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**Jung-Tae Kim, Ill-Min Chung, Mi-Jung Kim, Jin-Seok Lee, Beom-Young Son, Hwan-Hee Bae, Young Sam Go, Sun-Lim Kim, Seong-Bum Baek, Seung-Hyun Kim, Gibum Yi. Comparison of antioxidant activity assays in fresh purple waxy corn (Zea mays L.) during grain filling. (2022) Appl. Biol. Chem. 65: 1**

Purple waxy corn is a good source of antioxidant compounds such as anthocyanins and polyphenols. Promotion of its use requires an appropriate assay to determine antioxidant activity. The aim of this study is to verify compatibility of the antioxidant activity assays by comparing five different assays in daily kernel samples of the fresh purple waxy corn during grain filling. The levels of antioxidants measured by 2,2-diphenyl-1-picrylhydrazyl, ferric reducing antioxidant power, and chemiluminescence showed significant positive correlations with the levels measured by the other assays (*r* = 0.761–0.893; *p* < 0.01) and with anthocyanin content (*r* = 0.798–0.924; *p* < 0.01). Reducing capacity of 2,2ʹ-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) and total phenolic content also showed significant correlations (*r* = 0.764; *p* < 0.01). However, during late grain filling, the levels of antioxidants measured by all methods showed strong correlations with each other (*r* > 0.699; *p* < 0.05). The inconsistencies among the measurements are largely dependent on the developmental stage of the corn kernel. The combination of at least two assays is required to ensure reliable antioxidant activity estimates, especially for early grain-filling stages. These results will inform efforts to promote fresh purple corn as a source of antioxidants.

**Chenghu Wu, Ailin Yu, Yue Chen, Mingbo Fan. Homologous tumor cell membrane vesicles active preferential self-recognition of tumor cells in vitro. (2022) Appl. Biol. Chem. 65: 3**

Cell membrane vesicles, as delivery carriers of drugs or biological agents in vivo, are an important therapeutic mode in the study of disease treatment. Tumor membrane-derived vesicles have been widely used in tumor therapy because of their good tumor enrichment effect. The most common method is the surface of nanoparticles coated with tumor cell membrane, which can effectively prolong the circulation time of particles in the blood and the enrichment of tumors. In this study, we prepared vesicles of different tumor cell membrane derivate and studied their targeting to tumors detailly. The results showed that homologous vesicles have high targeting to homologous tumor cells. The fluorescence of vesicles in homologous tumor cells was significantly higher than that in other tumor cells. This study will provide a new strategy and guidance for the clinical treatment of cancer based on the tumor cell membrane system.

**Minsu Park, Tae Young Um, Geupil Jang, Yang Do Choi, Chanseok Shin. Targeted gene suppression through double-stranded RNA application using easy-to-use methods in *Arabidopsis thaliana*. (2022) Appl. Biol. Chem. 65: 4**

RNA interference (RNAi) is an RNA-dependent gene silencing process that is regulated by the interaction between the RNA-induced silencing complex (RISC) and double-stranded RNA (dsRNA). Exogenous dsRNAs are imported directly into the cytoplasm, where they are cleaved by Dicer into short dsRNA fragments of 20–25 base pairs. These short dsRNA fragments, called small interfering RNAs (siRNAs) have sequence-specific interaction with target genes. The guide strand, onto which siRNAs are incorporated in the RISC interacts with the target mRNA sequence, thereby inducing cleavage and degradation of target messenger RNAs (mRNAs) by ribonucleases. Recent studies have shown that plant dsRNA treatments can induce RNAi. However, the dsRNA application methods and delivery systems involved have not been well examined. In this study, dsRNA was introduced to *Arabidopsis thaliana* by two methods: dipping and spray. We synthesized two dsRNAs designed to target mRNAs encoding enhanced green fluorescent protein (*EGFP*). After applying dsRNAs that target *EGFP*, we found an obvious reduction in GFP expression. This was determined using fluorescence microscopy and quantitative reverse transcription PCR to assess the mRNA levels of the auxin-sensitive reporter *DR5-EGFP Arabidopsis thaliana*. Our data revealed that applying target gene-specific exogenous dsRNAs can induce suppression of target genes of interest whether the dipping or spray method is used. This study therefore provides a foundation for understanding how to apply and deliver dsRNAs in plants.

**Eun-Seo Lim. Influence of bacteriocin-producing *Bacillus* strains on quality characteristics of fermented soybean product with biogenic amine-forming lactic acid bacteria. (2022) Appl. Biol. Chem. 65: 5**

This study evaluated the antibacterial activity of bacteriocin produced by *Bacillus* strains against biogenic amines (BA)-forming lactic acid bacteria (LAB), and the applicability of desirable strains as *Bacillus* starters for safety and quality improvement of fermented soybean products. The BA-forming ability of the tested LAB in amino acid decarboxylase broth was mostly strain specific rather that species specific. The bacteriocin produced by specific *Bacillus* strains: *Bacillus* sp. DB407, *Bacillus licheniformis* DB612, and *Bacillus subtilis* DB821 may play a contributory role in the inhibiting the growth of BA-forming strains. In fermented soybean products manufactured using different starter cultures, there was a significant reduction (*p* < 0.05) in the cell counts of LAB by application of a mixed culture containing *B. subtilis* DB821, which showed the highest antimicrobial activity. Specifically, *B. subtilis* DB821 was highly efficient in reducing accumulation of cadaverine, tryptamine, and tyramine produced by *Enterococcus faecium* D12 and *Enterococcus faecalis* D51 during soybean fermentation. In conclusion, the bacteriocin-producing *Bacillus* strains such as *Bacillus* sp. DB 407, *B. licheniformis* DB612, and *B. subtilis* DB821 can be used as a starter culture for the production of BA-controlled soybean products and for the enhancement of the quality and safety of fermented foods.

**Jin Woo Lee, Lei Peng, Hyun Jegal, No-June Park, Sim-Kyu Bong, Joon Won Lee, Jeong Joo Pyo, Yongsoo Choi, Su-Nam Kim. The soybean cultivar SCEL-1 shows potent anti-photoaging effects in a UV-induced three-dimensional human skin and hairless mouse model. (2022) Appl. Biol. Chem. 65: 6**

Soybean (*Glycine max*) is one of the major sources of nutrition and is used as a raw material for food and as a source of feed for livestock. The efficacy of soybeans on skin health includes their ability to reduce wrinkles and pigmentation and increase skin elasticity and moisture content. Black soybean has been consumed worldwide for a long time, especially in Korea, and is used as a medicinal food against several disorders related to the skin. To evaluate whether its effect on the skin is different based on the cultivar of soybeans, three black soybean cultivars collected in Korea, Soybean Core collection Elite Line-1 (SCEL-1), Chung Ja-3 (CJ-3) and Won Heuk (WH), were selected to compare their effect on improving photoaging induced by ultraviolet rays (UVs). We found that SCEL-1 exhibited the best efficacy among the three cultivars tested, and treatment with this soybean extract significantly reduced the expression of matrix metalloproteinase-1 (MMP-1), preventing the degradation of collagen in a 3D human skin model. In addition, SCEL-1 application improved wrinkle- and photoaging-related symptoms, such as epidermal thickening, collagen deficiency and immune cell infiltration, in an animal model established by UV irradiation. Procyanidin B2 and epicatechin isolated from the SCEL-1 cultivar inhibited MMP-1 biosynthesis in UVB-irradiated human dermal fibroblasts, and these two major components are likely related to more significantly attenuated skin photoaging. Therefore, our results indicated that SCEL-1 exhibits good anti-wrinkle effects compared to the other two black soybean cultivars, suggesting that it represents an excellent agent for anti-photoaging.

**Jinhuang Shen, Yifan Zhang, Nana Yang, Xinhua Ma, Tianhua Zhong, Yonghong Zhang. Limonoids from the barks of *Chukrasia tabularis* and their anti-inflammatory activity. (2022) Appl. Biol. Chem. 65: 7**

Two novel phragmalin type limonoids, Tabularisin Q (**1**) and Chuktabularin Y (**18**), together with 22 known limonoids have been separated from bark of *Chukrasia tabularis*. The structures of **1–24** were identified with spectroscopic method (NMR, IR and mass spectrometry) and comparison with literature. The anti-inflammatory activity of all limonoids was assayed in RAW264.7 cells in vitro by evaluating the production of nitric oxide induced by lipopolysaccharide. Limonoids **8, 9, 1**, and **18** showed significant anti-inflammatory activity with the inhibitory rates of 4.32, 11.28, 13.13, and 10.40 μm, respectively, and likely to be useful for the development as therapeutic agents for inflammatory diseases.

**Jeong Hun Cho, Yong Deog Hong, Donghyun Kim, Si Jun Park, Jung Soo Kim, Hyun-Min Kim, Eun Jeong Yoon, Jin-Seong Cho. Confirmation of plant-derived exosomes as bioactive substances for skin application through comparative analysis of keratinocyte transcriptome. (2022) Appl. Biol. Chem. 65: 8**

Plant exosomes are nanosized (30–150 nm) membrane vesicles that contain biomolecules and influence the development of a plant and protect the plant from pathogens. Recently, plant exosomes are in the spotlight as a new biologically active substance. However, whether plant exosomes have similar efficacy to conventional secondary metabolites of plants is unknown. In this study, the difference in efficacy between plant exosomes and conventional secondary metabolites was analyzed with three or four types of plant extracts, including ginseng (*Panax ginseng*) and green tea (*Camellia sinensis*). After 6 h of treatment, the analysis of gene expression pattern of each sample showed that the exosome treatment group and the extract treatment group were clearly distinguished. After selecting the genes that showed differential expression of > twofold change, the number of genes that were up- or downregulated appeared to be 398 or 438 for the extract and 861 or 648 for the exosome, on average. This suggests that the change in transcriptome is more expressed in the exosome treatment group than in the extract treatment group. In addition, in the comparative analysis of expression of genes that are known to affect aging, regeneration, skin barrier, and moisturization—*MMP12*, *MMP13*, *NOTCH3*, *FGF12*, *HS3ST3A1*, *LOX*, *VIM*, *ELOVL3*, and *KRTI*—the exosome treatment group was predicted to more effectively contribute to maintaining a healthy skin when compared to the extract treatment group. The number of genes that were identified to specifically react to the *Panax ginseng* or *Camellia sinensis* treatment group during the transcriptome change phase was 11 and 8, respectively. This suggests that exosomes bear its specific effect according to the plant it is derived from. In conclusion, the results of this study indicate that plant exosomes, as natural biologically active substances, have different effects from conventional plant extracts, and have the potential to be commercialized as a cosmeceutical product.

**Vuong Vu, Karthika Muthuramalingam, Vineet Singh, Changmin Choi, Young Mee Kim, Tatsuya Unno, Moonjae Cho. *Schizophyllum commune-*derived β-glucan improves intestinal health demonstrating protective effects against constipation and common metabolic disorders. (2022) Appl. Biol. Chem. 65: 9**

It has been proven that β-glucan produced by *Schizophyllum commune* has beneficial effects on obesity, obesity-associated constipation, and colitis conditions; however, the protective effect of the compound on host at basal state is yet to be investigated. C57BL/6 J mice were fed with a normal diet (ND), normal diet supplemented with 3 g/kg (BG\_low), and 5 g/kg (BG\_high) of β-glucan (BG) for 12 weeks. Body weight, food and water intake and fecal status were monitored weekly. Intestine was stained by Periodic acid–Schiff (PAS) and Alcian Blue to evaluate the mucin layer thickness and goblet cell population. Morphological changes in internal organs and intestinal motility were also assessed, while serum biomarkers for liver injury and glucose level were analyzed. On the other hand, fecal microbiota and associated metabolic activities were also investigated. β-glucan bulked feces, decreased fecal moisture, and proliferated goblet cells resulted in a thickened lubricating mucin layer; however, the intestinal transit rate did not increase. The abundance of beneficial bacteria was increased while the harmful strains was decreased in a dose-dependent manner by the effect of β-glucan. Specific short chain fatty acid (SCFA)-producing strains, such as *Roseburia*, *Ruminococcus*, and *Bifidobacteria*, were selectively increased by β-glucan. In addition, consumption of β-glucan lowered level of obesity-associated biomarkers. *Schizophyllum commune* β-glucan showed an insignificant change in transit rate in healthy conditions when compared with obesity, despite similar effects on increasing mucus production and bulked feces. Nonetheless, the outcomes proposed protective effects against obesity, diabetes, inflammatory bowel diseases (IBD), and constipation, in which the modification of the gut microbiota by β-glucan is the largest contributor.

**Zhili Wang, Xin Wang, Weiye Xiu, Yongqiang Ma. Characteristics of selenium polysaccharide from sweet corncob and its effects on non-enzymatic glycosylation in vivo. (2022) Appl. Biol. Chem. 65: 10**

Selenium polysaccharide is an organic selenium compound, which has attracted much attention because of its unique biological properties. In the current study, a new selenium polysaccharide (Se-SCP) was synthesised, and its structure and effects on non-enzymatic glycosylation in vivo were studied. The molecular weight of Se-SCP was 9.02 g/mol, and its molecule was mainly composed of Man, Gal, GluA, Ara, Glu, Fuc, Rha, GalA, and Xyl. Selenium was present both as C–O–Se and Se=O. Compared with non-selenised sweet corncob polysaccharide (SCP) treatment, the blood glucose level in mice decreased considerably after Se-SCP treatment. Additionally, it improved oral glucose tolerance (OGTT) and significantly reduced the production of advanced glycation end-products (AGEs) (*P* < 0.05), compared with the model group. In addition, Se-SCP exhibited a positive effect on the morphology of the pancreas and liver. The current study elucidated the physiological and pharmacological effects of selenium polysaccharides and provided a basis for future studies on selenium polysaccharides.

**Hong-Guang Jin, Kwan-Woo Kim, Jing Li, Dae Young Lee, Dahye Yoon, Jin Tae Jeong, Geum-Soog Kim, Hyuncheol Oh, Ren-Bo An, Youn-Chul Kim. Anti-inflammatory components isolated from *Atractylodes macrocephala* in LPS-induced RAW264.7 macrophages and BV2 microglial cells. (2022) Appl. Biol. Chem. 65: 11**

The phytochemical investigation on the methanol extract of the rhizomes of *Atractylodes macrocephala* resulted in the discovery of one new compound 9α-hydroxyatractylenolide (**1**) and 21 known compounds including atractylone (**2**), 3β-acetoxyatractylon (**3**), atractylenolide I (**4**), atractylenolide II (**5**), 8-epiasterolid (6), atractylenolide III (**7**), atractylenolide VII (**8**), 8-epiatractylenolide III (**9**), eudesm-4(15)-ene-7α,11-diol (**10**), linoleic acid (**11**), myristic acid (**12**), 3-*O*-caffeoyl-1-methyquinic acid (**13**), (2*E*,8*E*,10*E*)-tetradecatriene-4,6-diyne-1,14-diol (**14**), 14-aceroxy-12-senecioyloxytetradeca-2*E*,8*Z*,10*E*-trien-4,6-diyn-1-ol (**15**), isoscopoletin (**16**), caffeic acid (**17**), protocatechic acid (**18**), 3-*O*-caffeoylquinic acid (**19**), 4-*O*-caffeoylquinic acid (**20**), 1,5-di-*O*-caffeoylquinic acid (**21**), and nicotinic acid (**22**). Their structures were identified using nuclear magnetic resonance (NMR) and mass spectroscopy, and by comparison with previously published data. Compounds **4**, **5**, **6**, **8**, and **10**–**22** significantly inhibited lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophages, and compounds **4**, **5**, **6**, **16**, and **17** showed those responses in BV2 microglial cells. Especially, compound **6** showed the second-best effect, and inhibited the LPS-induced production of prostaglandin E2 (PGE2), the protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, and the production of cytokines including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in both cells. These inhibitory effects were mediated by the inactivation of nuclear factor kappa B (NF-κB) signaling pathway.

**Jiyoung You, Hyanggi Ji, Kyung-Baeg Roh, Eunae Cho, Hanane Chajra, Mathilde Frechet, Deokhoon Park, Eunsun Jung. Anti-acne effects of *Castanea crenata* bur extract and identification of active compound. (2022) Appl. Biol. Chem. 65: 12**

Acne vulgaris is a common disease of the pilosebaceous unit. Hyperseborrhea, a follicular colonization by *Cutibacterium acnes* and a complex inflammatory state are pathogenic factors of acne vulgaris. In the present study we investigated the anti-acne efficacy of *Castanea crenata* bur extract (CBE) in vitro and searched active compound for mitigating hyperseborrhea. In sebocytes, CBE inhibited the sebum synthesis through downregulation of sterol response element-binding protein-1 and peroxisome proliferator-activated receptor γ expression. CBE also inhibited the 5-alpha reductase activity which is associated with androgen-induced sebum production. Moreover, CBE showed anti-inflammatory effect in *C. acnes* and free fatty acid-induced inflammatory condition through suppressing Toll-like receptor 2 activity. Anti-inflammatory effect was also observed in keratinocytes via inhibition of NF-κB translocation into nuclei. Finally, we identified the ellagic acid as an active compound for inhibiting sebum production in CBE. These findings suggest that CBE have potential to be a multi-target agent for acne vulgaris and a good source of ellagic acid as an anti-sebum compound.

**So Hee Yang, Euyeon Kim, Hyosun Park, Yeonjong Koo. Selection of the high efficient sgRNA for CRISPR-Cas9 to edit herbicide related genes, PDS, ALS, and EPSPS in tomato. (2022) Appl. Biol. Chem. 65: 13**

Herbicide resistance is one of the main crop traits that improve farming methods and crop productivity. CRISPR-Cas9 can be applied to the development of herbicide-resistant crops based on a target site resistance mechanism, by editing genes encoding herbicide binding proteins. The sgRNAs capable of editing the target genes of herbicides, *pds* (phytoene desaturase), *ALS* (acetolactate synthase), and *EPSPS* (5-Enolpyruvylshikimate-3-phosphate synthase), were designed to use with the CRISPR-Cas9 system in tomato (*Solanum lycopersicum* cv. Micro-Tom). The efficiency of the sgRNAs was tested using *Agrobacterium* mediated transient expression in the tomato cotyledons. One sgRNA designed for editing the target site of *PDS* had no significant editing efficiency. However, three different sgRNAs designed for editing the target site of *ALS* had significant efficiency, and one of them, ALS2-P sgRNA, showed over 0.8% average efficiency in the cotyledon genome. The maximum efficiency of ALS2-P sgRNA was around 1.3%. An sgRNA for editing the target site of *EPSPS* had around 0.4% editing efficiency on average. The sgRNA efficiency testing provided confidence that editing of the target sites could be achieved in the transformation process. We confirmed that 19 independent transgenic tomatoes were successfully edited by ALS2\_P or ALS1\_W sgRNAs and two of them had three base deletion mutations, which are expected to have altered herbicide resistance. In this study, we demonstrated the usefulness of performing an sgRNA efficiency test before crop transformation, and confirmed that the CRISPR-Cas9 system is a valuable tool for breeding herbicide-resistant crops.

**Somin Lee, Rakdo Ko, Kwanghun Lee, Jinchan Kim, Seokhyeon Kang, Jiho Lee. Dissipation patterns of acrinathrin and metaflumizone in *Aster scaber*. (2022) Appl. Biol. Chem. 65: 14**

The establishment of preharvest residue limits (PHRLs) is important to minimize damage to producer and consumers caused by agricultural products which pesticide residue exceeds maximum residue limits (MRLs). Dissipation patterns of acrinathrin and metaflumizone in *Aster scaber* in greenhouse were studied during 10 days in order to determine a pre-harvest interval after application. Acrinathrin and metaflumizone were applied in two different greenhouse, located in Taean-gun (field 1) and Gwangyang-si (field 2). Samples were collected at 0, 1, 2, 3, 5, 7, and 10 days after insecticides application. The recoveries of two insecticides analyzed by LC–MS/MS and HPLC–DAD were ranged from 77.1 to 111.3%. The half-lives of acrinathrin and metaflumizone residues respectively were 3.8 and 5.9 days in field 1 and 9.2 and 4.5 days in field 2. The PHRLs 10 days before harvesting *A. scaber* were 0.610 mg/kg (field 1), 0.946 mg/kg (field 2) for acrinathrin, and 5.930 mg/kg (field 1), 5.147 mg/kg (field 2) for metaflumizone. This results can be used as basic data for the establishment of PHRL in *A. scaber*.

**Yaran Wu, Lei Cai, Xingzi Xie, Shuying Yang, Qing Shi, Hongzhe Jia, Xuqiang Gu, Jingmin Deng, Mingzhao Shi, Qiuping Chen, Shaoqian Cao, Shuangfeng Cai. Resistant starch (RS), a novel endogenous inert marker for detecting glucose absorption of small intestine with sweeteners administration in mice. (2022) Appl. Biol. Chem. 65: 15**

Resistant starch could be degraded by the fermentation of colonic microorganisms in the large intestine of mammals, but not in the small intestine. In this study, we established a novel strategy by using resistant starch as an endogenous marker to determine the glucose absorption of the small intestine of laboratory animals. By optimization of the classical enzymatic method of starch measurement, the demand for the sample weight was reduced by 90%. Moreover, the amount of resistant starch in normal feed was detectable without any extra addition. The value of small intestine glucose absorption of mice was similar when using resistant starch and titanium dioxide as inert markers. The fermentation of resistant starch by intestinal microorganisms in the small intestine was demonstrated not disturbing the detection of glucose absorption significantly. Artificial sweeteners exposed ICR mice showed different glucose absorption which indicated, first, resistant starch can be used as a novel endogenous marker in the small intestine of small animals; second, although glucose tolerance did not change in mice after short-term exposure to artificial sweeteners, there were significant changes in glucose absorption associated with it; third, the short-term exposure resulted in no significant change in glucose tolerance.

**Ji-Won Choi, Sang Yoon Choi, Hyun Hee L. Lee, Guijae Yoo, Sang-Hoon Lee, In-Wook Choi, Chi Heung Cho, Jinyoung Hur. *Glycyrrhiza uralensis* attenuates TNF-α-induced muscle atrophy in myoblast cells through the Nrf2 and MAFbx signaling cascades. (2022) Appl. Biol. Chem. 65: 16**

Muscle atrophy is associated with chronic diseases, such as heart failure diabetes, and aging-related diseases. *Glycyrrhiza uralensis* (GU) extract is widely used in traditional medicine. However, no studies have evaluated the effects of GU on muscle atrophy. Thus, in this study, we assessed the effects of GU on prevention of muscle atrophy. GU reduced the levels of the TNF-α-induced muscle atrophy markers, muscle RING-finger protein-1(Murf-1) and muscle atrophy F-box (MAFbx), and upregulated myosin heavy chain expression (MyHC). It also reduced the phosphorylation of nuclear factor kappa B, and downregulated Smad3 proteins, which are involved in protein ubiquitination. When we examined whether GU exhibits antioxidant activities. GU suppressed TNF-α-induced muscle atrophy by increasing the translocation of nuclear factor erythroid 2-related factor 2 (Nrf2), which regulates the expression of antioxidant factors such as heme oxygenase-1 (HO-1) as well as apoptosis-related factors, such as caspase-3/7. These results suggest that GU extract is potentially an important agent in the regulation of TNF-an induced muscle atrophy.

**Seunghyun Ahn, Vi Nguyen-Phuong Truong, Beomsoo Kim, Miri Yoo, Yoongho Lim, Somi Kim Cho, Dongsoo Koh. Design, synthesis, and biological evaluation of chalcones for anticancer properties targeting glycogen synthase kinase 3 beta. (2022) Appl. Biol. Chem. 65: 17**

Chalcones compounds have been investigated to exhibit anticancer activity through various physiological modes of action. In order to develop chalcone compounds with novel anticancer-related modes of action, diverse chalcone compounds were designed and synthesized. Variously substituted poly-methoxy chalcone compounds **1**–**17** were prepared, and their structures were identified using high-resolution mass spectrometry (HR/MS) and nuclear magnetic resonance (NMR) experiments. Long-term survival clonogenic assay was applied to evaluate their anti-cancer abilities and revealed that their GI50 values ranged between 1.33 and 172.20 μM. When MCF-7SC cells were treated with various concentrations of compound **14**, reduced cell viability and induced apoptosis in MCF-7SC cells were observed in a dose-dependent manner. Wound healing assay demonstrated that compound **14** prevented the MCF7-SC migrated cells at non-lethal concentrations after 12 and 24 h of exposure. The efficiency of compound **14** on the levels of Epithelial-mesenchymal transition (EMT) markers was accessed by the western blot analysis. For the concrete understanding of anticancer properties at the molecular level, in vitro kinase assays on 12 cancer related proteins were carried out. Glycogen synthase kinase 3 beta (GSK3β) was most effectively inhibited by compound **14** with 89% inhibitory activity at 10 µM against GSK3β. The binding mode of compound **14** with GSK3β was reinforced through in silico experiments, which demonstrated compound **14** binds with GSK3β at binding affinity ranged between − 7.5 kcal/mol and − 6.8 kcal/mol. SwissADME analysis provided the druggability and leadlikeness of compound **14**, which unveiled drug development possibilities of chalcone compound **14**.

**Ngoc Minh Ha, Son Hung Tran, Yhong-Hee Shim, Kyungsu Kang. *Caenorhabditis elegans* as a powerful tool in natural product bioactivity research. (2022) Appl. Biol. Chem. 65: 18**

In addition to synthetic and semisynthetic compounds, natural products have received much attention as prolific sources of lead compounds with therapeutic effects on various diseases. In the process of screening the bioactivities of potential candidates, an in vivo assay is very important for providing meaningful insight into the efficacy, adverse effects, and modes of action that are relevant to humans. Among the many experimental models, *Caenorhabditis elegans* is particularly efficient due to its advantages in morphology, behavior, and genetic aspects. This review summarizes some basic and useful techniques commonly used in screening the bioactivities of natural products. Recent studies of naturally occurring extracts as well as bioactive compounds in various areas, namely, anti-aging, anti-neurodegeneration, anti-obesity, anti-infection, and gut health, are reviewed as examples of the applicability of the *C. elegans* model. Technological developments that incorporate *C. elegans* in other fields, such as instrumental analysis and emerging methods, are also discussed in this paper.

**Hairul-Islam Mohamed Ibrahim, Emad A. Ahmed, Abdullah M. Alzahrani, Abdullah A. Sayed, Hamza Hanieh. Swertiamarin ameliorates inflammation and oxidative damage in autoimmune encephalomyelitis mouse model. (2022) Appl. Biol. Chem. 65: 19**

Multiple sclerosis (MS) is a degenerative autoimmune disease attacks the myelin sheath of the central nervous system (CNS) neurons causing different disabilities. According to recent evidence, the main bioactive component in *Enicostema axillare*, the Swrtiamarin (SM) has been found to exert anti-inflammatory and antioxidant activities against several diseases. However, SM activities in treatment of autoimmune diseases remain to be explored. Herein, we used a murine model of MS, to show that SM treatment ameliorates the severity of experimental autoimmune encephalomyelitis (EAE). This occurs through reducing the levels of pro-inflammatory cytokines and infiltration of CD4+CD45+ cells into CNS. That was associated with a reduction in the expression of STAT3 and NFkB in CD4+ T cells under Th17 and LPS-stimulated macrophages. Furthermore, in silico studies revealed that SM interacts with NF-E2-related factor 2 (NRF2), and therefore, suppressed oxidative stress by inducing formation NRF2-antioxidant response element (ARE) complex. We found that SM is an agonist of NRF2 complex regulating the total CD4 population and antioxidant markers in EAE mice. Molecular docking analysis showed a stable and higher binding affinity between SM and NRF2. Results revealed that SM treatment increased the complex formation between ARE and NRF2 where immunoprecipitation methods showed a higher binding affinity of ARE to NRF2 in SM treated animals. Complex formation triggered ARE cascade of antioxidant gene clusters and reduces the MS pathological alterations in EAE mice model. Current data proposed SM as an effective biomolecule in treatment of MS and controlling neuronal damage through inhibiting oxidative stress markers and targeting NRF2.

**Zhehao Jin, Dae-Kyun Ro, Soo-Un Kim, Moonhyuk Kwon. Piperonal synthase from black pepper (*Piper nigrum*) synthesizes a phenolic aroma compound, piperonal, as a CoA-independent catalysis. (2022) Appl. Biol. Chem. 65: 20**

Piperonal is a simple aromatic aldehyde compound with a characteristic cherry-like aroma and has been widely used in the flavor and fragrance industries. Despite piperonal being an important aroma in black pepper (*Piper nigrum*), its biosynthesis remains unknown. In this study, the bioinformatic analysis of the *P. nigrum* transcriptome identified a novel hydratase-lyase, displaying 72% amino acid identity with vanillin synthase, a member of the cysteine proteinase family. In in vivo substrate-feeding and in vitro enzyme assays, the hydratase-lyase catalyzed a side-chain cleavage of 3,4-methylenedioxycinnamic acid (3,4-MDCA) to produce 3,4-methylenedioxybenzaldehyde (piperonal) and thus was named piperonal synthase (PnPNS). The optimal pH for PnPNS activity was 7.0, and showed a *K*m of 317.2 μM and a *k*cat of 2.7 s−1. The enzyme was most highly expressed in the leaves, followed by the fruit. This characterization allows for the implementation of PnPNS in various microbial platforms for the biological production of piperonal.

**Sung Jim Kim, Myoung-Sook Shin. IC261 inhibits the epithelial-mesenchymal transition induced by TGF-β in A549 lung cancer cells. (2022) Appl. Biol. Chem. 65: 21**

Despite rapid advances in cancer diagnosis and therapy, lung cancer continues to be the primary cause of cancer-related mortality. Epithelial mesenchymal transition has been implicated in drug resistance and cancer metastasis. IC261 mediates various pathophysiological processes, including inflammation and tumorigenesis. Therefore, we analyzed the involvement of IC261 in epithelial mesenchymal transition. Pretreatment with IC261 significantly inhibited the expression of transforming growth factor (TGF)-β1-induced mesenchymal cell markers, including N-cadherin (N-cad), vimentin (Vim), and β-catenin (β-cat), at the mRNA and protein levels in A549 lung cancer cells, which was confirmed using immunofluorescence staining. A migration assay revealed that IC261 treatment strongly inhibited TGF-β1-induced migration activity at 24 and 48 h. Additionally, IC261 treatment suppressed the activation of the TGF-β1 signaling pathway in A549 cells and phosphorylation of Smad2 and Smad3. Our findings demonstrate that IC261, a selective inhibitor of casein kinase 1, inhibits the TGF-β1-induced migration of A549 cells by inhibiting Smad2/3 phosphorylation and downregulating the expression of N-cad, Vim, and β-cat.

**Dahae Lee, Hak-Dong Lee, Hyukjin Kwon, Hye Lim Lee, Gwi Seo Hwang, Sungyeol Choi, Hyun Young Kim, Sanghyun Lee, Ki Sung Kang. Insulin secretion and α-glucosidase inhibitory effects of dicaffeoylquinic acid derivatives. (2022) Appl. Biol. Chem. 65: 22**

In this study, we investigated the effects of dicaffeoylquinic acid derivatives, including 1,4-di-O-caffeoylquinic acid (1,4-DCQA), 3,4-di-O-caffeoylquinic acid (3,4-DCQA), 3,5-di-O-caffeoylquinic acid (3,5-DCQA), 4,5-di-O-caffeoylquinic acid (4,5-DCQA), and 1,5-di-O-caffeoylquinic acid (1,5-DCQA) on glucose-stimulated insulin secretion (GSIS) activity and α-glucosidase activity were compared in rat INS-1 pancreatic β-cells. The α-glucosidase inhibitory activities of dicaffeoylquinic acid derivatives were as follows: 1,4-DCQA > 1,5-DCQA > 3,4-DCQA > 4,5-DCQA > 3,5-DCQA. In INS-1 cells, dicaffeoylquinic acid derivatives showed no cytotoxic effect at any concentration (2.5–10 μM). In addition, the GSIS activities of dicaffeoylquinic acid derivatives were as follows: 4,5-DCQA > 3,4-DCQA > 1,4-DCQA > 3,5-DCQA > 1,5-DCQA. Treatment of INS-1 cells with 4,5-DCQA resulted in a marked increase in protein expression of extracellular signal-regulated protein kinases (ERK), insulin receptor substrate-2 (P-IRS-2), Akt, phosphoinositide 3-kinase (P-PI3K), and pancreatic and duodenal homeobox-1 (PDX-1), which might be related to its GSIS activity in INS-1 cells. These findings indicate that the location of the dicaffeoyl functional group influences the anti-diabetic activity of quinic acid.

**Ming Tan, Xin Zhang, Maohui Xing. Propofol suppresses non-small cell lung cancer progression by modulating circ\_0001727/miR-516b-5p/LRRC1 axis. (2022) Appl. Biol. Chem. 65: 23**

Background

Propofol plays an anti-cancer role in diverse cancers, including non-small cell lung cancer (NSCLC). We aimed to study the function and underlying mechanism of propofol in NSCLC.

Methods

Cell Counting Kit-8 (CCK-8) and colony formation assays were used to detect cell proliferation. The expression of circ\_0001727, microRNA-516b-5p (miR-516b-5p) and leucine-rich repeat-containing protein 1 (LRRC1) mRNA was tested via quantitative real-time polymerase chain reaction (qRT-PCR). Cell migration and invasion were assessed by transwell assay. Angiogenesis and cell apoptosis were determined by tube formation assay and flow cytometry, respectively. Western blot (WB) assay was performed to measure all protein levels. In vivo experiments were conducted via establishing mice xenograft model. Dual-luciferase reporter and RNA Immunoprecipitation (RIP) assays were carried out to verify the relationship between miR-516b-5p and circ\_0001727 or LRRC1.

Results

Circ\_0001727 was overexpressed in NSCLC, and propofol treatment reduced circ\_0001727 level in NSCLC cells. Propofol could repress proliferation, migration, invasion, and angiogenesis while accelerated apoptosis of NSCLC cells, while these effects were augmented by circ\_0001727 knockdown. Moreover, circ\_0001727 depletion in combined with propofol also inhibited tumorigenesis in vivo. MiR-516b-5p was targeted by circ\_0001727, and miR-516b-5p downregulation counteracted the suppressive influence of circ\_0001727 deficiency on the malignant behaviors of NSCLC cells. LRRC1 was targeted by miR-516b-5p, and miR-516b-5p exerted its anti-tumor function in NSCLC cells by targeting LRRC1. Additionally, circ\_0001727 regulated LRRC1 expression via sponging miR-516b-5p.

Conclusion

Propofol inhibited NSCLC progression by regulation of circ\_0001727/miR-516b-5p/LRRC1 axis, which might offer an effective therapeutic target for NSCLC therapy.

**Gayani Udeshika Chandrasiri, Kariyawasam Ranaweerage Ranjith Mahanama, Kushani Mahatantila, Pavithra Sajeewani Pitumpe Arachchige, Roshan Chathuranga Midigama Liyanage. An assessment on toxic and essential elements in rice consumed in Colombo, Sri Lanka. (2022) Appl. Biol. Chem. 65: 24**

Being the dietary staple in most Asian countries, the concentrations of toxic and essential element content in rice is an important aspect in terms of both toxicological and nutritional standpoints. This study aimed to analyse trace elements (As, Cd, Pb, Hg, Cr, Ni, Zn, Cu, Mn, Fe, and Mo) in improved, traditional, and imported rice varieties consumed in Colombo district**,** Sri Lanka during 2018 and 2019. Further, the potential health risks were assessed in terms of maximum levels and provisional tolerable daily intake. Among the toxic elements analysed, As, Cd, Cr, and Pb were detected in certain rice varieties. Arsenic was detected in all three rice categories and the number of As detected samples were higher compared to other toxic elements in 2018 and 2019. In 2018, 4.2% of traditional rice exceeded As maximum level (0.2 mg/kg) whereas 2.1% of improved and 4.2% of traditional rice exceeded Pb maximum level (0.2 mg/kg). However, none of the toxic elements in rice exceeded the respective maximum levels in 2019. Only mean estimated daily intake of Pb through *Kaluheenati* exceeded the provisional tolerable daily intake value (0.0015 mg/kg bw/d) in 2018. Rice varieties that reported the highest toxic elements were *Basmathi* (imported), *Samba* (improved), and *Kaluheenati, Madathawalu, Pachchaperuman*, and *Suwadel (*traditional*)*. With regard to essential elements, concentrations of Fe, Zn, Cu, Mn, and Mo in rice were positively correlated (*p* < 0.05) to each other, and the highest essential element concentration were found in traditional rice with red pericarp (i.e., *Kaluheenati, Madathawalu,* and *Pachchaperuman*).

**Hyuk-Woo Kwon, Muhammad Irfan, Yuan Yee Lee, Man Hee Rhee, Jung-Hae Shin. Artocarpesin acts on human platelet aggregation through inhibition of cyclic nucleotides and MAPKs. (2022) Appl. Biol. Chem. 65: 25**

The cardiovascular diseases (CVDs) are becoming a critical threat to our lives in these years. It is now widely accepted that platelets play an important role in cardiovascular disease as they have a fundamental role in thrombosis. Therefore, many drugs or natural substances have been developed to treat CVDs. *Cudrania tricuspidata* is a regional plant containing various constituents, such as xanthones, flavonoids, organic acids, and polysaccharides. It has been widely used in East Asia as an important ethnomedicine for the treatment of many diseases such as eczema, mumps, tuberculosis and acute arthritis. Therefore, we evaluated antiplatelet effects using artocarpesin isolated from *C. tricuspidata.* Confirmation of the antiplatelet function of artocarpesin was made according to the following analyzes. Artocarpesin inhibited collagen-induced human platelet aggregation, calcium mobilization, glycoprotein IIb/IIIa activation and thrombin-induced clot retraction through the regulation of associated signaling molecules. Artocarpesin increased the phosphorylation of vasodilator-stimulated phosphoprotein (VASP) and inositol 1, 4, 5-triphosphate receptor I (IP3RI). On the other hand, the phosphorylation of cytosolic phospholipase A2 (cPLA2), mitogen-activated protein kinases p38, JNK and phosphoinositide 3-kinase (PI3K)/Akt decreased. Thus, the study highlights that artocarpesin has an inhibitory effect on platelet activity and thrombus formation, showing its potential value in preventing platelet-induced cardiovascular disease.

**Son Giang Nguyen, Shahbaz Raza, Loan Thi Ta, Lan-Anh Thi Le, Cuong Tu Ho, Tatsuya Unno. Metagenomic investigation of the seasonal distribution of bacterial community and antibiotic-resistant genes in Day River Downstream, Ninh Binh, Vietnam. (2022) Appl. Biol. Chem. 65: 26**

In this study, we use high-throughput sequencing-based metagenomic methods to investigate the differences in seasonal structures of the bacterial community and the abundance and diversity of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in both shrimp ponds and river water samples downstream of the Day River, Ninh Binh, Vietnam. The structure of the central bacterial community, ARGs, and MGEs was found to be regardless of the seasons and locations. The predominant phyla found in all samples was *Proteobacteria*, *Bacteroidetes, and Actinobacteria*. Multi-drug resistance (MDR) genes and transposases are the most dominant ARG types and MGEs, respectively. Our data showed a higher abundance of bacterial communities, ARGs, and MGEs in the river water during the rainy season. There is a significant correlation between the abundance of ARGs, MGEs, and environmental factors. Our results indicate that water environments containing ARGs/MGEs carrying bacteria pose a risk to shrimp and human health, especially during the rainfall-polluted water season.

**Hyun Young Hwang, Sang Min Lee, Cho Rong Lee, Nan Hee An. Addition of earthworm castings reduces gas emissions and improves compost quality in kitchen waste composting. (2022) Appl. Biol. Chem. 65: 27**

In this study, we demonstrate that the addition of earthworm castings (EC) in kitchen waste composting reduces ammonia and greenhouse gas (GHG) emissions and improves compost maturity. Kitchen waste (KW) was mixed with sawdust at a ratio of 7:3 as the compost stock. Four treatments with different proportions of EC added (0%, 2.5%, 5.0%, and 7.5% on the basis of the initial kitchen waste mass) were designed and utilized in a composting process lasting 85 days. The results showed that the GHG and ammonia emissions were considerably reduced in the treatments with EC added. In addition, EC amendment prolonged the thermophilic stage and shortened the composting period. The addition of EC reduced ammonia, methane, and nitrous oxide emissions by 61%, 48%, and 94%, respectively, also indicating that nitrogen in the compost was conserved. Nitrogen and major nutrients were best preserved in the EC 7.5% treatment, which produced a compost product with a better nutrient profile. Furthermore, the total global warming potential of the KW composting process was reduced by 74% by using the mixture with EC. An effective reduction in GHG emissions was observed already with the addition of 2.5% EC, but a significant reduction in ammonia emissions was observed for the EC 7.5% treatment. Therefore, the results of this study suggest that EC is an effective additive in KW composting. More specifically, addition of EC at 7.5% of the initial KW mass was most recommendable for mitigating potential global warming effects and improving compost quality.

**Kihwan Kim, Jooeun Lee, Byeonggyu Kim, Juhyung Shin, Tae-An Kang, Won-Chan Kim. GATA25, a novel regulator, accelerates the flowering time of *Arabidopsis thaliana*. (2022) Appl. Biol. Chem. 65: 28**

Intrinsic and exogenous signals in conjunction precisely regulate the initiation of flowering. Both signals influence flowering time, which is an integral part of plant reproduction. The signals converge through different pathways, and their coordinated action leads to the onset of flowering. Genetic pathways related to the regulation of flowering time are well-known from research into the molecular genetics of *Arabidopsis thaliana*. Specifically, crucial components of the photoperiodic pathway and floral integrators play a critically significant role in flowering. In this study, we found that GATA25 is a novel transcription factor that accelerates flowering time under long days. GATA25 encodes C-X2-C-X20-C-X2-C conserved cysteine residues of the zinc-finger domain and CCT domain which process photoperiodic flowering and regulate circadian rhythms. Flowering was accelerated by overexpression of GATA25 throughout the *Arabidopsis thaliana*. In contrast, GATA25 fused to SRDX (SUPERMAN repressive domain X)-motif plants showed delayed flowering. We also demonstrated that GATA25 induced the expression of floral integrator genes and photoperiodic pathway-related genes. Together, these results suggest that GATA25 might act to accelerate flowering time.

**Yifan Zhang, Jinhuang Shen, Xinhua Ma, Mingshuang Yao, Yonghong Zhang, Dairong Cao. Anti-infammatory and antioxidant activities of acteoside isolated from *Acanthus ilicifolius var. xiamenensis*. (2022) Appl. Biol. Chem. 65: 29**

*Acanthus ilicifolius var. xiamenensis* (Acanthaceae), a mangrove found in southeastern China, is an herb with strong antiinflammatory property. Phytochemical study of the mangrove showed that the plant has a high content of phenylethanol glycoside acteoside (AC). In this research, the anti-inflammatory activity of acteoside on dextran sodium sulphate (DSS)-mediated mouse ulcerative colitis model were studied. For DSS- mediated colitis in mice, AC can reduce weight loss and DAI score in UC mice, suppress colon shortening and alleviate colon pathological injury. Moreover, AC treatment notably up-regulates IL-10, down-regulates the levels of IL-1*β* and TNF-*α*, and inhibits the protein expression of JAK2/STAT3, NF-*κ*B p65, IKK*α/β* and IKB of colon. In addition, after AC treatment, the level of MDA and NO in colonic tissue were remarkably decreased, while the levels of GSH, SOD, and Nrf2 and HO-1 protein expression levels were significantly increased. These results indicate that AC can activate the Nrf2 signaling pathway by inhibiting the JAK/STAT, iNOS/eNOS and NF-*κ*B signaling cascades, enhance the intestinal barrier function, and effectively reduce DSS-induced UC in mice.

**Abdullah Sheikh, Faisal Almathen, Mohammed Alfattah. The impact of dromedary camel milk on mice gut microbiota. (2022) Appl. Biol. Chem. 65: 30**

The gut microbiota plays an important role in the health and disease resistance of the host. Host health depends on the microbiota of the gastrointestinal tract, and imbalance in its composition may leads to certain diseases. This study analyzed the influence that dromedary camel milk has on the gut microbiota of mice. This study characterized the bacterial populations of untreated [Control (C) and camel milk-treated Raw (R), Pasteurized (P), and Fermented (F)] C57BL/6 J mice feces using high-throughput 16S rDNA sequencing on the Illumina MiSeq platform. In total, 286,606 tags were generated, with an average of 71,651 tags being generated per group, and these tags were clustered to the operational taxonomic units (OTUs) at 97% sequence similarity, resulting 1090 OTUs. Significant weight gain was observed among all of the groups, and the total cholesterol level declined in F group followed by in group P compared to group C. The F and P groups demonstrated a correlation between the beneficial microbiota structures that corresponded with lower cholesterol levels than those observed in the other groups. The major dominant bacteria correspond to the phyla Bacteroidetes and Firmicutes. The dromedary camel milk propagated the beneficial bacteria (Allobaculum and Akkermansia) and reduced harmful bacteria such as *Proteobacteria, Erysipelotrichaceae*, and *Desulfovibrionaceae*. This study provides a comparative analysis of the gut microbiota of mice based on camel milk, which may be helpful in understanding host health and diverse gut microbial conditions.

**Gholamali Sharifiarab, Abbas Mehraie, Majid Arabameri, Nabi Shariatifar, Amir M. Mortazavian, Mojtaba Moazzen, Sara Sohrabvandi. Evaluation of polycyclic aromatic hydrocarbons (PAHs) in bottled water samples (non-carbonated, mineral, carbonated and carbonated flavored water) in Tehran with MSPE-GC/MS method: a health risk assessment. (2022) Appl. Biol. Chem. 65: 32**

Polycyclic aromatic hydrocarbons (PAHs) are dangerous environmental compounds that are sometimes found in food. The objective of present study was to measure the level of 16 PAHs in bottled water samples (non-carbonated or drinking, mineral, carbonated and carbonated flavored water) in Tehran by using magnetic solid-phase extraction and gas chromatography–mass spectrometry (MSPE/GC–MS) method. The limit of detections (LOD), limit of quantifications (LOQ) and recovery of PAH compounds were 0.010–0.210, 0.03–0.700 μg/L and 92.5–103.4%, respectively. The results showed that the mean of total PAHs in samples was 2.98 ± 1.63 µg/L and the mean of Benzo[a]pyrene (BaP) was 0.08 ± 0.03 µg/L, which were lower than standard level of the US-EPA (0.2 µg/L, BaP in drinking water). Also, our results showed that carbonated flavored water had maximum mean of total PAHs (4.95 ± 0.8 µg/L) and mineral water had minimum mean of total PAHs (1.24 ± 0.8 µg/L). The Monte Carlo method was applied to calculate the Estimated Daily Intake (EDI) and Incremental Life Cancer Risk (ILCR) indexes. In all samples, the rank order of the estimated CDI values based on the 95 percent percentile was F > B(a)A > Ace > Fl > Na > Ph > B(b)F > B(k)F > B(a)P > P > Ac > A. The cancer risk and uncertainty analysis of 95th Percentile for bottled waters studied gave values lower permissible limit of 10−6, indicating not pose a serious concern to humans.

**Chang Geun Choi, Deok Jae Lee, Namhyun Chung, Yong Hoon Joo. Anti-obesity effects of isoacteoside on 3T3-L1 adipocytes. (2022) Appl. Biol. Chem. 65: 33**

Isoacteoside is a caffeoyl phenylethanoide glycoside found in various plant parts, such as the flower of *Magnolia denudata*. In particular, magnolia has been studied for its anti-obesity, anticancer, and anti-inflammatory effects. However, isoacteoside has not been extensively studied, except for its anti-inflammatory effects. In this study, the anti-obesity effects of isoacteoside were investigated in 3T3-L1 mouse pre-adipocytes. Isoacteoside treatment did not induce cytotoxicity in 3T3-L1 cells up to a concentration of 1000 μM. The anti-obesity effects on 3T3-L1 cells were confirmed using oil red O staining. In addition, the expression of obesity-related proteins and genes, such as peroxisome proliferator-activated acceptor gamma (*PPARγ*), CCAAT/enhancer-binding protein alpha (*C/EBPα*), and perilipin (*PLIN1*), was determined by western blotting and qRT-PCR assays to confirm the anti-obesity effects of isoacteoside. The results of this study suggest that isoacteoside, a natural substance isolated from plant extracts, is not highly toxic to normal cells, such as pre-adipocytes, and displays anti-obesity effects in vitro.

**Ji Soo Kim, Do Manh Cuong, Yu Bin Bae, Somi Kim Cho. Antioxidant and antiproliferative activities of solvent fractions of broccoli (*Brassica oleracea* L.) sprout. (2022) Appl. Biol. Chem. 65: 34**

Crude methanol extract (ME) of broccoli (*Brassica oleracea L.*) sprout was fractioned by hexane, chloroform, ethyl acetate, butanol, and water. The contents of total polyphenols (19.89 mg GAE/g) and flavonoids (10.06 mg RE/g) were significantly higher in the butanol fraction (BF) than in the other fractions. The BF showed the highest DPPH (EC50 = 0.524 mg/mL) and ABTS (EC50 = 0.180 mg/mL) radical scavenging activities. High-performance liquid chromatography (HPLC) of crude ME showed that the most abundant phenolic compounds were rutin, quercetin, chlorogenic acid, catechin, and *p*-coumaric acid. The contents of quercetin, chlorogenic acid and *p*-coumaric acid were higher in the ethyl acetate fraction (EF) and BF than in the other fractions. Antioxidant activity and phenolic compound contents were correlated, suggesting that phenolics were responsible for the antioxidant activity. The hexane fraction (HF) and chloroform fraction (CF) decreased the viability of breast cancer stem cells (BCSCs), and the CF had the highest antiproliferative activity (IC50 = 69.47 mg/mL). The CF also suppressed the stemness characteristics of BCSCs and induced apoptotic cell death. The most abundant characteristic peak in CF was identified as oleic acid (area = 35.05%) by gas chromatography-mass spectrometry (GC–MS). Therefore, the broccoli sprout BF contained high levels of phenolic compounds that contributed to its antioxidant activity, and CF had a marked anti-proliferative effect on BCSCs.

**Aisha M. H. Al-Rajhi, Rana Yahya, Marwah M. Bakri, Reham Yahya, T. M. Abdelghany. In situ green synthesis of Cu-doped ZnO based polymers nanocomposite with studying antimicrobial, antioxidant and anti-inflammatory activities. (2022) Appl. Biol. Chem. 65: 35**

The use of eco-friendly methods for the synthesis of nanoparticles and its nano-composite has become a public demand nowadays to reduce the risks of chemical methods. In the current study, green synthesis of Cu-doped ZnO based polymers nan-ocomposite was performed. Various instrumental analysis including UV–vis, ATR-FTIR spectroscopy, XRD, SEM coupled with energy dispersive X-ray analysis, TEM and Thermal gravimetric were used to characterize nano-composite. Highly antibacterial activity of the synthesized nano-composite was recorded against tested microorganisms with promising efficacy against bacteria namely; *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus vulgaris, Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium* and yeast (*Candida albicans*) but unfortunately not against black fungus (*Mucor circinelloides*) and filamentous fungi *Aspergillus flavus* and *A. niger.* Anti-inflammatory of nano-composite represented by hemolysis inhibition was observed at using low concentration (100 µg/mL) with enhancing 23.85% compared with free nano-composite while at high concentrations 500 and1000 µg/mL the anti-inflammatory activity was approximately similar with enhancing 3.91% and 1.99%, respectively. Antioxidant of the nano-composite was better than the antioxidant of free nano-composite at all tested concentrations, moreover the IC50 of the nano-composite (91.16 µg/mL) was less than the IC50, (203.65 µg/mL) of the free nano-composite.

**Yang Fu, Yi-Fei Dong. 5-Methoxytryptophan pretreatment alleviates lipopolysaccharide-induced cardiac injury and dysfunction. (2022) Appl. Biol. Chem. 65: 36**

Reducing inflammation is a promising therapeutic approach for sepsis-induced cardiomyopathy (SIC). The 5-Methoxytryptophan (5-MTP) is a tryptophan metabolite that demonstrates anti-inflammatory, anti-fibrosis, anti-tumorigenesis, and anti-senescence features. Current investigations aimed to assess the 5-MTP pretreatment impacts on lipopolysaccharide (LPS)-induced cardiac injury and dysfunction. For in vivo studies, the mice were categorized randomly into four groups: control, LPS, LPS+5-MTP (25 mg/kg) and LPS+5-MTP (50 mg/kg). The mice in the LPS+5-MTP groups were given 5-MTP intraperitoneally once a day for 7 days. LPS (10 mg/kg) was then administered intraperitoneally for 24 h. Echocardiography, cardiac injury biomarkers, and H & E staining evaluated heart anatomy and function. The findings indicate that 5-MTP pretreatment significantly reduced LPS-induced heart dysfunction and morphological alterations. Western blot assay was used for investigating molecular mechanisms. After LPS stimulation, the pro-inflammatory cytokines (IL-1β, IL-6, TNF-α and NLRP3) protein levels increased while anti-inflammatory cytokine (IL-10) decreased; however, 5-MTP pretreatment mitigated this response by suppressing the stimulation of the NF-κB signaling pathway. Furthermore, 5-MTP administration reduced LPS-induced cardiac apoptosis, as demonstrated by increased protein levels of cleaved-Casepase-1, cleaved-Casepase-3 and Bax, and decreased protein level of Bcl-2 after LPS stimulation, whereas LPS-induced cardiac apoptosis was reversed by 5-MTP pretreatment. In vitro, 5-MTP pretreatment had a similar cardioprotective effect on cultured cardiac fibroblasts challenged with LPS. In conclusion, 5-MTP pretreatment can reduce LPS-induced cardiac inflammation and apoptosis, implying that 5-MTP is a possible therapeutic option for SIC.

**Ying-Hao Han, Ying-Ying Mao, Yao-Yuan Feng, Hong-Yi Xiang, Hu-Nan Sun, Mei-Hua Jin, Taeho Kwon. Identification of peroxiredoxin II and its related molecules as potential biomarkers of dermal mesenchymal stem cell homing using network analysis. (2022) Appl. Biol. Chem. 65: 37**

In this study, we performed RNA sequencing of Prx II+/+ and Prx II−/− dermal mesenchymal stem cells (DMSCs) to identify differentially expressed genes (DEGs). To explore the role of Prx II in DMSCs, we performed Gene Ontology analysis of the DEGs. The results showed that the DEGs were mainly involved in the biological processes of cell migration, intercellular adhesion, and coordination of the regulation of stem cell homing. Through the construction of protein–protein interaction network, four hub genes *Cd274*, *Ccl5*, *Il1b*, and *Stat1* involved in cell adhesion and cell homing were screened. Quantitative reverse transcription PCR analysis showed that *Cd274*, *Ccl5*, *Il1b*, and *Stat1* were down regulated in Prx II−/− DMSCs. miRwalk and Starbase databases were further used to screen the upstream molecules miRNA and lncRNA regulating hub gene. Prx II was found to be involved in the regulation of stem cell homing via the Tctn2/miR-351/Stat1/Il1b axis. Thus, we demonstrated that Prx II is a key molecule in the regulation of the homing ability of DMSCs. Our results provide a theoretical foundation for improving the homing ability of DMSCs by targeting Prx II.

**Ye-eun Kim, Hanseob Shin, Youri Yang, Hor-Gil Hur. Geographical distribution and genetic diversity of *Bradyrhizobium* spp. isolated from Korean soybean root nodules. (2022) Appl. Biol. Chem. 65: 39**

This study investigated the distribution and genetic diversity of the indigenous *Bradyrhizobium* spp. in the Korean agricultural field. A total of 254 *Bradyrhizobium* strains were isolated from 97 soybean samples (9 cultivars) collected in 14 regions. *B*. *elkanii* dominated in the southern regions, while *B*. *diazoefficiens* dominated in most central and northern regions. Through non-parametric multidimensional scaling (NMDS) analysis, we confirmed the possibility that environmental factors such as annual average temperature and soybean cultivars might affect the distribution of *Bradyrhizobium* spp. in some regions. The DNA fingerprint using repetitive DNA sequences showed the genetic diversity among the *Bradyrhizobium* strains isolated from the different regions. Clustering the strains based on the genetic diversity indicated that *Bradyrhizobium* spp. grouped into different clusters depending on geographic location. This study suggests that the Korean indigenous *Bradyrhizobium* spp. distribute differently according to the geographical feature, and the high genetic diversity of each strain attribute to the geographic location.

**Xin Wang, Yu Luo, Rui Ma, Zhili Wang, Shiyou Yu, Chenchen Li, Chunran Han. Effect of *Lonicera edulis* polysaccharide on reducing oral dyeing of *lonicera edulis* juice. (2022) Appl. Biol. Chem. 65: 40**

Fluorescence spectroscopy, particle size determination, and potential analysis were exploited to elucidate the effect of *Lonicera edulis* polysaccharide on polyphenol protein. The results revealed that *Lonicera edulis* polysaccharides mediated the binding of polyphenols and proteins through competition and formation of ternary complexes and were also able to enhance the stability of the polyphenol-protein complex solution system. A certain electrostatic effect was also present in the process simultaneously. As confirmed by the dyeing test, to improve oral dyeing, the optimum conditions of adding polysaccharide, pectin, and casein were as follows: the dosage of the polysaccharide group was 1.2 mg/mL, coloring time was 100 min, pH value was 4.0. Pectin group added 0.8 mg/mL with coloring time 80 min, pH 5.0. The addition of casein was 1.2 mg/mL; the coloring time was 100 min with pH 5.0. The sample juice substantiated a significant improvement in the dyeing of porcine tongue mucosa. Under the optimal conditions, microscopic observation validates that the mucosal color of the porcine tongue epidermis was closer to that of unstained porcine tongue epidermis, which significantly improved astringency and oral staining.

**Mohamed E. El-Beeh, Ashraf A. El-Badawi, Sameer H. Qari, Mohamed Fawzy Ramadan, Wessam M. Filfilan. Protective and health-promoting impact of *Washingtonia filifera* oil on the kidney of STZ-induced diabetic mice. (2022) Appl. Biol. Chem. 65: 41**

Diabetes kidney damage (DKD) is a chronic inflammatory disease of the kidney induced with continuous hyperglycemia as the most prevalent consequence of diabetes. *Washingtonia filifera* seed oil (WFO) was used as a traditional medicine to cure various diseases in ancient Saudi. This work was carried out to investigate the potential protective impact of WFO against DKD on streptozotocin (STZ)-induced type 2 diabetic mice (C57BL/6 mice). The mice were randomly split into groups: C, C + WFO (200 mg/Kg B.W.), T2D, and T2D + WFO (200 mg/Kg B.W.). Diabetes was created in mice groups except for the control group after 6 weeks of high-fat diet (HFD) feeding. Treatments with STZ (60 mg/kg body weight) were administered three times for 6 weeks, and after that, mice were sacrificed. Kidney tissues and serum were obtained to analyze levels of insulin, metabolism of lipids [triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and free fatty acids (FFA)], antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)], creatine, and blood urea nitrogen (BUN). In addition, H&E staining had been used to investigate the histological changes of the kidneys. In T2D mice, WFO corrected aberrant serum lipids (TG, TC, HDL, LDL, and FFA), elevated antioxidative enzyme levels (CAT, SOD, and GPx), and inhibited GST to various degrees. In addition, WFO improves kidney pathological traits such as fibrosis of the kidney, hypertrophy of glomeruli, and basement membrane thickness of glomeruli. Through hypoglycemic, hypolipidemic, antioxidative, and anti-inflammatory actions, WFO might ameliorate diabetic alterations in T2D mice. WFO could significantly reduce AGE buildup in the T2D mice kidneys, therefore alleviating kidney oxidative stress and inflammatory kidney damage.

**Liping Huang, Xiaoqin Zhong, Zhongliu Zhou, Yuanliang Cai, Minzhen Deng. Piperine increases striatal levels of DA and TH and decreases α-syn and Aβ42 deposition in PDD mice by regulting autophagy: downexpression Beclin-1 and LC3B and upexpression p62. (2022) Appl. Biol. Chem. 65: 42**

Piperine, the major pharmacological ingredient of pepper, can delay the procession of neuropharmacological effects, but its effects and mechanisms on Parkinson's disease dementia (PDD) mice is still unclear, we investigated whether piperine could help treating PDD mice. Here, PDD mice were randomly divided into eight groups (n = 12/group): a normal control group, a PDD model group, a madopar group, an autophagy inhibitor group, an autophagy activator group, and groups receiving low, medium or high doses of piperine respectively. The normal control and PDD model mice were injected with saline. Treatments were administered to the mice once per day continuously for 30 days. The behavioral tests were assessed. Dopamine (DA), Monoamine Oxidase-B (MAO-B), DOPA decarboxylase (DDC), β-secretase, acetylcholinesterase (AChE), amyloid β42 (Aβ42), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels were detected. α-synuclein (α-syn), tyrosine hydroxylase (TH), HSP90, Beclin-1, LC3B, p62 mRNA levels and miRNA-99a-5p expression were determined. Neuronal histology was observed. The behavior of PDD mice improved significantly after peperine treatment compared with the PDD model mice. In addition, our results also showed that peperine treatment increased DA, TH, DDC and p62 levels, decreased MAOB, β-secretase, AChE, Aβ42, TNF-α, IL-6, Beclin-1 and LC3B levels, and down-regulated α-syn, HSP90, Beclin-1, LC3B mRNA levels and miR-99a-5p expression. These findings suggest that piperine may reduce the expression of mmu-miR-99a-5p and autophagy-related factors (HSP90, Beclin-1, LC3B and p62) to alleviate the neurological impairment of PDD mice, which is shown to slow down the process of DA metabolism and Aβ production and resist neuroinflammation.

**Sullim Lee, Quynh Nhu Nguyen, Sung Jin Kim, Joohwan Lee, Myoung-Sook Shin. Estrogenic activity of freeze-dried silkworm extracts through the activation of estrogen receptors in MCF-7 cells. (2022) Appl. Biol. Chem. 65: 43**

Silkworm is an insect that feeds on mulberry leaves only, and silkworm powder has been reported to have antioxidant, hypoglycemic, and anticholesterol activities. In this study, we measured the content of 1-deoxynojirimycin (1-DNJ) in 24 different extracts from silkworm cocoons and also investigated the estrogenic activities of these extracts and their effects on the activation of intracellular signaling pathways in MCF-7 breast cancer cells. Among the 24 silkworm extracts, relatively high 1-DNJ content and estrogenic activity were shown by Extract 11 (30E3H20-1), which was prepared by a single extraction of silkworm powder with 20 times the volume of 30% ethanol against each powder weight for 3 h. The estrogen receptor activity of this extract was confirmed based on its promoting effects on the phosphorylation of the estrogen receptor α (ERα) and mRNA expression of the *ESR1* and *ESR2* genes. In addition, treatment with Extract 11 (30E3H20-1) increased the phosphorylation of AKT, p38, and JNK, which are downstream proteins of ERα. Based on our findings, a silkworm extract could be developed as a natural estrogen supplement in the future.

**Fei Gao, Jianjun Han, Li Jia, Jun He, Yun Wang, Mi Chen. CircCEP85 upregulates IGF1 expression to promote breast cancer progression via sponging miR-1193. (2022) Appl. Biol. Chem. 65: 44**

Background

Increasing evidence has suggested that circular RNAs (circRNAs) play critical roles in breast cancer (BC) progression. However, the expression level and potential functional role of circRNA centrosomal protein 85 (circCEP85) in BC remains largely unknown. Here, we aimed to explore the role of circCEP85 in BC.

Methods

The levels of circCEP85, insuline-like growth factor I (IGF1) mRNA and microRNA-1193 (miR-1193) were examined by quantitative real-time polymerase chain reaction. The protein level was measured by Western blot. Cell proliferation, migration, apoptosis, angiogenesis and stemness were assessed by cell counting kit-8, 5-ethynyl-2’-deoxyuridine assay, transwell assay, flow cytometry, tube formation and sphere formation assays. Xenograft mouse models were conducted to evaluate the effect of circCEP85 in BC in vivo. Moreover, dual-luciferase reporter, RNA pull-down, and RNA immunoprecipitation (RIP) assays were preformed to confirm the interaction between miR-1193 and circCEP85 or IGF1.

Results

CircCEP85 was upregulated in BC tissues and cells. Silencing of circCEP85 inhibited proliferation, invasion, angiogenesis and stemness, but promoted apoptosis in BC cells in vitro. In addition, circCEP85 silencing inhibited tumor growth in vivo. Mechanistically, circCEP85 elevated IGF1 expression via sponging miR-1193 to promote breast cancer progression.

Conclusion

The circCEP85-miR-1193-IGF1 axis regulated BC progression via the competitive endogenous RNA (ceRNA) mechanism. CircCEP85 might be a prognostic biomarker and therapeutic target for BC.

**Ae Sin Lee, Jinyoung Hur, Sang Yoon Choi. Isobavachalcone attenuates TNF-α-induced ICAM-1 and VCAM-1 expression in human umbilical vein endothelial cells by regulating the NF-κB signaling pathway. (2022) Appl. Biol. Chem. 65: 45**

Vascular inflammation activated by pro-inflammatory cytokines is an inflammatory response that occurs in the early stages of atherosclerosis. Endothelial dysfunction in vascular inflammation begins with the expression of cell surface adhesion molecules by pro-inflammatory cytokines. The purpose of this study was to evaluate and verify the vascular inflammatory effects of isobavachalcone. In this study, we investigated the effects of isobavachalcone on inflammatory responses in vascular inflammation induced by the tumor necrosis factor-α (TNF-α) in human umbilical vein endothelial cells (HUVECs). TNF-α stimulation significantly increased the expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) proteins, and concentration-dependently decreased by isobavachalcone in HUVECs. Isobavachalcone suppressed TNF-α-induced ICAM-1 and VCAM-1 expression in HUVECs, thereby inhibiting TNF-α-induced increase in monocyte adhesion. In addition, isobavachalcone decreased the phosphorylation of the NF-κB (necrosis factor κB) p65 subunit. The findings of this study demonstrate that isobavachalcone prevents TNF-α-induced vascular inflammation and has the potential to protect against the early progression of atherosclerosis.

**Jianming Fang, Jianxin Huang, Xiaodong Zhang. Depressing hsa\_circ\_0058092 functions an integrated anti-proliferation and anti-motility role in gastric cancer partially through targeting miR-1294/SIX1 axis. (2022) Appl. Biol. Chem. 65: 46**

Fibronectin 1-derived circular RNA hsa\_circ\_0058092 is a novel potential oncogene in gastric cancer (GC). Yet, previous studies have not determined the role of hsa\_circ\_0058092 GC progression and the underlying mechanism. Herein, we investigated its role and competing endogenous RNA (ceRNA) pathway in the development of GC. The results showed that hsa\_circ\_0058092 was substantially upregulated in GC patients’ tissues and cells, allied with upregulated SIX1 and downregulated miR-1294 compared with normal gastric tissues and cells. There were linear correlations among hsa\_circ\_0058092, miR-1294 and SIX1 levels in GC tumors. Moreover, hsa\_circ\_0058092 acted as a miR-1294 sponge, and miR-1294 targeted SIX1. Functionally, colony formation, EdU positive rate, tumor growth of GC cells, as well as ki-67 expression in xenograft tumors was greatly suppressed by depressing hsa\_circ\_0058092. Besides, hsa\_circ\_0058092 knockdown repressed GC cell migration and invasion, accompanied with increased E-cadherin expression and descended N-cadherin expression. Moreover, inhibiting miR-1294 expression could counteract hsa\_circ\_0058092 knockdown-mediated effects in GC cells. The inhibitory effects of miR-1294 mimics on GC cell malignancy were relieved by increasing SIX1 expression. Further, hsa\_circ\_0058092 depletion repressed SIX1 protein expression by interacting with miR-1294. Hsa\_circ\_0058092 was oncogenic in GC cell proliferation and motility via ceRNA pathway of hsa\_circ\_0058092/miR-1294/SIX1.

**Dorsilla Anono Katimbwa, Jisun Oh, Chan Ho Jang, Jinkyu Lim. Orlistat, a competitive lipase inhibitor used as an antiobesity remedy, enhances inflammatory reactions in the intestine. (2022) Appl. Biol. Chem. 65: 47**

Alterations in secondary gut metabolites derived from the microbial fermentation of food in the gut have significant effects on various aspects of host physiology. Our recent studies on obese mice treated with Orlistat, an antiobesity treatment, revealed a significantly altered gut microbial profile marked by an over-abundance of Proteobacteria and alterations in secondary gut metabolites. In this study, we determined effect of fecal metabolites from high-fat diet fed mice treated with Orlistat (HFDOrl) on colonic epithelial cells in relation to inflammation, barrier function, mitochondrial activity, reactive oxygen species (ROS) levels, and oxidative stress. Quantitative PCR was used to measure intestinal mRNA expression of oxidative stress, inflammation, apoptosis, and gut barrier function genes in mice on a high fat diet with and without Orlistat treatment versus those fed a low-fat diet (HFDOrl, HFD, Normal diet-fed [ND] respectively). Alterations to antioxidant function in HCT-116-ARE-luciferase stable cell line and mitochondrial function in Caco-2 cells was analyzed under oxidative stress with exposure to aqueous fecal extracts from HFDOrl, HFD, and ND groups. The results of this study indicate that a significant increase in anti-oxidative response was observed based on the luciferase activity of HCT-116-ARE-luciferase stable cells. Increased maximal respiration and mitochondrial ROS under oxidative stress was also detected in confluent Caco-2 cells resulting from exposure to fecal extracts from the HFDOrl group compared with the HFD group and pure Orlistat. Furthermore, mice from the HFDOrl group exhibited a significant increase in colonic epithelial expression of oxidative markers (Nrf-2 and SOD-2), inflammation-related markers (IL-6 and TNF-α), and gut barrier function markers (Muc-2 and Occludin). Taken together, the results suggest that Orlistat treatment in the HFD group causes changes in secondary gut metabolites which affect the colonic redox state and may eventually lead to the development of inflammatory, oxidative, and mitochondrial dysfunction at the cellular level.

**Hongchun Zhu, Shihong Lv, Baijing Yang, Zhuoxi Liu, Dan Zhang. CircGAK inhibits cell growth, migration, invasion, and angiogenesis of hepatocellular carcinoma via miR-1323/HHIP axis. (2022) Appl. Biol. Chem. 65: 48**

Increasing evidence demonstrates that circular RNA (circRNA) plays a pivotal role in the development of disease, especially in Cancer. A previous circRNA microarray study showed that circGAK (hsa\_circ\_0005830) was remarkably down-regulated in hepatocellular carcinoma (HCC) tissues. However, the role of circGAK in HCC remains largely unclear. The candidate circRNAs were screened via integrating the Gene Expression Omnibus (GEO) database (GSE164803) analysis with the online program GEO2R. Quantitative real-time PCR (qRT-PCR) was employed to measure the expression of circGAK miR-1323, and hedgehog-interacting protein (HHIP) in HCC tissues and cells. The biological function of circGAK in HCC was examined using colony formation assay, 5-ethynyl-2'-deoxyuridine (EdU) assay, wound healing assay, transwell cell invasion assay, endothelial tubular formation assay, western blot assay, and xenograft mouse model. Bioinformatics analysis, RNA immunoprecipitation (RIP) assay, and dual-luciferase reporter assay were utilized to test the interaction between miR-1323, and circGAK or HHIP. The expression of circGAK was abnormally down-regulated in HCC tissues and was associated with the tumor-node-metastasis (TNM) stage. Overexpression of circGAK remarkably impeded HCC cell proliferation, migration, invasion, and endothelial tube formation in vitro, and tumor growth in vivo. Bioinformatics predicted that circGAK interacted with miR-1323, which targeted the HHIP mRNA 3'untranslated regions (UTR). Furthermore, upregulation of miR-1323 or shRNA-mediated HHIP suppression could recover circGAK-mediated malignant behaviors of HCC cells and tube formation of endothelial cells. Taken together, the circGAK/miR-1323/HHIP axis could suppress the progression of HCC and may provide potential new targets for the diagnosis and therapy of HCC.

**Chul-Min Park, Bong-Hwa Jeong, Raj Bhesh Sharma, Hyun-Jung Kim, Dong-Wook Kim, Dong-Young Rhyu. *Crateva unilocularis* Buch-Ham leaf extract improves glucose metabolism via regulation of insulin secretion and sensitivity in vitro and in vivo. (2022) Appl. Biol. Chem. 65: 49**

*Crateva unilocularis* Buch-Ham has traditionally been used in Nepal for the treatment and prevention of diabetes. However, scientific verification through studies on the preventive effect of *C. unilocularis* on diabetes has not been done properly. In this study we investigated the effect of *C. unilocularis* leaf extract (CULE) on glucose uptake and insulin resistance. The effect of CULE on glucose uptake in an in vivo system was measured using zebrafish. In the cell-free system, enzymes activities related to diabetes were measured. Moreover, in the cell-cultured system, RIN-m5F pancreatic beta cells, 3T3-L1 adipocytes, and L6 myotubes were used to measure the effect of CULE on insulin secretion and glucose metabolism. CULE effectively enhanced glucose uptake in zebrafish larvae, and inhibited the activity of dipeptidyl peptidase-IV (DPP-IV) and protein tyrosine phosphatase-1B (PTP-1B) enzymes related to insulin secretion and insulin signaling activation. Also, CULE significantly increased insulin secretion with suppression of NO production in RIN-m5F pancreatic beta cells. In L6 myotubes and TNF-α-induced insulin resistance model of 3T3-L1 adipocytes, CULE significantly increased glucose uptake and immunofluorescence staining of glucose transporter (GLUT)4 protein. Furthermore, the regulatory response of glucose metabolism by CULE was a close correlation with the activation of insulin signaling (IRβ, PI3K, AKT) and 5′-AMP-activated protein kinase (AMPK)α and the reduction of p38 mitogen-activated protein kinase (MAPK) in TNF-α-induced insulin resistance model of 3T3-L1 adipocytes. Thus, our results suggest that CULE may act as a potential agent for the prevention and treatment of metabolic syndrome associated with type 2 diabetes.

**Min-Kyoung Kang, Ji-Young Kim, Young-Im Choi, Lujie Hu, Chaodong Yang, Zhehao Jin, Yun Ji Park, Soo-Un Kim, Sang-Min Kim. Enhanced metabolic flux of methylerythritol phosphate (MEP) pathway by overexpression of *Ginkgo biloba* 1-Hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate Reductase 1 (GbHDR1) gene in poplar. (2022) Appl. Biol. Chem. 65: 50**

Terpenoids are of great interests in a broad range of health-beneficial biological activities and various industrial applications. In plants, terpenoids are synthesized by two distinct pathways, methylerythritol phosphate (MEP) and mevalonate pathways in a separate location. MEP pathway supplies isoprene precursors isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) of terpenoid biosynthesis in plant plastids. The MEP pathway has been an engineering target to increase the metabolic flux towards higher terpenoid production in plants. 1-Hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate reductase (HDR) is the terminal step of the MEP pathway to regulate the terpenoid biosynthesis and is encoded by three paralogous genes in *Ginkgo biloba.* In this study, we assessed the effect of overexpression of *GbHDR1* on terpenoid metabolism in poplar plants. Overexpression of *GbHDR1* in poplar plants accelerated growth and delayed winter-bud formation. Transcript levels of gibberellin, chlorophylls, and carotenoid biosynthetic genes in *GbHDR1-*overexpressing (*GbHDR1*ox) poplars were up-regulated, suggesting metabolic flux enhancement. Moreover, enhanced contents of chlorophylls and carotenoids in the leaves of the *GbHDR1*ox plants resulted in a higher photosynthetic rate as a consequence. Therefore, we expect the *GbHDR1* overexpression will be a desirable engineering point of the MEP pathway for enhancing terpenoid metabolic flux and production in plants.

**Min-Kyoung Kang, Ji-Young Kim, Young-Im Choi, Lujie Hu, Chaodong Yang, Zhehao Jin, Yun Ji Park, Soo-Un Kim, Sang-Min Kim. Enhanced metabolic flux of methylerythritol phosphate (MEP) pathway by overexpression of *Ginkgo biloba* 1-Hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate Reductase 1 (GbHDR1) gene in poplar. (2022) Appl. Biol. Chem. 65: 50**

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**Ying Yu, Siqi Huan, Xiaodan Wang, Cong Yang, Dengyong Liu. An electrochemical sensor based on [Ru(bpy)2dpp]2+/SMWCNTs/Au modified glassy carbon electrode for the detection of 5ʹ-GMP. (2022) Appl. Biol. Chem. 65: 51**

A sensitive electrochemical sensor for the selective detection of 5ʹ-guanylic acid (5ʹ-GMP) was prepared by combining sulfonated-multiwalled carbon nanotubes (SMWCNTs) and [Ru(bpy)2dpp]Cl2, which were dripped on the surface of a glass carbon electrode (GCE) immobilized with gold nanoparticles. The 5ʹ-GMP electrochemical biosensor was fabricated using [Ru(bpy)2dpp]2+/SMWCNTs/Au/GCE as working, Ag/AgCl as reference and Pt as auxiliary electrode connected by an electrochemical workstation. The modified electrode was characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The results showed the sensor’s response current had the best peak shape and maximum peak when the pH of electrolyte was 3, scan speed of CV was in the range of 100 to 180 mV/s, and the enrichment time was in the range of 200 to 300 s. Under the optimum conditions, a linear analytical curve was obtained for 5ʹ-GMP concentrations in the range of 0.01 to 0.5 mmol L−1, with a detection limit of 0.0014 mmol L−1. The analytical results of the 5ʹ-GMP sensor were exhibited good consistent with the data from liquid chromatography. The sensor has good reproducibility, long-term stability and strong immunity to interference, and may be a powerful device for 5ʹ-GMP detection, with great advantages such as simple preparation and operation, low equipment cost.

**Jun Young Ha, Gibum Yi, Hwan Hee Bae, Young Sam Go, Yu Jin Kim, Kwang Min Lee, Chang Oh Hong, Keun Ki Kim. Isolation, identification, and apoptosis activity of the photosensitizer methyl pheophorbide A from *Perilla frutescens* leaves. (2022) Appl. Biol. Chem. 65: 52**

Photodynamic therapy (PDT) is a promising cancer treatment with fewer side effects, and it eliminates tumors in target tissues with reactive oxygen species produced by photosensitizers (PS) and light. In this study, we isolated methyl pheophorbide A, which induces photodynamic cell death in the U937 and SK-HEP-1 cells, from perilla leaves. Its potential as a material for the development of a new PS was also evaluated. The methyl pheophorbide A is a dark green porphyrin compound isolated from methanol extract of perilla leaves. Apoptosis occurred was methyl pheophorbide A treated and irradiated with light, and there was no significant change where light not treated. In both U937 and SK-HEP-1 cells, apoptotic body, vesicle formation, and DNA ladder were confirmed in the light-irradiated. The caspase-3/7 activity an important factor in apoptosis, was 101.50 ± 14.24% when treated with 0.25 μg/ml methyl pheophorbide A in U937, and 91.32 ± 16.23% when treated with 1.00 μg/ml in SK-HEP-1 cells. The apoptotic phenomenon appeared more strongly when the methyl pheophorbide A concentration was increased with irradiating light. This study demonstrates the isolation, identification, and phototoxic activity of methyl pheophorbide A in perilla leaves. We expect that this study will be useful in the search for PS candidates using natural products.

**Yun-Jeong Shin, Jiyoung Shin, Hyewon Jang, Hokyoung Son, Yonghoon Kwon. Decursinol chloroacrylates useful as fungicides. (2022) Appl. Biol. Chem. 65: 53**

Natural products decursin and decursinol angelate were recently reported as benign fungicides for controlling rice blast. Inspired by the structural similarity of the cumarin compounds and gained hint from the skeletal motifs, we designed and prepared synthetic compounds to increase the natural product efficacy and evaluated their antifungal activities against various plant disease pathogens in vitro. Synthetically prepared compound **4** and **5** indeed suppressed the mycelial growth of *B. cinerea*, *F. oxysporum*, *P. italicum*, and *R. quercus-mongolicae*. Additionally, compound **5** effectively prevents the growth of *C. coccodes* and *C. parasitica*. Furthermore, both **4** and **5** possess better inhibitory activities on spore germination of *F. oxysporum and M. oryzae* than the natural product decursin and commercial pesticide Iprodione. These results suggest that the effect of the lead compound for plant disease protection can be improved by tuning the structure of the original natural product and decursinol chloroacrylates **4** and **5** are candidates for the control of *F. oxysporum* and *M. oryzae*.

**Ahmad E. Mohamed, Walid E. Elgammal, Aya M. Dawaba, Ahmed G. Ibrahim, Amr Fouda, Saber M. Hassan. A novel 1,3,4-thiadiazole modified chitosan: synthesis, characterization, antimicrobial activity, and release study from film dressings. (2022) Appl. Biol. Chem. 65: 54**

Herein, two new polymers designated as Cs-EATT and Cs-BATT have been synthesized via linking the chitosan with the synthesized 1,3,4-thiadiazole compounds. They were characterized using 1H,13C-NMR, FT-IR, TGA, Elemental analysis, Mass spectrum, and UV–vis spectrophotometer. The synthesized polymers exhibit high activity to control the growth of pathogenic bacteria (*S. aureus*, *B. subtilis*, *E. coli,* and *P. aeruginosa*), and unicellular fungi (*C. albicans*). The MIC values were in the range of 25–100 µg mL–1 for Cs-EATT and 25–200 µg mL–1 for Cs-BATT with varied clear zones. The new polymers were mixed with three film-forming agents: polyvinyl alcohol, hydroxyethyl cellulose, and carboxymethyl cellulose to form six film dressings designated as E1, E2, and E3 for Cs-EATT, and B1, B2, and B3 for Cs-BATT, respectively. The evaluation of film dressings showed that the formed films had transparency, uniformity, homogeneity, elasticity, and non-irritation pH values for skin within the normal range. The maximum percentages of Cs-E/B-ATT content were recorded for film dressings E2 and B2, with values of 92.5% and 94.9%, respectively. Also, the release percentages varied according to film dressing formulation, with values in the ranges of 83.88–93.2% for Cs-EATT and (87.7–97.35%) for Cs-EATT after 9 h.

**Nagham H. Kamal, Ahmed Essmat, Hesham I. El Askary, Hala M. El Hefnawy, Samia M. Abdel Wahab, Meselhy R. Meselhy. Chemical profile and beneficial effect of standardized extract of *Stevia rebaudiana* Bertoni leaves on metabolic syndrome in high fat diet streptozotocin-induced diabetic rats. (2022) Appl. Biol. Chem. 65: 55**

Stevia (*Stevia rebaudiana* Bertoni) is a natural zero calorie sweetener with significant economic and medicinal values due to its high contents of steviosides (SVGs) in the leaves. The aqueous extract of *Stevia* leaves (TAqE) was standardized to contain 8.5% w/w of SVGs (HPLC), total phenolics (164.63 ± 1.39 µg Gallic acid/mg extract) and total flavonoids of 100.5 ± 0.79 µg QE/mg extract. Twenty-one compounds were tentatively identified in the leaves via UPLC-Orbitrap HRMS and stevioside, rebaudioside A, and quercetrin were isolated from TAqE by repeated column chromatography. Stevioside showed significant inhibition of pancreatic lipase, α-amylase, and α-glucosidase enzymes. The effect of a standardized TAqE on high fat diet (HFD)-streptozotocin (STZ)-induced diabetic rats was investigated. Thirty-six animals were divided into 6 groups (each of 6). Rats in group I (control) and group II (control/HFD-STZ) received distilled water, and rats in groups III and IV received TAqE for 4 weeks in two doses; 300 mg/kg b.wt., and 500 mg/kg b.wt., respectively. Rats in group V received metformin (200 mg/kg), while those in group VI received statin (1 mg/kg). Body weight, fasting blood glucose, lipid profile (total cholesterol and triglycerides), liver enzymes (alanine transaminase and aspartic transaminase), and serum kidney parameters (urea and creatinine) were decreased in rats treated with TAqE (300 mg/kg b.wt.), while insulin sensitivity was enhanced, when compared to that in group II. These findings could justify the use of *Stevia* as a complementary medicine for the prevention and treatment of metabolic changes associated with diabetes mellitus type 2.

**Wei Dai, Yanqun Duan, Wenkang Yuan, Siyu Wang. circTUBGCP5 promotes liver cancer progression and glycolysis by up-regulating the expression of ACSL4. (2022) Appl. Biol. Chem. 65: 56**

A large number of researches have shown that circular RNA (circRNA) is new hope for the diagnosis or treatment of tumors, including liver cancer (LCa). However, it remains largely unclear the role of circRNA in the progression of LCa and its molecular mechanism. GSE164803 microarray dataset was applied to identify dysregulated circRNAs in LCa and noncancerous tissues. CircTUBGCP5 (hsa\_circ\_0034049) was selected for further research. Biological functions of circTUBGCP5 were investigated by EdU, colony formation, flow cytometry, glucose consumption and lactate production assay, and in vivo tumorigenesis. RNA pull-down assay and dual-luciferase reporter assay were used to investigate the interaction between circTUBGCP5, miR-144-3p, and ACSL4. We demonstrated that circTUBGCP5 was significantly up-regulated in LCa tissues and cells. CircTUBGCP5 promoted LCa cell proliferation, anti-apoptotic ability, glycolysis, and tumorigenesis at least partially by sponging miR-144-3p to regulate ACSL4 protein level. In conclusion, circTUBGCP5 is a forceful contributor to malignant behaviors and glycolysis of LCa via modulating the circTUBGCP5/miR-144-3p/ACSL4 axis, which has provided a target for the diagnosis and treatment of LCa patients.

**Shuirong Lu, Jinlai Lu, Lang Liu, Yilong Sun, Yixuan Zhao, Xi Tan, Jingze Li. Circ\_0026359 induces HOXA9 to regulate gastric cancer malignant progression through miR-140-3p. (2022) Appl. Biol. Chem. 65: 57**

Recent researches indicate the key role of circRNA in gastric cancer (GC) progression. However, the role of circ\_0026359 in GC progression remains unclear. This study aims to analyze the role of circ\_0026359 in GC development and the underlying mechanism. The results showed that compared with controls, GC tissues and cells displayed high circ\_0026359 and HOXA9 expression, and low miR-140-3p expression. Depletion of circ\_0026359 repressed cell proliferation, migration, invasion and glycolysis, and induced cell apoptosis. Circ\_0026359 knockdown inhibited neoplasm growth in vivo. Under-expression of miR-140-3p, a target miRNA of circ\_0026359, relieved the effects of circ\_0026359 knockdown on GC progression. Additionally, HOXA9 was regulated by the circ\_0026359/miR-140-3p axis. Thus, circ\_0026359 absence inhibited GC progression by miR-140-3p/HOXA9 pathway, which provided an effective therapeutic target for GC.

**Zhihui Li, Yawei Guan, Jingfei Teng, Zhuomin Jia, Guohui Zhang, Xing Ai. CircTRRAP (hsa\_circ\_0081234) participates in prostate cancer progression and glycolysis by HOXA1 via functioning as a miR-515-5p sponge. (2022) Appl. Biol. Chem. 65: 58**

Dysregulated circular RNAs (circRNAs) are implicated in prostate cancer (PCa) progression. Hsa\_circ\_0081234 (circTRRAP) has been revealed as a facilitator in PCa, but the mechanisms associated with circTRRAP in PCa progression are largely unclear. The present study was to explore the regulatory mechanism of circTRRAP-mediated PCa progression. A total of 50 PCa tissues and normal tissues were collected. RNA levels of circTRRAP, microRNA (miR)-515-5p and homeobox A1 (HOXA1) were detected by quantitative real-time polymerase chain reaction (qRT-PCR) or western blot. Cell viability, proliferation, migration, and invasion were estimated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, 5-ethynyl-2ʹ-deoxyuridine (EdU) and transwell assays, respectively. Cell glycolysis was assessed by measuring glucose uptake and lactate production. The target interaction between circTRRAP or HOXA1 and miR-515-5p was investigated by the dual-luciferase reporter assay. We observed the overt upregulaiton of circTRRAP in PCa samples and cells. Silencing of circTRRAP lowered tumor growth in vivo and restrained PCa cell viability, proliferation, migration, invasion, and glycolysis in vitro. miR-515-5p was negatively regulated by circTRRAP and its deficiency reversed the inhibiting effects of circTRRAP knockdown on PCa cell malignancy and glycolysis. HOXA1 was confirmed as a miR-515-5p target and miR-515-5p overexpression lessened PCa cell malignancy and glycolysis by decreasing HOXA1 expression. Importantly, circTRRAP mediated HOXA1 expression by functioning as a miR-515-5p sponge. In conclusion, circTRRAP took part in PCa progression and glycolysis through mediating the miR-515-5p/HOXA1 axis, suggesting that circTRRAP can serve as a potential therapeutic target for PCa patients.

**Ju-Yeong Kang, Won-Jung Park, Youngdae Yoon, Bong-Gyu Kim. Production of isoquercitrin from quercetin by biotransformation using *Bascillus* sp. CSQ10 isolated from *Camellia sinensis* cultivation soils. (2022) Appl. Biol. Chem. 65: 59**

Microorganisms are widely used to produce biologically active substances owing to their versatile ability to convert inexpensive compounds into physiologically active compounds. In this study, we isolated a microorganism capable of converting quercetin to isoquercitrin, a substance with various biological functions, from tea cultivation soils. A *Bacillus* strain was isolated and verified as *Bacillus* sp. CSQ 10 using 16sRNA gene analysis. When quercetin was fed as a substrate for *Bacillus* sp. CSQ10, isoquercitrin was produced through biotransformation. Furthermore, *Bacillus* sp. CSQ10 was able to biotransform isoquercitrin to quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside when the medium for biotransformation was replaced with yeast extract–peptone–dextrose (YPD) medium. Based on these findings, the biotransformation performance of *Bacillus* sp. CSQ10 was verified by optimizing the experimental conditions for the culture system at the laboratory scale in terms of temperature, cell density, biotransformation medium, and substrate concentration. The best biotransformation yields were achieved at 37 °C, 6.0 OD600, with YPD, and 181.0 mg/L of quercetin supply. Conclusively, 193.3 mg/L and 198.8 mg/L of isoquercitrin and quercetin-3-*O*-(6″-*O*-acetyl)-β-D-glucoside, respectively, were produced by *Bacillus* sp. CSQ 10 under these optimized experimental conditions.

**Geun-Hyoung Choi, Deuk-Yeong Lee, A-Reum Song, Bo-Yeon Moon, Jin-Hyo Kim. The dietary risk assessment of perfluorooctanoic acid (PFOA) and perfluorosulfonic acid (PFOS) in the root crops from the survey of the residue in agricultural soil and the crops. (2022) Appl. Biol. Chem. 65: 60**

Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are persistent organic pollutants (POPs) that are prohibited from being produced and used. However, they remain in the agricultural environment and are transferred to crops. In addition, PFOA is classified as possibly carcinogenic to humans. To fully understand the exposure and risk of PFOA and PFOS for human in crops, a residue survey and estimation of dietary exposure assessment are needed. Therefore, we investigated the residues of PFOA and PFOS in upland soil and cultivated root crops. The average residues of PFOA and PFOS in the soil were < 0.054–0.541 μg kg–1 and 0.024–0.111 μg kg–1, and 0.067–0.193 μg kg–1 and < 0.02 μg kg–1 in the crops, respectively. The average PFOA residues were higher than PFOS in the soil and crops. The estimated daily intakes of PFOA and PFOS in the crops were 0.284 ng kgbw–1 day–1 and 0.023 ng kgbw–1 day–1, and the estimated hazard quotients were 0.355 and 0.013, respectively. In addition, the excess cancer risk of PFOA was calculated to be 1.99 × 10–8. Thus, the non–carcinogenic and carcinogenic risks of PFOA and PFOS were not notable from the surveyed residues in the crops. However, the risks may be higher when the residues in other food crops are considered.

**Tamer Ismail, Attia Keratum, Lamia El-Hetawy. Formulation of abamectin and plant oil-based nanoemulsions with efficacy against the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae) under laboratory and field conditions. (2022) Appl. Biol. Chem. 65: 61**

Due to the harmful effects of synthetic chemical acaricides on ecosystems and human health, there is increasing interest in the use of nanotechnology to fabricate eco-friendly nanoemulsions based on plant oils in the field of spider mite control. In this study, nanoemulsions of abamectin, garlic, and neem oils were prepared by a high-energy approach and characterized by transmission electron microscopy. The droplet sizes of all tested nanoemulsions were less than 100 nm. The acaricidal activities of the prepared nanoemulsions compared to abamectin were evaluated against a susceptible laboratory strain of *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory and field conditions. The results showed that abamectin nanoemulsion was the most toxic compound against adult females of *T. urticae* followed by abamectin emulsion. Neem nanoemulsion had moderate toxicity and garlic nanoemulsion had the lowest toxicity. The effects of tested compound residues on egg deposition and egg hatching in descending order were as follows: abamectin nanoemulsion > abamectin emulsion > neem oil nanoemulsion < garlic oil nanoemulsion. In the field experiment, all tested compounds were effective in reducing the population density of *T. urticae* in the motile stage, with mean reductions ranging between 66.08% and 95.24% for all compounds. The most effective compound was abamectin nanoemulsion. The results of the present study demonstrate that nanoemulsion enhanced the biological activity of abamectin. Further, neem and garlic oil nanoemulsions have potential utility as environmentally friendly acaricides in integrated pest management programs.

**Ying-Hao Han, Xu-Dong Lian, Seung-Jae Lee, Wei-Long Li, Hu-Nan Sun, Mei-Hua Jin, Taeho Kwon. Regulatory effect of peroxiredoxin 1 (PRDX1) on doxorubicin-induced apoptosis in triple negative breast cancer cells. (2022) Appl. Biol. Chem. 65: 63**

Patients with triple negative breast cancer (TNBC) lack the estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2; thus, conventional hormone and targeted therapies have minimal effect on them. Therefore, clinical treatment of TNBC is still based on chemotherapy and supplemented by other methods. Doxorubicin (DOX), a common drug used in TNBC chemotherapy, has high affinity for cardiolipin, and the nematosomes are rich in cardiolipin; therefore, DOX has high mitochondria-targeting ability. DOX accumulates and plunders the electrons of nicotinamide adenine dinucleotide phosphate (NADPH) and cytochrome C in mitochondria to produce semiquinone DOX. Under the action of oxygen molecules, semiquinone DOX is reduced to DOX and reactive oxygen species (ROS) are generated. The accumulation of ROS can cause mitochondrial dysfunction and lead to mitochondrial dependent apoptosis. Bioinformatic analysis of samples from TNBC patients revealed that peroxiredoxin 1 (PRDX1) was highly expressed in TNBC tissues, and the poor prognosis of patients with high PRDX1 expression was considerably increased. Previous studies determined that DOX can upregulate the expression of the PRDX1 protein in the human TNBC cell line (MDA-MB-231). Thus, we speculate that PRDX1 plays an important role in the process of DOX-induced TNBC cell apoptosis. In this study, we aimed to explore the role of PRDX1 in the process of DOX-induced TNBC cell apoptosis. We found that PRDX1 deletion increased the sensitivity of MDA-MB-231 cells to DOX, which was mainly due to mitochondrial oxidative stress caused by intracellular ROS accumulation, leading to mitochondria-dependent apoptosis. Deletion of PRDX1 promotes the PI3K/Akt signaling pathway to mediate the expression of GSK3β. Gsk3β is an upstream signal of mitochondria-dependent apoptosis, and is also an important target of ROS. PRDX1 participates in adriamycin-induced apoptosis of TNBC cells by regulating the expression level of GSK3β. Our findings present new insights to treat breast cancer and TNBC, outlines the clinical use of DOX, and provides a basic theory to develop PRDX1 gene function.

**Jinlong Luo, Hua Yang, Xuefeng Peng, Faqiang Zhang, Shilong Shu, Ke Lan, Shengjin Tu, Kai Lu, Xiaoying Cha. Circ\_0000554 is identified as a cancer-promoting circRNA in colorectal cancer by regulating the miR-1205/LASP1 axis. (2022) Appl. Biol. Chem. 65: 64**

Background

Colorectal cancer (CRC) is a prevalent malignant tumor with poor prognosis. Circular RNAs (circRNAs) are key regulators in the progression of CRC. Our study aimed to disclose the role of circ\_0000554 in CRC.

Methods

The expression of circ\_0000554, miR-1205 and LIM and SH3 protein 1 (LASP1) was measured by quantitative real-time polymerase chain reaction (qRT-PCR). Cell proliferation, invasion and migration were monitored using cell counting kit-8 (CCK-8) assay, EdU assay, transwell assay and wound healing assay respectively. The protein levels of C-myc, matrix metallopeptidase 2 (MMP-2) and LASP1 were detected by western blot. Tumor formation assay in nude mice was conducted to explore the role of circ\_0000554 in vivo. The association between miR-1205 and circ\_0000554 or LASP1 was identified by dual-luciferase reporter assay and RNA immunoprecipitation (RIP) assay.

Results

circ\_0000554 was upregulated in CRC tissues and cells, high circ\_0000554 expression was significantly linked to shorter overall survival. Downregulation of circ\_0000554 restrained cell growth and metastasis while promoted apoptosis in vitro, and suppressed tumorigenesis of CRC in vivo. Furthermore, mechanism study and rescue experiments confirmed miR-1205 could be sponged by circ\_0000554 and its inhibitor reversed the inhibitory effect of circ\_0000554 silencing on CRC progression. LASP1 was a target gene of miR-1205 and the upregulation of LASP1 overturned miR-1205-induced effects on CRC cells. Circ\_0000554 could elevate LASP1 expression via interacting with miR-1205.

**Yeo-Jin Park, Gyu‑Sik Choi, Shin-Won Lee, Joong-Hoon Ahn. Production of quinolone derivatives in *Escherichia coli*. (2022) Appl. Biol. Chem. 65: 65**

Alkyl-4-quinolones (AQs) are natural compounds synthesized by bacteria. Members of this group are known quorum-sensing molecules. Other biological functions, such as anti-bacterial, anti-algal, antifungal, and anti-malaria activities have also been reported. The synthetic pathways of AQs have been validated in *Pseudomonas aeruginosa*. Five genes (*pqsA–E*) are involved in the synthesis of 2-heptyl-4(1H)-quinolone (HHQ). To synthesize HHQ in a microbial system, *pqsA–E* genes were introduced into *Escherichia coli* and HHQ and 2-methyl-4(1H)-quinolone (MHQ) were synthesized. After the copy number, construct promoters, and substrate supplements were optimized, 141.3 mg/L MHQ and 242.8 mg/L HHQ were synthesized.

**Joon-Yung Cha, Gyeong-Im Shin, Gyeongik Ahn, Song Yi Jeong, Myung Geun Ji, Aliya Alimzhan, Min Gab Kim, Woe-Yeon Kim. Loss-of-function in *GIGANTEA* confers resistance to PPO-inhibiting herbicide tiafenacil through transcriptional activation of antioxidant genes in Arabidopsis. (2022) Appl. Biol. Chem. 65: 66**

Herbicides play a crucial role in maintaining crop productivity by reducing competition between weeds and crops. Protoporphyrinogen oxidase (PPO)-inhibiting herbicides trigger the photooxidative damage that destroys cell membranes. Tiafenacil is a recently developed pyrimidinedione-type PPO-inhibiting herbicide that has low IC50 values in plants and is less toxic in humans compared to other PPO inhibitors. Previous reports confirmed that mutations in Arabidopsis circadian clock-controlled gene *GIGANTEA* (*GI*) were insensitive to phytooxidants, including chloroplast biogenesis inhibitors and herbicides. Here, we examined whether GI regulates the resistance to tiafenacil. Both *gi* mutant alleles, *gi-1* and *gi-2*, were resistant to tiafenacil with survival rates of 97% and 83%, respectively, under 1 µM tiafenacil treatments, while 56% of wild-type and GI-overexpressing plants (GI-OX) survived. Both *gi* mutants were insensitive to tiafenacil-induced inhibition of photosystem efficiency and alleviated photooxidative damage. The *gi* mutants showed significant increases in transcriptional expressions and enzyme activities of antioxidants compared to wild-type and GI-OX. Moreover, loss-of-function in *GI* enhanced resistance to tiafenacil-containing commercial herbicide Terrad’or Plus®. Collectively, based on our results together with previous reports, mutations in *GI* confer resistance to herbicides with different MoAs and would be a crucial molecular target for non-target-site resistance strategies to develop herbicide-resistant crops.

**Xiaoqi Yang, Shuhua Wang, Xianjun Zhang, Xiangbin Gao, Pengfei Xu. Circ-NT5C2 stimulates FZD4 expression to promote the malignant progression of osteosarcoma by targeting miR-488-3p. (2022) Appl. Biol. Chem. 65: 67**

Background

Circ-NT5C2 has been confirmed to be highly expressed and associated to the progression of osteosarcoma (OS). However, the behind mechanism of circ-NT5C2 involvement in OS remains unclear.

Methods

The expression of circ-NT5C2, miR-488-3p and FZD4 was measured by quantitative real-time PCR, and the protein expression of E-cadherin, N-cadherin and FZD4 was detected by western blot. Cell counting kit 8 assay, colony formation assay and 5-ethynyl-2-deoxyuridine assay were performed to assess the cell proliferation. The cell apoptosis was measured by flow cytometry and Caspase3/Caspase9 Activity Assay Kits. Cell migration and invasion were detected by transwell assay. Dual-luciferase reporter assay and RIP assay were carried out to determine the binding relation among circ-NT5C2, miR-488-3p and FZD4. Animal experiment and immunohistochemistry analysis were conducted to explore the role of circ-NT5C2 in tumor growth in vivo.

Results

Comparing with controls, the expression of circ-NT5C2 and FZD4 was upregulated and miR-488-3p expression was downregulated in OS tumor tissues and cells. Circ-NT5C2 overexpression facilitated the cell proliferation and motility and induced cell apoptosis of OS cells, whereas circ-NT5C2 knockdown had the opposite effect. Besides, we also found and confirmed that circ-NT5C2 regulated cell malignant behaviors via modulating miR-488-3p/FZD4 axis in OS. Moreover, circ-NT5C2 silencing repressed the growth of xenografts in vivo.

Conclusion

Circ-NT5C2 upregulated FZD4 expression via sponging miR-488-3p, thus facilitating cell malignant behaviors in OS.

**Farid M. Sroor, Wahid M. Basyouni, Hanan F. Aly, Eman A. Younis, Karima F. Mahrous, Ahmed A. Haroun. Biochemical and histopathological studies of sulfonylurea derivative as a new chemotherapeutic agent against liver cancer in free- and nano-coated forms. (2022) Appl. Biol. Chem. 65: 68**

The most frequent type of primary liver cancer is hepatocellular carcinoma (HCC), accounting for approximately 90% of primary liver cancers and a third leading cause of cancer deaths. In the current study, the synthesized compound **3** was re-formulated using tetraethyl orthosilicate (TEOS) with weight ratio (1:1) via sol-gel technique. The prepared material has been examined using Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray elemental analysis (EDX), and scanning and transmission electron microscopes (SEM and TEM). Herein, we investigate the mode of action of **3** as potent anti-liver cancer in vivo as normal and nano-forms. Rats were given a single dosage of 50 mg/kg b.wt. of HCC through an intraperitoneal injection (ip). A single dosage of CCl4 (2 ml/kg IP) was also given to rats 2 weeks later. Several liver, tumor and oxidative stress biomarkers were detected including liver enzymes; alanine and aspartate aminotransferases (ALT and AST), alkaline phosphatse (ALP), gamma glutamyl transferase (GGT), glutathione (GSH), lipid peroxide (MDA), catalase (CAT), superoxide dismutase (SOD), total antioxidant capacity (TAC), α-fetoprotein and α-L-Fucosidase. Hepatic pathological pictures were also performed for the documentation of the presence of HCC and supported the biochemical results. Moreover, the DNA damage in liver tissues of male rats using comet assay was studied. The results showed that the HePG2 (− ve) group of rats exhibited a significant reduction (P < 0.05) in DNA damage values (9.30 ± 0.89) relative to other treatment groups. Nevertheless, the DNA damage values in the HePG2 (+ ve) and 5-flurouracil groups were significantly higher (P < 0.01) compared to the HePG2 (− ve) group. Additionally, HePG2 (coated **3**) and HePG2 (**3**) groups exhibited significant decrease in the DNA damage compared to those in HePG2 (+ ve) group.

**Hwan-Hee Bae, Jun Young Ha, Young Sam Go, Jae-Han Son, Beom-Young Son, Jae-Hong Kim, Seonghyu Shin, Tae-Wook Jung, Gibum Yi. High phytosterol levels in corn cobs point to their sustainable use as a nutritional source. (2022) Appl. Biol. Chem. 65: 69**

Phytosterols are important structural components of plant cells that affect membrane fluidity, permeability, and membrane-related metabolic regulation. These compounds, which are abundant in vegetable oils and corn kernel oil, are also beneficial for human health. Cultivation of corn (*Zea mays* L.) produces huge amounts of cobs as a by-product, but efforts to utilize cobs are still limited. Here, we investigated phytosterol, crude oil, and fatty acid contents in the kernels and cobs of four major corn cultivars in South Korea and explored the potential use of cobs as a source of phytosterols. Total phytosterol levels were two times higher in cobs (68.0–217.1 mg 100 g−1 DW) than in kernels (43.8–89.5 mg 100 g−1 DW) and were highest in the kernels and cobs of Sinhwangok at 60 days after pollination. We showed that not only kernels but also cobs can be a rich source of phytosterols. The results also revealed that the amount of phytosterol is depending on a genetic background as well as developmental stages suggesting further investigation would enhance the utilization of corn cobs as a phytosterol source.

**Emad Mohamed El-Sherbiny, Eman Ismail Abdel-Gawad, Hala Fawzy Osman. Impact of nano silver composite structure on cadmium neurotoxicity in albino rats. (2022) Appl. Biol. Chem. 65: 70**

The present study was planned to investigate the possible therapeutic effects of silver/hydroxyapatite nanocomposite (nAg/HAp) on neurotoxicity induced by cadmium chloride (CdCl2) in albino rats. The nanocomposite has been formulated by a chemical route and characterized by scanning electron microscope (SEM), Transmission Electron Microscopy (TEM), and energy-dispersive X-ray Analysis spectroscopy (EDAX). A population of rats was randomly assorted into three groups; the animals were subjected to intraperitoneal CdCl2 administration every 2 days at a dose level of 1.0 mg/kg b.wt. for 3 months while the treatment with nAg/HAp was performed via intravenous injection at a dose level of 50 mg/kg b,wt. once a week for 4 weeks. Quantitative DNA fragmentation and biochemical analysis including the content of γ-aminobutyric acid (GABA), noradrenaline (NA), dopamine (DA), caspase-3, calmodulin (CaM), calcium adenosine 5′-triphosphatase (Ca++ATPase), tau protein, glutathione (GSH) and malondialdehyde (MDA) were measured in brain tissue. The results revealed the potent efficacy of nAg/HAp in attenuating DNA fragmentation and partially recovering most of the investigated parameters manifested by a significant elevation in GABA, NA, DA, Ca++ATPase, and GSH levels and a decrease in tau protein, caspase-3, CaM and MDA tissue content in comparison with Cd—intoxicated groups. Accordingly, the synthesized nAg/HAp at the selected dose can be used as a biosafe intravenous injection in neurodegenerative diseases.

**Huiji Kim, Minjoo Han, Seong-Ah Shin, Jangeun An, Mi-Jeong Ahn, Jun Hyuck Lee, Hyun Ho Park & Chang Sup Lee. Impact of nano silver composite structure on cadmium neurotoxicity in albino rats. (2022) Appl. Biol. Chem. 65: 71**

Melanoma is a deadly skin cancer with high mortality, and its incidence is increasing every year. Although numerous anticancer drugs have been developed, these treatments have various side effects, such as skin rash, fatigue, diarrhea, cough, and muscle pain. Therefore, there is a need for research on novel anticancer drugs with low cytotoxicity and few side effects. In this study, we investigated whether afrormosin (7-hydroxy-4′,6-dimethoxyisoflavone), a member of the isoflavonoid family, could have the potential as a novel anticancer drug. Afrormosin decreased the viability of B16F10 melanoma cells in a time- and dose-dependent manner. We also found that the afrormosin-induced decrease in cell viability was caused by the reduction of cell proliferation through Go/G1 arrest and the induction of apoptosis in B16F10 melanoma cells. Furthermore, afrormosin decreased the metastatic activity (cell invasion and migration) of B16F10 melanoma cells. At the molecular level, afrormosin reduced the levels of Bcl-2, an anti-apoptotic protein, and augmented the levels of Bax, a pro-apoptotic protein, and p53, a tumor suppressor. Additionally, procaspase-3 levels were reduced by afrormosin treatment. When we examined the signaling pathways affected by afrormosin, we found that the AKT/ERK pathways were inhibited and the p38/JNK pathway was activated by afrormosin. Collectively, these results suggest the potential anticancer effect of afrormosin, making it a prospective candidate for development as an anticancer drug.

**Ie Byung Park, Min Hee Kim, Jung-Soon Han, Woo-Jae Park. *Gryllus bimaculatus* extract protects against palmitate-induced β-cell death by inhibiting ceramide synthesis. (2022) Appl. Biol. Chem. 65: 72**

Type I diabetes mellitus is an autoimmune disease characterized by the destruction of β-cells, leading to severe insulin deficiency. Environmental factors and genetic predisposition are implicated in β-cell destruction, which is the final step in a cascade of complex events. Possible triggers of β-cell destruction are activation of Fas, activation of perforin, increased generation of reactive oxygen species, increased production of inflammatory cytokines, and endoplasmic reticulum (ER) stress. In this study, we examined whether *Gryllus bimaculatus* (GB) extract could prevent palmitate-induced β-cell apoptosis. Exposure to GB extract prevented palmitate-induced death of MIN6 cells, a mouse pancreatic β-cell line. Palmitate increased total ceramide levels with the elevation of ceramide synthase (CerS)1, CerS4, and CerS6 expressions. Treatment with GB extract decreased the levels and expressions of ceramides related to insulin resistance. CerS4 and CerS6 overexpression, but not CerS1 overexpression, increased palmitate-induced MIN6 cell death by increasing ceramide synthesis. Oppositely, inhibition of ceramide synthesis by fumonisin B1 treatment partially recovered palmitate-induced MIN6 cell death. Furthermore, GB extract reduced ER stress (phosphorylation of PERK and eIF2α), NF-κB–iNOS signaling, and the phosphorylation of MAP kinase (JNK, p38). GB extract reduced pro-apoptotic Bax protein expression but increased anti-apoptotic Bcl2 expression. In addition, CerS4 and CerS6 overexpression aggravated impairment of insulin secretion by palmitate, but GB extract recovered it. In conclusion, GB could be a functional food that improves palmitate-induced β-cell death and insulin secretion.

**Jae Ho Choi, Gyung Min Go, Tatsuya Unno. Diatom frustule silica extracted from *Melosira nummuloides*ameliorates acetaminophen-induced acute liver injury in mice. (2022) Appl. Biol. Chem. 65: 73**

*Melosira nummuloides* is a type of diatom in the family Melosiraceae. Diatoms are unicellular microalgae widely distributed in aquatic environments. Diatoms are known to be suitable for many industrial and biomedical applications because of their high biocompatibility and ease of use. In this study, we investigated the hepatoprotective effect of diatom frustule silica (DFS) extracted from *Melosira nummuloides* on hepatotoxicant-induced liver injury. Hepatoprotective effects of DFS were examined using acetaminophen-induced acute liver injury (ALI) mouse model. We evaluated the hepatoprotective effects through hepatotoxicity, pro-inflammatory cytokines, transcriptional factors, upstream signaling pathways, and histopathological analysis by DFS in an animal model of acetaminophen-induced ALI. Our results showed serum alanine aminotransferase/aspartate aminotransferase activity and hepatic malondialdehyde formation were significantly attenuated upon DFS administration. DFS also ameliorated glutathione depletion and down-regulated acetaminophen-induced CYP2E1. DFS administration also down-regulated expressions of pro-inflammatory cytokines through preventing NF-κB activation by JNK1/2 phosphorylation inhibition. These findings demonstrate that the hepatoprotective effect of DFS is associated with suppression of inflammatory responses in an animal model of acetaminophen-induced ALI.

**Su Jung Lee, Han Sol Lee, Sun Young Gu, Hye-Sun Shin, Sung Eun Kang, Sang Soon Yun, Yong-Hyun Jung, Hye-Jung Youn. Development of simultaneous analytical method for the determination of fluazaindolizine and its seven metabolites in agricultural products by liquid chromatography tandem mass spectrometry. (2022) Appl. Biol. Chem. 65: 74**

A simultaneous analytical method has been developed for quantification and confirmation of the nematicide fluazaindolizine and its seven metabolites (IN-A5760, IN-F4106, IN-QEK31, IN-QZY47, IN-TMQ01, IN-UNS90 and IN-UJV12) in agricultural products. The compounds were extracted with acetonitrile/water (80/20, v/v) and purified using C18 cartridge, and analysis was conducted by liquid chromatography-tandem mass spectrometry in the electrospray positive and negative ion mode. The method has been validated by verifying the performance characteristics such as selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision. To prevent the matrix effects, all analytes were quantified with matrix-matched calibration assessed by the determination coefficient (R2) of the range from 0.9988 to 1.0000. The LOD and LOQ were satisfactory to determine the low residual level in agricultural products. The accuracy and precision of the method were evaluated by recoveries with five replicates at three fortification levels (LOQ, 10 × LOQ and 50 × LOQ). The mean recoveries of fluazaindolizine and seven metabolites in agricultural products were 75.6–110.0% with the CV% of 0.2–9.1%. All optimized results were displayed excellent results assessed by the Ministry of Food and Drug Safety guidelines and the Codex Alimentarius Commission guidelines for pesticide residue analysis. This study could use as basic data for setting of residue definition and maximum residue limits of fluazaindolizine in agricultural products.

**Jong-Hwan Park, Ah-Young Choi, Su-Lim Lee, Jae-Hoon Lee, Jun-Suk Rho, Seong-Heon Kim, Dong-Cheol Seo. Removal of phosphates using eggshells and calcined eggshells in high phosphate solutions. (2022) Appl. Biol. Chem. 65: 75**

This study was conducted to evaluate the phosphate sorption properties of eggshell (ES) and calcined ESs (C-ESs) in a high-concentration phosphate solution. The C-ESs yield decreased rapidly at 900 °C, indicating that the CaCO3 constituting the ES was converted to CaO by the high calcination temperature. The optimum calcination temperature for phosphate removal using C-ES was 900 °C. The actual sorption amount of phosphate by ES and C-ES900 was in agreement with the Langmuir isothermal sorption equation, and the maximum sorption capacities derived from this equation were 178.6 and 270.3 mg/g, respectively. The sorption rate of phosphate by ES and C-ES900 was divided into two stages: an initial fast sorption stage, followed by a slow sorption stage. The sorption of phosphate by ES was dominantly influenced by the initial pH and salt concentration, whereas C-ES900 exhibited a constant sorption capacity regardless of environmental changes. The SEM–EDS and XRD results demonstrated that phosphate was successfully adsorbed on the ES and C-SE900 surfaces. In this study, it was found that the sorption of phosphate by ES occurred via ion exchange and precipitation reactions and that the sorption of phosphate by C-ES900 was dominantly affected by precipitation. Above all, C-ES can be applied as an effective adsorbent for removing high concentrations of phosphate under a wide range of environmental conditions.

**Teshome Degfie, Milkyas Endale, Tarekegn Tafese, Aman Dekebo, Kebede Shenkute. In vitro antibacterial, antioxidant activities, molecular docking, and ADMET analysis of phytochemicals from roots of *Hydnora johannis*. (2022) Appl. Biol. Chem. 65: 76**

*Hydnora johannis* is a medicinal plant traditionally used to treat various ailments. Chemical investigation of the dichloromethane (DCM)/methanol (MeOH) (1:1) roots extract of *Hydnora johannis* afforded three compounds (**1–3**), reported herein for the first time from the species. The structures of the isolated compounds **1–3** were elucidated using 1D and 2D NMR spectroscopic analysis and comparison with literature data. The highest zone of inhibition value was measured for DCM/MeOH extract (10.75 ± 0.25 mm) against *Staphylococcus aureus* at concentration of 0.25 mg/mL, promising in comparison to the standard amoxicillin (16.0 ± 0.0 mm, 0.25 mg/mL). At concentration of 0.25 mg/mL, the largest mean inhibition zone of 12.0 ± 0.0 mm was measured for compound **2** against *Pseudomonas aeruginosa*, comparable to the standard drug amoxicillin (16.0 ± 0.0 mm, 0.25 mg/mL). Compound **2** displayed better binding affinity with minimum binding energy of − 8.7 kcal/mol (PqsA), − 7.6 kcal/mol (DNA gyrase), and − 7.4 kcal/mol ( *S aureus* PK) than amoxicillin (− 7.3, − 6.1, and − 7.0 kcal/mol, respectively). This suggests that compound **2** may act as potential inhibitor of the tested bacterial proteins. Compound **1** satisfies the Lipinski’s rule of five with zero violations. Compound **2** obey the MW (452.4 g/mol) and iLogP (< 5) rules, and compound **3** obey the NHD (4) and NHA (6) rules. Compounds **2** recorded iLogP value less enough than five (1.55), implying its optimal lipophilicity. Compounds **1** and **3** satisfy the veber’s rule (NRB < 12, and TPSA < 140 unit). Compound **2** and **3** exhibited negligible acute toxicity (LD50 > 5000, Toxicity class > 5. Compound **2** demonstrated maximum scavenging activity (67.87%) with IC50 value of 0.190 µg/mL, compared to ascorbic acid (78.21%) with IC50 value of 0.014 µg/mL at concentration of 12.5 µg/mL. Overall, the in vitro antibacterial activity of the extracts and compounds, molecular docking analysis and radical scavenging activity results of the isolated compounds suggest DCM/MeOH crude extract and compound **2** are promising antibacterial agents whereas compound **2** and **3** are promising antioxidants which corroborates with the traditional uses of the roots of *H. johannis.*

**Dong Hwan Lee, Youngki Park, Jun Hyuk Jang, Yonghwan Son, Ji Ah Kim, Sun-Young Lee, Hyun-Jun Kim. The growth characteristics and lignans contents of *Schisandra chinensis* fruits from different cultivation regions. (2022) Appl. Biol. Chem. 65: 77**

We developed and validated an optimized method for quantifying lignans using ultra-performance liquid chromatography (UPLC) and performs correlation analysis of growth characteristics and contents of lignans. The methods for determining lignans were validated by measuring the linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision using UPLC. All calibration curves showed good linearity (*r*2 ≥ 0.9998) within the tested ranges. The limit of detection (LOD) and limit of quantification (LOQ) were 0.003–0.02 µg/mL and 0.01–0.07 µg/mL, respectively. The precision of analysis was less than 3%. The recoveries of quantified compounds ranged from 98.4 to 101.68%. Growth characteristics of fruits were negatively correlated with content of total marker compounds. The results of this study can be used to quality-control of *S. chinensis* fruits used as medicinal raw materials.

**Yongqiang Ma, Weiye Xiu, Xin Wang, Qiuhui Yang. Extraction of raspberry ketone from red raspberry and its intervention in the non-alcoholic fatty liver disease. (2022) Appl. Biol. Chem. 65: 78**

Nonalcoholic fatty liver disease (NAFLD) is characterized by diffused hepatocyte bullous fat in the liver, which is not caused by alcohol or drugs like amiodarone and tamoxifen. Presently, no drug is approved for NAFLD treatment. Therefore, it’s important to extract effective components from natural plants to alleviate NAFLD. In this study, we extracted and purified raspberry ketone, a natural phenolic compound from red raspberry (*Rubus ideaus* L.) by an ultrasonic-assisted ethanol extraction method. The structure of red raspberry ketone (RRK) was determined using Fourier-transform infrared spectroscopy and the purity of RRK was found as 80.06 ± 1.19%. After 28 days of intragastric administration of RRK, the bodyweight of NAFLD model rats decreased significantly (p < 0.05). Besides, the levels of low-density lipoprotein cholesterol, total cholesterol, and total triglyceride (TG) decreased and the content of high-density lipoprotein cholesterol in serum increased drastically. Moreover, the level of liver damage indicators (aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) and the levels of glucose, insulin, free-fatty acid, tumor necrotic factor-α in the liver decreased distinctly. The levels of TG and malondialdehyde in the liver decreased, whereas the levels of superoxide dismutase, total glutathione, and glutathione peroxidase drastically increased. We also found that RRK reduced the uneven size of liver cells and blurred boundaries of hepatic lobules, and alleviated hepatic steatosis and inflammation caused by NAFLD. We inferred that RRK could relieve NAFLD progression by regulating glucose and lipid metabolism and alleviating oxidative stress in vivo. This study sheds new light on the use of RRK as a functional food for NAFLD prevention.

**Nuri Oh, Jun Sung Seo, Pil Joong Chung, Jimin Lee, Jang-Kyun Seo, Jin-Ho Kang, Hye Sun Cho, Choonkyun Jung. Identification and characterization of drought-induced long noncoding RNAs (*DRIL*s) in rice. (2022) Appl. Biol. Chem. 65: 79**

Long noncoding RNAs (lncRNAs) act as transcriptional regulators in plants and animals. To date, they have been reported to regulate various biological processes, such as phosphate homeostasis, grain yield, and fertility in rice (*Oryza sativa* L.). However, the lncRNAs involved in abiotic stress responses remain poorly identified in rice. In this study, we analyzed the expression profiles of lncRNAs using public rice transcriptome datasets derived from abiotic stress-treated samples. We found that the expression of thousands of rice lncRNAs was significantly altered in the shoot and root tissues under different abiotic stresses (drought, high salinity, low temperature, and abscisic acid). We selected six novel drought-induced lncRNAs (*DRIL*s, specifically *DRIL1* to *DRIL6*) for further study. Real-time polymerase chain reaction analysis revealed the differential expression patterns of these *DRIL*s under various stress conditions. The expression of abiotic stress-responsive genes was upregulated in the protoplasts by transiently overexpressed *DRIL1* and *DRIL4*. Therefore, *DRIL*s may be involved in the transcriptional regulation of abiotic stress-responsive genes in rice.

**Jung Min Kim, Han Yong Lee, Jinho Jung. *Escherichia coli* methionine-tRNAi/methionyl tRNA synthetase pairs induced protein initiation of interest (PII) expression. (2022) Appl. Biol. Chem. 65: 80**

The precise regulatory role in protein synthesis by facilitating interactions with mRNA codons for various tRNA modifications is unclear. We previously reported that enhanced green fluorescent protein (GFP) reduced enhanced GFP mRNA expression in human methionine-conjugated initiator tRNA (tRNAi)/tRNA synthetase pairs under methionine-deficient conditions. Here, we investigated the effect of non-formylated methionine-conjugated *Escherichia coli* tRNAi on the synthesis of the protein initiation of interest (PII) in HeLa cells under intracellular L-methionine levels. We found that *E. coli* methionine-tRNAi counteracts human methionine-tRNAi, indicating that *E. coli* methionyl tRNA synthetase can induce enhanced GFP expression due to increased stability of enhanced GFP mRNA. Both complexes could support translation initiation without being employed to introduce methionine residues in the subsequent elongation steps. The results indicated that *E. coli* methionine-tRNAi could offset human methionine-tRNAi, and *E. coli* methionine-tRNAi/methionyl tRNA synthetase pairs can drive enhanced GFP mRNA expression. Unlike the human methionine-tRNAi/methionyl tRNA synthetase pairs that were used as a positive control, the non-formylated *E. coli* methionine-tRNAi/methionyl tRNA synthetase pairs reduced the expression of enhanced GFP mRNA, resulting in reduced HeLa cell survival. Using tRNAs functions causes of heterologous origin, such as from prokaryotes, and modified, to enhance or suppress the synthesis of specific proteins in eukaryotic organisms into the potential may possess a more prominent advantage of *E. coli* methionine-tRNAi as approaches that can control PII. This study provides new insights on the *E. coli* methionine- tRNAi/methionyl tRNA synthetase pair induced PII synthesis and the relative viability of cells could pave the way to regulate ecological/biological systems.

**Lijuan Hou, Wenwen Wang, Jianjun Zhai, Huafang Zhao. Circular RNA pyridoxal kinase (circPDXK) involves in the progression of ovarian cancer and glycolysis via regulating miR-654-3p and hexokinase II. (2022) Appl. Biol. Chem. 65: 81**

Background

Circular RNA pyridoxal kinase (circPDXK; hsa\_circ\_0061893) is newly identified to be aberrantly expressed in ovarian cancer (OVCA); however, its functional role in OVCA cells remains to be expounded.

Methods

Real-time quantitative polymerase chain reaction, western blotting, and immunohistochemistry quantified RNA and protein expression levels. MiRNA binding site prediction tools predicted direct interaction between two RNAs, and dual-luciferase reporter and RNA immunoprecipitation assays further confirmed that prediction. Cell-counting kit-8, colony formation, and 5-ethynyl-2ʹ-deoxyuridine assays measured cell growth; nude mice xenograft tumor experiment detected tumor growth. Transwell and Annexin V-fluorescein isothiocyanate/propidium iodide staining assays evaluated cell motility and apoptosis. Glycolysis process was determined by glucose uptake, lactate, and ATP assay kits.

Results

CircPDXK is highly expressed in OVCA patients’ tumor tissues and cells, concomitant with microRNA (miR)-654-3p downregulation and hexokinase II (HK2) upregulation. RNA interference of circPDXK could restrain cell viability, colony formation, DNA synthesis, migration, invasion, and glycolysis of OVCA cells, but also retard xenograft tumor growth. Allied with those are higher apoptosis rate, elevated Bax and E-cadherin levels, and depressed ki67 and HK2 levels. Compared to circPDXK inhibition, restoration of miR-654-3p functions analogical effects in OVCA cells in vitro. Mechanistically, there are direct interactions between miR-654-3p and circPDXK or HK2; moreover, miR-654-3p inhibition could weaken the functional roles of circPDXK interference in OVCA cells, and either HK2 ectopic expression abrogates the effects of miR-654-3p overexpression.

Conclusion

CircPDXK/miR-654-3p/HK2 axis could be a novel molecular mechanism of OVCA progression and glycolysis, and targeting circPDXK might overcome OVCA.

**Jin Hee Park, Gyung Min Park, Eun Jeong Kim, Yu Min Jeon. Monitoring of an electrically induced signal in melon in relation to different environmental conditions in a smart farm. (2022) Appl. Biol. Chem. 65: 82**

A smart farm that automatically regulates environmental conditions such as temperature, humidity and nutrient supply will maximize crop production per unit area by using ICT-based technology. To control the environment in a smart greenhouse, plant growth should be monitored in real time. The physiological activity of a plant was monitored by receiving an electrical signal from inside the plant stem which changed when the plant absorbed nutrients and water. In this study, the environmental conditions in different areas of a smart farm were not much different, and growth parameters were not much affected by the environmental conditions of the area. However, a plant induced electrical signal (PIES) was associated with the atmospheric and media temperature and relative humidity although there was time lag of 6 ~ 7 h for the peaks of the PIES and other environmental conditions. Therefore, monitoring a PIES will make it possible to improve the growth environments in smart farms.

**Toluwase Oreoluwa Adegoke, Tae-il Moon, Hyun-Hwoi Ku. Ammonia emission from sandy loam soil amended with manure compost and urea. (2022) Appl. Biol. Chem. 65: 83**

Urea is a renowned nitrogen (N) fertilizer widely used in plant N nutrition owing to its high N concentration (46%). The effect of the N application rate on ammonia (NH3) emission into the environment and soil properties is yet to be unraveled. The rate of NH3 emission from sandy loam soil amended with manure compost (T1: 5000 kg ha−1) and urea at different application rates (100, 200, and 400 kg N ha−1 designated as T2, T3, and T4, respectively) with 3 replications was evaluated in an incubation experiment. Ammonia emission was rapid at the early stage of the experiment followed by an equilibrium decrease that tends toward zero at the end of the incubation. Ammonia emissions of 16.50, 52.15, and 75% of applied N in T2, T3, and T4 were observed on day 49. Ammonia emission increased linearly (y = 0.7823x−32.169, R2 = 0.95\*\*) in response to N application rates. T1 conserves more NH4+ (20.79 mg kg−1) in the soil, followed by T2 (11.8 mg kg−1) and T3 (11.7 mg kg−1). T2 and T3 reduced the soil pH to optimal for microbial activities and nutrient availability, while T4 promoted soil acidity. A balanced combination of compost and urea mitigates NH3 volatilization and improves soil fertility. In conclusion, applying compost (5000 kg ha−1) with urea at 100 kg N ha−1 or 200 kg N ha−1 is recommended for NH3 volatilization mitigation, nitrification inhibition, and efficient NH4+ retention in the soil.

**Zhuang Yao, Hyeon Jeong Seong, Yu-Sin Jang. Degradation of low density polyethylene by *Bacillus* species. (2022) Appl. Biol. Chem. 65: 84**

Since its invention, polyethylene (PE) has brought many conveniences to human production and life. In recent years, however, environmental pollution and threats to human health caused by insufficient PE recycling have attracted widespread attention. Biodegradation is a potential solution for preventing PE pollution. In this study, *Bacillus subtilis* and *Bacillus licheniformis*, which are widespread in the environment, were examined for their PE degradation abilities. Biodegradation of low-density polyethylene (LDPE) was assessed by weight loss, Fourier transform infrared spectroscopy (FTIR), and high performance liquid chromatography (HPLC) analyses. Weight losses of 3.49% and 2.83% were observed for samples exposed to strains *B. subtilis* ATCC6051 and *B. licheniformis* ATCC14580 for 30 days. Optical microscopy revealed obvious structural changes, such as cracks, pits, and roughness, on the surfaces of the microorganism-exposed LDPE sheets. Oxidation of the LDPE sheet surfaces was also demonstrated by the FTIR-based observation of carbon-unsaturated, –OH, –NO, –C=C, and –C–O bonds. These results support the notion that *B. subtilis* ATCC6051 and *B. licheniformis* ATCC14580 can degrade PE and could potentially be used as PE-biodegrading microorganisms. Further research is needed to examine potential relevant degradation mechanisms, such as those involving key enzymes.

**Tae Jin Kim, Ye Jin Kim, Woo Duck Seo, Sang Un Park, Jae Kwang Kim. Improved quantification of catechin and epicatechin in red rice (*Oryza sativa* L.) using stable isotope dilution liquid chromatography-mass spectrometry. (2022) Appl. Biol. Chem. 65: 85**

Epimerization can change the catechin content and composition of samples during extraction and analytical analyses. To control the effect of epimerization, we developed a novel and stable isotope dilution liquid chromatography-mass spectrometry (LC–MS) method using catechin-2,3,4-13C3 and epicatechin-2,3,4-13C3 as stable-isotope-labeled internal standards (SIL-ISs). When the SIL-ISs were used, the catechin and epicatechin contents were stable (104–109% and 100–109% of the initial concentration, respectively) despite long storage times. In contrast, when L-2-chlorophenylalanine was used as an internal standard, catechin and epicatechin concentrations of 88–97% and 164–277% of the initial concentration, respectively, were obtained after long storage times. Furthermore, the least significant epimerization effect and highest extractability were observed when extraction was performed at 70 ℃ for 30 min. The recoveries for red rice using the developed isotope dilution LC–MS method at two different concentrations were between 100.72 and 118.67%, with relative standard deviations less than 3.67%.

**Minji Kim, Sangoh Kwon, Sueongmok Cho, Min Young Um. *Ishige foliacea* ameliorates depressive-like behaviors in stress hormone treated mice. (2022) Appl. Biol. Chem. 65: 86**

Chronic stress is an important factor to several physical and mental diseases. *Ishige foliacea*, an edible brown seaweed, has various biological properties; however, its role in alleviating stress-induced depression remains unclear. Thus, the antidepressant-like effects of *I. foliacea* ethanolic extract (IE) were investigated using corticosterone (CORT)-treated HT22 cells and CORT-induced depressive mice. IE significantly reduced the production of reactive oxygen species (ROS) and inhibited monoamine oxidase (MAO) activity and protein expression; furthermore, it up-regulated the extracellular signal-regulated kinase (ERK)**-**cAMP response element-binding protein (CREB)**-**brain derived neurotrophic factor (BDNF) signaling pathway. In the CORT-induced depressive mice, IE administration (500 mg/kg, bodyweight) exerted antidepressant effects according to behavioral tests. In addition, IE activated the ERK**-**CREB**-**BDNF signaling pathway owing to CORT exposure in the hippocampus. Therefore, IE inhibited ROS production and MAO activity and improved depressive behaviors caused by stress hormones, which demonstrates the therapeutic potential of IE in the treatment of depression.

**Yujin Kweon, Miao Fang, Sang-Yoon Shin, Dowhan Lee, Kook-Hyung Kim, Chanseok Shin. Sequence optimization and multiple gene-targeting improve the inhibitory efficacy of exogenous double-stranded RNA against pepper mottle virus in *Nicotiana benthamiana***

**. (2022) Appl. Biol. Chem. 65: 87**

Double-stranded RNA (dsRNA)-induced RNA interference is a promising agricultural technology for crop protection against various pathogens. Recent advances in this field have enhanced the overall efficiency with which this approach inhibits pathogenic viruses. Our previous study verified that treatment of *Nicotiana benthamiana* plants with dsRNAs targeting helper component-proteinase (*HC-Pro*) and nuclear inclusion b (*NIb*) genes protected the plant from pepper mottle virus (PepMoV) infection. The aim of this study was to improve the inhibitory efficacy of dsRNAs by optimizing the target sequences and their length and by targeting multiple genes via co-treatment of dsRNAs. Each of the two targeting dsRNAs were divided into three shorter compartments and we found that *HC-Pro*:mid-1st and *NIb*:mid-3rd showed significantly superior antiviral potency than the other fragments, including the parent dsRNA. In addition, we confirmed that the co-treatment of two dsRNAs targeting *HC-Pro* and *NIb* produced a greater inhibition of PepMoV replication than that obtained from individual dsRNA treatment. Complementing our previous study, this study will provide future directions for designing dsRNAs and enhancing their efficiency in dsRNA-mediated RNA interference technologies.

**Gaeun Kim, Heejae Jo, Hyung-Sub Kim, Minyoung Kwon, Yowhan Son. Earthworm effects on soil biogeochemistry in temperate forests focusing on stable isotope tracing: a review. (2022) Appl. Biol. Chem. 65: 88**

Earthworms (Oligochaeta) are globally distributed soil-dwelling invertebrates that alter soil properties through feeding, casting, and burrowing behaviors. Soil physicochemical modification, which may directly influence the availability and dynamics of organic and inorganic nutrients in the soil, such as carbon and nitrogen, includes soil texture, porosity, and pH. Temperate forests produce year-round plant litter, the primary food source for earthworms, and litter processed by earthworms significantly contributes to soil organic material storage. In recent decades, studies on temperate forest ecosystems have attempted to elucidate and quantify the earthworm impact on soil organic material dynamics, mainly targeting carbon and nitrogen, using isotope analysis methods. This paper summarizes studies on the following topics: (1) effect of earthworm modification on soil property to understand these alterations’ interaction with carbon and nitrogen dynamics, and (2) isotope tracing method, used to elucidate the earthworm effect on carbon and nitrogen transformation and movements in temperate forests. The particular emphasis on the isotope method is based on its capability of time-adjusted quantification of organic materials in the ecosystem compartments. Also, isotopic labeling in biomass has a broad range of applications, such as tracing assimilated food sources, identifying trophic interactions in soil food webs, and addressing material dynamics in complex linkages between earthworms and their environment. In addition, we provide perspectives on other methodologies, such as chronology and population ecology, as feasible options to further assist the isotope tracing of earthworms’ impact on soil nutrient dynamics.

**Yinglong Zhang, Bo Li, Guangliang Wu. Dandelion (*Taraxacum mongolicum* Hand.-Mazz.) suppresses the decrease in physical strength and exercise capacity caused by insufficient liver function. (2022) Appl. Biol. Chem. 65: 89**

In this study, D-galactose was used to establish a model of liver dysfunction caused by oxidative stress in mice, and the effect of dandelion on improving the exercise capacity of mice with liver dysfunction was observed and its mechanism was expounded. This study examined the role and mechanism of dandelion in improving running ability, swimming endurance, blood biochemical indices, histopathological changes, and tissue mRNA expression changes. The animal results showed that dandelion extended the running and swimming time to exhaustion in liver dysfunctional mice, reduced the serum levels of blood urea nitrogen (BUN), blood lactic acid (BLA) and malondialdehyde (MDA) in the liver, and increased hepatic glycogen (HG) and muscle glycogen (MG) levels as well as uperoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities. Histopathological observations suggested that dandelion alleviated lesions in the liver. The quantitative polymerase chain reaction (qPCR) analysis results showed that dandelion downregulated inducible nitric oxide synthase (iNOS) and tumor necrosis factor-alpha (TNF-α) mRNA expression and neuronal nitric oxide synthase (nNOS), copper/zinc-superoxide dismutase (Cu/Zn-SOD), manganese-superoxide dismutase (Mn-SOD), and catalase (CAT) expression in the liver and skeletal muscle of the liver-dysfunctional mice. In contrast, dandelion downregulated syncytin-1 mRNA expression in skeletal muscle of mice with a dysregulated liver. The positional analysis showed that the main components of dandelion were gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, p-coumaric acid, rutin, myricitrin, isoquercitrin, isochlorogenic acid A, and luteolin.

**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.