits for several metabolic diseases, including obesity, type 2 diabetes, and metabolic dysfunction-associated steatotic liver disease (MASLD), by modulating the gut microbiota. In this study, we evaluated the safety and efficacy of *Lactobacillus delbrueckii* subsp. *lactis* CKDB001 as a potential therapeutic candidate for the treatment of MASLD. We evaluated antibiotic resistance, hemolytic, gelatinase, and bile salt hydrolase activities, and the production of biogenic amines and D-lactate using in vitro analyses. We found that *L. lactis* CKDB001 treatment resulted in significant anti-adipogenic properties in the HepG2 cell line, reducing lipid accumulation and improving lipid profiles through mechanisms involving the upregulation of SIRT1 and PPARα, and downregulation of CD36 and ELOVL6. These results suggest that *L. lactis* CKDB001 is a safe and effective probiotic for managing MASLD. Further in vivo studies and clinical trials are required to validate these effects and fully elucidate their therapeutic potential and safety profiles.

**Nguyen Khoi Song Tran, Nhu Quynh Nguyen, Sullim Lee, Seung Hyun Kim, Daesik Jeong, Eunjeong Seo, Jin Ju Park, Jaejin Cho, Ki Sung Kang. Anticancer effects of aloe-emodin from *Rheum undulatum* L. through activation of the p53 pathway in human prostate cancer cells. (2024) Appl. Biol. Chem. 67: 102**

Aloe-emodin, an anthraquinone compound naturally derived from *Rheum undulatum* L., has gained extensive research attention owing to its various pharmacological effects, including its potential as an anticancer, antivirus, anti-inflammatory, antibacterial, and anti-parasitic agent. It has demonstrated notable inhibitory effects against various types of cancer and cancer cells. Prostate cancer is among the most commonly identified cancers globally and remains a leading cause of cancer-associated deaths in men, often presenting challenges in early detection due to its asymptomatic nature during initial stages. The aim of present study was to determine the biological activity of aloe-emodin obtained from *Rheum undulatum* L. involving activation of the p53-dependent pathway in certain human prostate cancer cell lines. We explored the mechanisms underlying the anticancer effects of aloe-emodin using LNCaP cells, which include p53-wild type and phosphatase and tensin homolog-deficient mutated genes, a widely studied model in genomic research. Aloe-emodin induced apoptosis in LNCaP cells through several mechanisms, including upregulation of the cleavage of caspase-8 (a cross-linked promoter of cell death signals), phosphorylation of p53 at serine 15, DNA fragmentation, cleavage of poly [ADP-ribose] polymerase, and promotion of cell death. These findings strongly indicated that aloe-emodin's anticancer properties in human prostate cancer involve the activation of p53-induced cellular senescence. Conclusively, the findings of this study imply that aloe-emodin extracted from *Rheum undulatum* L*.* is a potential therapeutic compound for adjuvant chemotherapy that induces apoptosis and pyroptosis, an innate immune response, in preventing the progression of precancerous lesions in patients with prostate cancer.

**Hiruni Sandunika kumarasinghe, Ji-Hyang Kim, Su-Lim Kim, Kyeoung Cheol Kim, Rambukkana Maggonage Thiruni Dananjana Perera, Seong-Cheol Kim, Dong-Sun Lee. Bioactive constituents from *Carica papaya* fruit: implications for drug discovery and pharmacological applications. (2024) Appl. Biol. Chem. 67: 103**

*Carica papaya*, commonly known as papaya, is a fruit recognized for its substantial medicinal potential, primarily due to its wide range of bioactive compounds. This review thoroughly examines the pharmacological implications of these constituents and highlights their potential applications in drug discovery and therapy. Papaya is abundant in vitamins (A, C, and E), minerals, enzymes, and phytochemicals such as flavonoids, phenolic acids, carotenoids, and alkaloids, all of which contribute to its antioxidant, anti-aging, anti-inflammatory, and anticancer effects. The fruit demonstrates significant activity against diabetes, obesity, cardiovascular diseases, and gastrointestinal disorders. This review also discusses how environmental factors, including temperature, light, soil quality, and rainfall, impact the phytochemical composition of papaya, thereby influencing its medicinal properties. Both in vitro and in vivo studies have highlighted the therapeutic potential of papaya-derived compounds in various health conditions, including cancer, diabetes, wound healing, and cardiovascular health. Additionally, we explore papaya's role in promoting gut health and its antimicrobial properties against bacterial and viral pathogens. In conclusion, the diverse pharmacological activities of papaya's bioactive compounds position it as a valuable candidate for further research and development in drug discovery and therapeutic applications.

**Shiyang Zhou, Gangliang Huang. The chemical composition and pharmacological activities of *Morinda citrifolia*. (2024) Appl. Biol. Chem. 67: 104**

*Morinda citrifolia* has been widely used by Polynesians as a folk medicine for more than 2000 years. It was reported that the main chemical components of *Morinda citrifolia* include nthraquinones, phenylpropanoids, flavonoids, terpenoids, glycosides, steroids, fatty acids and their esters, etc., it has a wide range of therapeutic properties, including anti-bacterial, anti-oxidant, anti-inflammatory and analgesic, hypoglycemic, hepatoprotective, protective cardiovascular and anti-tumor effects. In order to reveal the nutritional and medicinal value of *Morinda citrifolia* and provide reference for further rational development and comprehensive utilization of *Morinda citrifolia* resources, the chemical composition, pharmacological activity and mechanism of *Morinda citrifolia* were reviewed in this review.

**Danny Arteaga, Angie Nathalia Ramírez, Carlos Acevedo, Jaime Martin Franco, Ricardo Benítez Benítez. Extraction of chontaduro oil (*Bactris gasipaes*) for the synthesis of isopropyl esters through transesterification reactions assisted by ultrasound using green solvents. (2024) Appl. Biol. Chem. 67: 105**

An experimental design study, using the Box-Behnken method with response surface methodologies (RSM), was conducted to evaluate the extraction process of chontaduro pulp (*Bactris gasipaes*) oil and its potential application in obtaining isopropyl esters through a transesterification process. The extraction of chontaduro oil and the transesterification reactions of the fatty esters were assisted by ultrasound techniques using *n*-hexane and isopropanol as environmentally friendly solvents. During the extraction process, the parameters evaluated were the sample to solvent ratio (w/w), power amplitude (%), and ultrasound pulse times (s). For the transesterification process, the parameters evaluated were ultrasound amplitude, ultrasound pulse time, and the amount of isopropanol (mol). The optimal conditions for the extraction process were found to be a sample to *n*-hexane ratio of 1:10 (w/w) and an ultrasound amplitude of 30%, with the pulse time not significantly affecting the oil yield of 15.02% achieved in a total time of 30 min. The predominant fatty acids in chontaduro oil were C18:1 *cis* oleic acid (54.31%) and C16:0 palmitic acid (20.40%). To synthesize isopropyl esters, we found that the ideal conditions are an ultrasound amplitude of 50%, an ultrasound pulse time of 20 s, and 0.4 moles of isopropanol. These conditions resulted in complete conversion within 45 min. The use of ultrasound radiation proved to be an alternative method to conventional techniques, reducing extraction and transesterification times, minimizing energy consumption, and increasing percentage yield. This contributes to green chemistry and the production of value-added products, potentially useful in the cosmetics industry.

**Ji Won Yang, Chanhyuk Park, Eun Hea Jho. Removal technologies of microplastics in soil and water environments: review on sources, ecotoxicity, and removal technologies. (2024) Appl. Biol. Chem. 67: 106**

Microplastics (MP) in the environment has attracted extensive attention due to their ubiquitous occurrence and potential toxic effects; but less attention has been given to the removal technologies of MP in different environmental media. This review covers the sources, ecotoxicities, and removal technologies of MP in soil and water environments reported in previous studies to derive the future research directions for MP removal technologies. Previous studies reported various sources contribute to MP present in soil and water environments, indicating that the source control may be a better strategy than contaminated media treatment for management of MP contamination. Similarly, different terrestrial and aquatic organisms have been used to determine the toxic effects of MP with different characteristics. Previous ecotoxicity studies of MP on soil organisms cover only a small number of species compared to that of MP on aquatic organisms. Therefore, further studies are necessary to investigate the ecotoxic effects of MP on a broader range of soil organisms. Also, since the characteristics of MP are diverse, more ecotoxicity studies in both water and soil environments are required. The review provides an overview of various removal technologies for MP in soil and water and identifies gaps in existing studies. Although more studies have been conducted for removal of MP in water than in soil, they remain in their early stages. More studies are needed for removal of MP removal in soil than in water. With MP in water, most studies have been carried out on a lab scale with artificial wastewaters, thus, studies with natural waters in a pilot or field scale are required. Overall, this review highlights the need for further studies on MP removal technologies for real world applications.

**Kwanyong Choi, Soyeon Lee, Sunyong Yoo, Hyoung-Yun Han, Soo-yeon Park, Ji Yeon Kim. Prediction of bioactive compounds hepatotoxicity using in silico and in vitro analysis. (2024) Appl. Biol. Chem. 67: 107**

The leading safety issue and side effect associated with natural herb products is drug-induced liver injury (DILI) caused by bioactive compounds derived from the herb products. Herein, in silico and in vitro analyses were compared to determine the hepatotoxicity of compounds. The results of in silico analyses, which included an integrated database and an interpretable DILI prediction model, identified calycosin, biochanin\_A, xanthatin, piperine, and atractyloside as potential hepatotoxic compounds and tenuifolin as a non-hepatotoxic compound. To evaluate the viability of HepG2 cells exposed to the selected compounds, we determined the IC50 and IC20 values of viability using MTT assays. For in-depth screening, we performed hematoxylin and eosin-stained morphological screens, JC-1 mitochondrial assays, and mRNA microarrays. The results indicated that calycosin, biochanin\_A, xanthatin, piperine, and atractyloside were potential hepatotoxicants that caused decreased viability and an apoptotic phase in morphology, while these effects were not observed for tenuifolin, a non-hepatotoxicant. In the JC-1 assay, apoptosis was induced by all the predicted hepatotoxicants except atractyloside. According to transcriptomic analysis, all the compounds predicted to induce DILI showed hepatotoxic effects. These results highlighted the importance of using in vitro assays to validate predictive in silico models and determine the potential of bioactive compounds to induce hepatotoxicity in humans.

**Byeonggyu Kim, Kihwan Kim, Won-Chan Kim. *AtGATA5* acts as a novel regulator in secondary cell wall biosynthesis by modulating NAC-domain transcription factors in *Arabidopsis thaliana*. (2024) Appl. Biol. Chem. 67: 108**

The plant cell wall is composed of a primary and secondary cell wall. The secondary cell wall (SCW) plays a crucial role in the movement of nutrients and water and serves as a barrier against pathogens and environmental stresses. However, the biosynthesis of the SCW is complex, involving a network of genes regulated by environmental factors, including light. In this study, we investigated the nuclear localization of AtGATA5 to determine its potential role as a transcription factor and its involvement in SCW formation. To explore changes in leaf phenotypes in overexpression *AtGATA5* and the thickening of interfascicular fiber cells, we conducted a transient activity assay using Arabidopsis protoplasts. The results demonstrated that *AtGATA5* can up-regulate NAC-domain transcription factors, which are master regulators of the SCW biosynthesis pathway. Furthermore, gene expression analysis in plants confirmed that as *AtGATA5* expression increased, the expression levels of NAC-domain transcription factors also increased. These findings suggest that *AtGATA5* plays a functional role in SCW formation by up-regulating master regulators in the SCW biosynthesis pathway. Overall, *AtGATA5* may act as a novel regulator of SCW biosynthesis, offering insights into potential application.

**Nusrat Jahan Methela, Mohammad Shafiqul Islam, Ashim Kumar Das, Hasan Uz Zaman Raihan, Md. Motiar Rohman, Abul Kashem Chowdhury, Bong-Gyu Mun. Antioxidant mechanisms in salt-stressed Maize (Zea mays L.) seedlings: comparative analysis of tolerant and susceptible genotypes. (2024) Appl. Biol. Chem. 67: 109**

Recent anthropogenic activities have spurred unparalleled environmental changes, among which elevated salinity levels emerge as a substantial threat to plant growth and development. This threat is characterized by oxidative stress, marked by the excessive generation of reactive oxygen species (ROS), proline accumulation, and lipid peroxidation. This study investigated the response of four maize (*Zea mays* L.) genotypes - two tolerant (9120 and Super Gold) and two susceptible (Pacific 984 and PS999) - to salinity-induced oxidative stress. Seedlings aged seven days were exposed to 12 dSm− 1 salinity stress for five days, with various parameters including relative water content (RWC), ROS accumulation, proline levels, lipid peroxidation, lipoxigenase (LOX) activity, enzymatic and non-enzymatic antioxidants, and glyoxalases evaluated in fully expanded leaves. Susceptible genotypes exhibited higher RWC loss compared to tolerant genotypes, while proline accumulation was elevated in the latter. Enhanced ROS production (hydrogen peroxide and superoxide), melondialdehyde (MDA) levels, and LOX activity were observed in susceptible genotypes under salinity stress, along with increased oxidation of glutathione (GSH) and ascorbate (ASA) compared to tolerant genotypes. Enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD), glutathione peroxidase (GPX), and monodehydroascorbate reductase (MDHAR) displayed higher activity in tolerant genotypes, while catalase (CAT) activity was significantly different between tolerant and susceptible genotypes under salinity stress in maize. Conversely, elevated activities of ascorbate peroxidase (APX), glutathione S-transferase (GST), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) were observed in both genotypes, indicating their crucial role in cellular protection against ROS and metabolites during salt stress. In short, plants have devised tactics to scavenge surplus Reactive Oxygen Species (ROS) and uphold cellular redox balance amidst oxidative stress. This study aims to offer basic knowledge regarding both enzymatic and nonenzymatic antioxidants, and the defense mechanisms they constitute against ROS detoxification upon salt stress conditions; furthermore, it also explores their interactions with cellular components.

**Nehala Sona Payanthoth, Nik Nurhidayu Nik Mut, Palas Samanta, Guanlin Li, Jinho Jung. A review of biodegradation and formation of biodegradable microplastics in soil and freshwater environments. (2024) Appl. Biol. Chem. 67: 110**

Plastic pollution is of critical environmental concern, thus biodegradable plastics (BPs) have emerged as a potential solution to limit plastic waste accumulation. However, the fate of BPs in the environment, particularly their degradation and the subsequent generation of biodegradable microplastic (BMP) particles, remains poorly understood. This review aims to provide comprehensive insights into the biodegradation process of BPs and their impacts on soil and freshwater environments. Microorganisms play a pivotal role in this process by dismantling polymer chains into smaller particles. Factors influencing biodegradation rates include polymer composition, environmental conditions (e.g., temperature, ultraviolet radiation (UV), and pH), and the presence of chemical additives. However, incomplete degradation can result in BMPs, potentially perpetuating their presence in the environment and posing risks to ecosystems and organisms. This review consolidates understanding the mechanisms governing biodegradation and BMP formation, which is imperative for evaluating their environmental consequences and devising effective strategies for managing plastic waste.

**Jung-Hwan Yoon, Mahesh Adhikari, Seok Soon Jeong, Sang Phil Lee, Hyuck Soo Kim, Geon Seung Lee, Duck Hwan Park, Heejung Kim, Jae E. Yang. Microbial diversity of soils under different land use and chemical conditions. (2024) Appl. Biol. Chem. 67: 111**

Soil microbial communities are crucial to ecosystem functionality, influencing soil fertility and health. Microbial diversity in soil is impacted by various land-use practices and environmental conditions, but the effects on both prokaryotic and eukaryotic communities remain insufficiently understood. This study investigates the influence of different land-use types and soil chemical properties on the composition and diversity of prokaryotic and eukaryotic microbes using next-generation sequencing (NGS). Soil samples were collected from seven distinct locations in South Korea, representing various land uses, including paddy fields, upland fields, forest areas, hydrocarbon- and heavy-metal-contaminated sites, greenhouse soils, and reclaimed tidal soils. Alpha diversity, assessed using Chao1 and Shannon indices, and beta diversity, evaluated through Bray-Curtis dissimilarity and Principal Coordinates Analysis (PCoA), were used to characterize microbial diversity. Soil chemical properties were analyzed, and their relationships with microbial community structure were examined. Results revealed significant variations in both prokaryotic and eukaryotic diversities across different land uses. Soils under conventional agricultural management (paddy and upland fields) showed higher microbial diversity compared to soils with high salinity, contamination, or low suitability for agriculture. Prokaryotic communities were dominated by *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, and *Bacteroidetes*, with variations in abundance linked to soil condition and quality. Eukaryotic communities predominantly consisted of *Opisthokonta*, SAR (*Stramenopiles*,*Alveolates* and *Rhizaria)*, and Amoebozoa, with distinct abundance patterns across different soils. In conclusion, land-use practices and soil chemical properties significantly influence microbial diversity and community composition. Soils subjected to less stress, e.g., agricultural soils, exhibited higher microbial diversity, while stressed soils, e.g., contaminated and saline soils, showed reduced diversity. These findings emphasize the importance of understanding the interplay between land management and microbial ecology for optimizing soil fertility and health.

**Yu Na Lee, Sin Sil Kim, Dong Won Lee, Jae Hong Shim, Sang Ho Jeon, Ahn Sung Roh, Soon Ik Kwon, Dong-Cheol Seo, Seong Heon Kim. Characterization and application of biochar derived from greenhouse crop by-products for soil improvement and crop productivity in South Korea. (2024) Appl. Biol. Chem. 67: 112**

The study examined the optimal production conditions and application rates of biochar derived from greenhouse crop by-products to enhance soil improvement and increase crop yield, thereby promoting sustainable agriculture in South Korea. The expansion of greenhouse cultivation has resulted in significant waste management challenges, and biochar production has emerged as a promising recycling solution for these by-products. Biochar was produced from red pepper stalks through pyrolysis at 200 to 600 °C, and its chemical properties, including pH, EC, T-C, and T-N, were analyzed. In this study, the chemical properties of biochar showed a significant increase in pH (from 5.8 to 10.3), EC (from 46.0 to 119.5 dS m⁻¹), and T-C (from 47.7 to 63.1%) with rising pyrolysis temperatures, while T-N decreased due to nitrogen volatilization above 300 °C. In the lettuce cultivation experiment, biochar application significantly improved fresh weight yield, with the biochar-treated group achieving a maximum of 83.3 g pot− 1 in the first cropping season, compared to 62.8 g pot− 1 in the NPK-only treatment group. However, excessive biochar application rates (≥ 800 kg ha⁻¹) led to yield reductions in the second cropping season, likely due to increased soil pH and EC. These results suggest the potential of recycling greenhouse crop residues into biochar to enhance soil fertility and crop productivity while indicating the need to manage application rates to minimize negative impacts from excessive use.

**Dongxu Song, Mingming Chen, Longbing Yang, Zhenlong Jiao, Jian Peng, Guo Guo. Mechanism of antimicrobial peptide AMP-17 for inhibition of *Aspergillus flavus*. (2024) Appl. Biol. Chem. 67: 113**

*Aspergillus flavus* is a pathogenic fungus with a broad host range, and its secondary metabolite, aflatoxin, recognized as the world’s first naturally occurring carcinogen. Nonetheless, the current control measures for *A*. *flavus* are inadequate, thus, it is imperative to seek alternative control methods for this species. In the present study, we identified an antimicrobial peptide AMP-17, which was found to effectively inhibit the conidial germination, growth, conidiation, and aflatoxin production of *A. flavus*. Additionally, our investigation revealed that the inhibition of *A. flavus* by AMP-17 is primarily attributed to increase cell membrane permeability, modify cell surface morphology, and compromise cellular integrity, as observed through flow cytometry and scanning electron microscopy. Transcriptome analysis indicated significant transcriptional changes in several genes associated with cell wall, cell membrane, cell cycle, detoxification, and aflatoxin biosynthesis in response to AMP-17 treatment, suggesting disruption of these cellular processes and pathways in *A. flavus*. Furthermore, AMP-17 exhibited a broad-spectrum antifungal activity against *Aspergillus* spp. These findings provide a strong theoretical basis for the potential use of AMP-17 as an effective antifungal agent against *A. flavus*.

**Youngshim Lee, Seunghyun Ahn, Euitaek Jung, Dongsoo Koh, Yoongho Lim, Young Han Lee, Soon Young Shin. Design, synthesis, and biological evaluation of (*E*)-2-benzylidene-1-indanones derivatized by bioisosteric replacement of aurones. (2024) Appl. Biol. Chem. 67: 114**

Thymic stromal lymphopoietin (TSLP) is a cytokine derived from epithelial cells and plays an essential role in the onset and activation of Th2-derived allergic inflammatory conditions, including atopic dermatitis. Despite their potential as drug targets, well-defined small molecules that effectively block TSLP expression are still lacking. A plant-derived secondary metabolite, aurone, was derivatized based on bioisosteric replacement to identify compounds that inhibit the promoter activity of TSLP. Thirteen (*E*)-2-benzylidene-1-indanones were designed and synthesized, and their structures were identified using NMR spectroscopy and mass spectrometry. Inhibition of the expression of TSLP triggered by interleukin-4 (IL-4) caused by (*E*)-2-benzylidene-1-indanones was measured using a TSLP gene promoter-reporter activity assay. Because compound **12**, (*E*)-5-methoxy-2-(3-methoxybenzylidene)-2,3-dihydro-1*H*-inden-1-one, showed the best activity, further biological experiments, including RT-PCR analysis, quantitative real-time PCR, and inhibitory effects on IL-4-induced early growth response-1 (EGR-1) expression, EGR-1 DNA-binding activity, and IL-4-induced phosphorylation of the mitogen-activated protein kinase (MAPK) signaling cascade were performed. This study demonstrated that compound **12** acts on MAPK to block IL-4-triggered mRNA expression of TSLP via the MAPK-EGR-1 signaling pathway in HaCaT keratinocytes.

**Priskila Tolangi, Jeehyoung Shim, Raña Mae Sumabat, Sunghan Kim, Hyun-Seung Park, Kyung Do Kim, Hyun Uk Kim, Sanghyun Lee, Joong Hyoun Chin. The genetics and genomics of milk thistle: unlocking its therapeutic potential through modern breeding and biotechnological innovations. (2024) Appl. Biol. Chem. 67: 115**

Milk thistle (*Silybum marianum*) is a Mediterranean herb renowned for its liver-protective, antioxidant, anti-inflammatory, and detoxifying properties, primarily attributed to the bioactive compound silymarin. Recent studies have also highlighted its potential efficacy against COVID-19, contributing to the growing demand for milk thistle dietary supplements, particularly for liver health and immunity support. Milk thistle seeds, rich in silymarin and unsaturated fatty acids, hold significant industrial value as both medicinal and oilseed crops. To meet the growing demand, it is essential to develop standardized seeds, cultivation practices, and extraction methods aimed at maximizing yields of silymarin and other valuable metabolites. Recent advancements in genetic and genomic research, including the development of the first reference genome of *S. marianum*, have played a pivotal role in elucidating the biosynthesis pathways of silymarin and optimizing phytochemical production. This review highlights recent advancements in the genetics, genomics, and biochemistry of milk thistle, with particular emphasis on the importance of diverse genetic resources and AI-driven phenomics strategies, such as hyperspectral and RGB imaging, for high-yield and chemotype breeding. Further, feasibility of developing elite cultivars through molecular approaches, such as genome editing and metabolic engineering, is also discussed as the new traits obtained this way would be key to enhancing the commercial value of milk thistle in light of mass production of phytochemicals to meet rising market demands.

**Bishnu Prasad Pandey, Jong Min Oh, Woong-Hee Shin, Abhimat Subedi, Ankita Dahal, Sumit Bhattarai, Hoon Kim. BACE1 inhibitory potential: screening of medicinal plants collected from Nepal high altitude regions. (2024) Appl. Biol. Chem. 67: 117**

Fifty-four plant extracts from thirty-two medicinal plants collected in Nepal were evaluated for their inhibitory potential against the enzyme beta-secretase-1 (BACE1), to identify potential therapeutic agents for Alzheimer’s disease (AD). Of the studied extracts, rhizome extract of *Rheum australe* D. Don showed the highest inhibitory potential, with an IC50 value of 0.872 ± 0.006 µg/mL. After BACE1 inhibitory activity check using 9 fractions collected from Prep-HPLC, further profiling of the metabolites of the best fraction 7 was performed using high-resolution mass spectrometry (HRMS). Results revealed the presence of diverse secondary metabolites, including aloe-emodin-8-O-β-D-glucoside, rhein-8-O-glucoside, piceatannol-3’-O-β-D-glucoside, emodin-8-glucoside, physcion 8-O-β-D-glucoside, desoxyrhaponticin, chrysophanol-8-O-glucoside, rhapontigenin, rhein, desoxyrhapontigenin, piceatannol, chrysophanol, physcion, and aloe-emodin. In-silico docking simulations were performed to identify potent compounds with high binding efficiencies to BACE1. Compound picetannol-3’-O-β-D-glucoside showed the best binding energy (-53.494 kcal/mol) and inhibitory potential with an IC50 value of 1.270 ± 0.130 µM for BACE1. These results suggested that the *R. australe* D. Don extract is a promising agent for the treatment of AD.

**Hyo Kyung Jee, Han Na Kim, Jin Hee Park. Immobilization of manganese in solution and soil contaminated with trivalent chromium using biochars. (2024) Appl. Biol. Chem. 67: 118**

Mn (manganese) exists in various oxidation states in soil, and Mn2+ is the most mobile species of Mn, which is toxic to plants and restricts their growth. When soil is contaminated with trivalent chromium (Cr3+), Mn oxides in the soil are reduced to Mn2+ by oxidizing Cr3+ while oxidized Cr is subsequently reduced back to Cr3+ by organic matter in soil, leaving Mn2+ and Cr3+ in the soil. Therefore, the objective of this study was to immobilize Mn2+ without altering the Cr species in the soil and to evaluate the effectiveness of biochar treatment in immobilizing both Mn2+ and Cr³⁺ in Cr³⁺-contaminated soil. Biochars derived from different sources including rice bran (RB), chicken manure (CM) and cow manure (WM) were tested for Mn adsorption and the chicken manure derived-biochar showed the highest removal efficiency (100%) for Mn in Mn solution. Moreover, 100% of both Mn²⁺ and Cr³⁺ were removed in Mn²⁺ and Cr³⁺ mixed solution without oxidizing Cr3+. In Mn2+ and Cr3+ mixed solution, initially 1.7% of Cr³⁺ was oxidized to Cr⁶⁺ by Mn, which was subsequently reduced back to Cr³⁺ by biochar, leading to its complete adsorption. In Cr3+ spiked soils treated with 5% and 10% CM biochar, bioavailable Mn and Cr concentrations were significantly reduced. Therefore, biochar is a promising amendment for reducing the bioavailability of Mn and Cr limiting Cr3+ oxidation in Cr3+ contaminated soils.