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**Jeongyeon Yoon, Miao Fang, Dowhan Lee, Minsu Park, Kook-Hyung Kim, Chanseok Shin. Double-stranded RNA confers resistance to pepper mottle virus in Nicotiana benthamiana. (2021) Appl. Biol. Chem. 64: 1**

RNA interference (RNAi) is a regulatory mechanism of gene expression mediated by small RNAs. By using the RNAi technique, exogenous double-stranded RNA (dsRNA) designed to target mRNA, suppresses target gene expression levels in plants. In this study, we adopted the RNAi mechanism as a tool to protect plants from viruses. We designed and synthesized several dsRNAs targeting the pepper mottle virus (PepMoV) genes HC-Pro and NIb. When used on *Nicotiana benthamiana* plants, these dsRNAs protected the plant against viral infection over a specific period. By optimizing dsRNA and virus injection time, the protection efficiency of dsRNA by targeting virus genes could be maximized. It seems that exogenous dsRNA-derived RNA-induced silencing complex was able to defend the host against viral infection instantly. Furthermore, each dsRNA designed to target different regions within a transcript had varying levels of effects on virus survival in the host plants. When targeting the middle part of both the NIb and HC-Pro genes using the dsRNAs, the highest viral growth inhibitory effect was observed. An RLM-5′ RACE was performed using plant leaves infected with PepMoV after dsRNA treatment and it was observed that most of the mRNA cleavages occurred close to the 3′ part within the dsRNA target position on the mRNA. These results suggest that the dsRNA tool can be used as a plant vaccine platform for crop protection.

**Seong Su Hong, Chun Whan Choi, Ji Eun Lee, Yeon Woo Jung, Jung A. Lee, Wonsik Jeong, Yun-Hyeok Choi, Hanna Cha, Eun-Kyung Ahn, Joa Sub Oh. Bioassay-guided isolation and identification of anti-obesity phytochemicals from fruits of Amomum tsao-ko. (2021) Appl. Biol. Chem. 64: 2**

*Amomum tsao-ko* (Zingiberaceae), an important traditional medicinal herb, possesses many biological activities, including anti-inflammatory effects. Though the anti-obesity properties of the crude ethanol extract of *A. tsao-ko* fruits have been reported, the anti-adipogenic properties of its phytochemical constituents have not been reported. Therefore, in the present study, we isolated the active constituents of *A. tsao-ko* and investigated their anti-adipogenic effects. The bioassay-guided isolation of the phytochemicals from the ethanol extract of *A. tsao-ko* fruits identified four bioactive compounds, comprising one fatty acid (**1**), one sesquiterpene alcohol (**2**), and two phenolic compounds (**3** and **4**). Their structures were elucidated by a combination of 1D and/or 2D nuclear magnetic resonance and mass spectrometry. The anti-adipogenic activities of the four compounds evaluated by Oil Red O staining in 3T3-L1 cells revealed that the treatment with the isolated compounds **1** and **3** reduced the lipid accumulation in 3T3-L1 adipocytes more strongly than the compounds **2** and **4**, in a dose-dependent manner.

**Hyeon Ji Yeo, Seung-A Baek, Ramaraj Sathasivam, Jae Kwang Kim, Sang Un Park. Metabolomic analysis reveals the interaction of primary and secondary metabolism in white, pale green, and green pak choi (Brassica rapa subsp. chinensis). (2021) Appl. Biol. Chem. 64: 3**

This study aimed to comprehensively analyze primary and secondary metabolites of three different-colored (white, pale green, and green) pak choi cultivars (*Brassica rapa* subsp. *chinensis*) using gas chromatography attached with time-of-flight mass spectrometry (GC-TOFMS) and high-performance liquid chromatography (HPLC). In total, 53 primary metabolites were identified and subjected to partial least-squares discriminant analysis. The result revealed a significant difference in the primary and secondary metabolites between the three pak choi cultivars. In addition, 49 hydrophilic metabolites were detected in different cultivars. Total phenolic and glucosinolate contents were highest in the pale green and green cultivars, respectively, whereas total carotenoid and chlorophyll contents were highest in the white cultivar. Superoxide dismutase activity, 2,2-diphenyl-1-picrylhydraz scavenging, and reducing power were slightly increased in the white, pale green, and green cultivars, respectively. In addition, a negative correlation between pigments and phenylpropanoids was discovered by metabolite correlation analysis. This approach will provide useful information for the development of strategies to enhance the biosynthesis of phenolics, glucosinolates, carotenoids, and chlorophyll, and to improve antioxidant activity in pak choi cultivars. In addition, this study supports the use of HPLC and GC-TOFMS-based metabolite profiling to explore differences in pak choi cultivars.

**Jung-Hae Shin, Muhammad Irfan, Man Hee Rhee, Hyuk-Woo Kwon. Antiplatelet effect of cudraxanthone B is related to inhibition of calcium mobilization, αIIbβ3 activation, and clot retraction. (2021) Appl. Biol. Chem. 64: 4**

*Cudrania tricuspidata* (*C. tricuspidata*) is widespread throughout Asia and has known to have various physiological activities such as, inflammation, diabetes, obesity and tumor. *Cudrania tricuspidata*, a rich source of xanthones and flavonoids, have been investigated phytochemically and biologically. However, research of these compounds on platelets is limited. Therefore, we searched for a new substance from various xanthones and flavonoids in *C. tricuspidata*. We confirmed the results that steppogenin and isoderrone suppress human platelets among the various components isolated from *C. tricuspidata*, and as a result of analyzing the antiplatelet effect using additional new samples, we found that cudraxanthone B (CXB) has the effect of suppressing human platelets. Therefore, we studied the potential efficacies of CXB on human platelet aggregation and its inhibitory mechanism. Inhibitory effects of CXB on platelet aggregation were assessed using washed platelets, followed by measurement of [Ca2+]i mobilization and dense granule release, fibrinogen binding, fibronectin adhesion assay, and clot retraction. Our data showed that CXB suppressed collagen-induced human platelet aggregation, [Ca2+]i mobilization, fibrinogen binding, fibronectin adhesion and clot retraction without cytotoxicity. Thus, our results show that inhibitory effects of CXB on human platelet activation and thrombus formation, suggesting its potential use as a natural substance for preventing platelet-induced thrombosis.

**Yeonjong Koo, R. Scott Poethig. Expression pattern analysis of three R2R3-MYB transcription factors for the production of anthocyanin in different vegetative stages of Arabidopsis leaves**

**. (2021) Appl. Biol. Chem. 64: 5**

Anthocyanin is a type of flavonoid that appears purple in plants. PAP1, PAP2, and MYB113 are the three major R2R3-MYB transcription factors that regulate flavonoid biosynthesis in *Arabidopsis thaliana*. In this study, we found that the three MYB genes regulate anthocyanin accumulation in different leaf stages. Under limited nutrient conditions, PAP1 and PAP2 genes were highly induced in juvenile leaves. Conversely, MYB113 was expressed mainly in adult leaves. In addition, we investigated the role of trans-acting siRNA4 (TAS4) in the post-transcriptional regulation of anthocyanin expression in Arabidopsis leaves. In plant growth, the inhibition of PAP1 and PAP2 gene expression by TAS4 was observed only in juvenile leaves, and MYB113 inhibition was observed in adult leaves. In conclusion, we found that transcription and transcript repression of the three MYB genes is differentially regulated by TAS4 in leaf developmental stages. Our results improve the understanding of the regulation of plant anthocyanin production under stress conditions.

**Hyojik Jung, Inhwan Kim, Sunghyeon Jung, Jihyun Lee. Oxidative stability of chia seed oil and flax seed oil and impact of rosemary (*Rosmarinus officinalis* L.) and garlic (*Allium cepa* L.) extracts on the prevention of lipid oxidation. (2021) Appl. Biol. Chem. 64: 6**

Chia seed and flax seed oils are rich in polyunsaturated fatty acids, but are susceptible to oxidative deterioration. The aim of this study was to determine the oxidative stability of chia seed and flax seed oils and enhance the stability using rosemary or garlic extracts. During accelerated storage at 65 °C for 14 days, the antioxidant abilities of rosemary or garlic extracts were evaluated and compared with those of butylated hydroxy toluene, ascorbyl palmitate, and α-tocopherol using peroxide value, conjugated dienoic acids, free fatty acid, thiobarbituric acid value analysis. The profile of volatiles, fatty acid composition, and the tocopherol contents in the treated and/or untreated oils were also determined. Active ingredients of rosemary and garlic extracts were also determined. Rosemary extract was found to provide higher oxidative stability than garlic extract after 14 days in most assays (e.g., the CDA values of 4.8% for rosemary extract and 5.2% for garlic extract in chia seed oil). The contents of γ-tocopherol, linoleic acid, and α-linolenic acid were well retained in the functional oils treated with the two extracts. After accelerated storage, the content of the major odor-active volatiles varied based on the type of oil. Our findings show the potential of natural aromatic plant extracts with respect to improving the oxidative stability of functional oils.

**Vineet Singh, Karthika Muthuramalingam, Young Mee Kim, Sanggyu Park, Sung Hong Kim, Jongdae Lee, Changlim Hyun, Tatsuya Unno, Moonjae Cho. Synbiotic supplementation with prebiotic *Schizophyllum commune* derived β-(1,3/1,6)-glucan and probiotic concoction benefits gut microbiota and its associated metabolic activities. (2021) Appl. Biol. Chem. 64: 7**

Synbiotics synergistically favors beneficial effects of prebiotics and probiotics towards host metabolic health by modulating gut ecosystem. In this study, we sought to examine the effects of prebiotics (*Schizophyllum commune* derived β-(1,3/1,6)-glucan), probiotics (concoction made of eight different bacterial strains) and synbiotics (prebiotics + probiotics) on gut microbiota and its associated metabolic functions through 16S rRNA gene sequences analysis. Results showed that probiotics strains used in this study were detected more in the synbiotic and probiotic treatments, while prebiotic dietary intervention increased the total bacterial abundance and metabolisms related to host immune strengthening. Probiotics and synbiotics dietary interventions enhanced similar metabolisms relating to butanediol and *s*-adenosyl-L-methionine biosynthesis. Probiotics treatment also showed depleted metabolic activities related to SCFA productions, that were not depleted in prebiotics treatment. With varying differential abundance patterns and metabolic activities across the treatments, our results suggest that synbiotic treatment provide more beneficial effects over probiotics and prebiotics.

**Adewale Adewuyi, Chiagoziem A. Otuechere, Nkechi H. Ellah, Shiva Shanker Kaki, Scott O. Fayemi, Charles B. Adeosun. Evaluation of the hepato-renal functions and antimicrobial activity of fatty amido benzoic acid synthesised from *Citrullus colocynthis* seed oil. (2021) Appl. Biol. Chem. 64: 8**

Rapid progression in resistance to antimicrobial agents by pathogenic organisms is a serious concern. This study aimed to synthesize fatty amido benzoic acid (FBA) from *Citrullus colocynthis* seed oil (CCO) and evaluate its safety profile as an alternative bioactive agent for combating drug-resistant pathogens. FBA was synthesised through simple chemical reaction route and examined for its antioxidant activity and antimicrobial capacity against selected drug-resistant microorganisms. Effect of FBA on hepato-renal function makers and oxidative stress was also examined using Wistar rats. Density functional theory (DFT) approach was employed to understand the action of FBA with the aid of lowest unoccupied molecular orbital (LUMO) and highest occupied molecular orbital (HOMO). Gas Chromatography (GC) revealed the most abundant fatty acid in CCO to be C18:2 (55.88%). Results from Fourier transformed infrared spectroscopy (FTIR), and proton nuclear magnetic resonance (1HNMR) confirmed the synthesis of FBA with a yield of 97.10%. FBA exhibited antioxidant potential (IC50 of 1.96 µg mL−1) as well as antimicrobial potency. Minimum inhibitory concentration (MIC) of FBA was 0.026 mg mL−1. Biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, hydrogen peroxide, and lipid peroxidation were significantly elevated in rats administered high dose FBA (100 mg kg−1). Histology of the liver and kidney confirmed the biochemical results. Furthermore, mechanism of action of FBA could be described by quantum chemical analysis to be via nucleophilic interaction, which may be viewed electronically as donor–acceptor interaction. The study presents FBA as a promising antimicrobial agent for combating drug-resistant pathogenic organisms.

**Chengfu Li, Wei Yan, Enji Cui, Changji Zheng. Anti-bacterial effect of phytoconstituents isolated from *Alimatis rhizoma*. (2021) Appl. Biol. Chem. 64: 9**

Five compounds including three triterpenoids and two sesquiterpenes were isolated from *Alimatis rhizoma*. Their chemical structures were determined to be alisol B 23-acetate (**1**), alisol C 23-acetate (**2**), alisol B (**3**), alismol (**4**) and alismoxide (**5**) by various spectroscopic analysis, including 1H-NMR, 13C-NMR, HMBC and MS spectra. Compounds **1**–**5** were evaluated for their antibacterial potential against 6 strains of bacteria including three drug-resistant bacteria (one methicillin-resistant *Staphylococcus aureus* strain CCARM 3506, two quinolone-resistant *Staphylococcus aureus* strains CCARM 3505 and CCARM 3519), two G+ bacteria (*Streptococcus mutans* KCTC 3289 and *Staphylococcus aureus* KCTC 209) and one G− bacterium (*Escherichia coli* KCTC 1924). Compounds **1**–**5** showed strong antibacterial effect against *S. mutans* KCTC 3289, their MIC values were 2, 64, 16, 32 and 32 μg/mL, respectively. The antibacterial activity results of compounds **1**–**5** against these bacteria were reported for the first time. The results indicate that *Alimatis rhizoma* are potential sources of new antibacterial material.

**Jun-Hyuk Yoo, Deogratius Luyima, Jae-Han Lee, Seong-Yong Park, Jun-Woo Yang, Ji-Young An, Yeo-Uk Yun, Taek-Keun Oh. Effects of brewer’s spent grain biochar on the growth and quality of leaf lettuce (*Lactuca sativa* L. var. crispa.). (2021) Appl. Biol. Chem. 64: 10**

The need for organic soil amendments is increasing in the Republic of Korea against the backdrop of increased soil acidification and nutrient losses. The pyrolysis of biomass produces biochar which not only increases soil productivity but also provides environmental benefits through carbon sequestration. The portion of the brewer’s spent grain (BSG) recycled is by far less than the amount generated, but pyrolysis can help to reverse this trend by turning BSG waste into a valuable soil amendment. The current study, therefore, evaluated the effects of brewer’s spent grain biochar (BBXXX) produced at three different temperatures of 300 °C, 500 °C and 700 °C on the yield and quality characteristics of the leaf lettuce as well as the effects on soil chemical properties through a pot experiment. Each of the BBXXX and BSG were added to the soil at two rates of 2% and 5% by weight. The pH and carbon content of the BBxxx increased with increasing pyrolysis temperatures and the trend was replicated in the soil upon biochar application i.e. the soil pH and carbon content increased alongside temperatures at which biochar was pyrolyzed. On the other hand, however, the soil electrical conductivity (EC) diminished with the increasing pyrolysis temperatures of the biochar applied. With regards to crop growth, the BB500 5% amendment produced the highest marketable yield of the leaf lettuce and while the lettuce grown on the control produced leaf lettuce with the lowest content of nitrate nitrogen, BB500 5% amendment generally produced the highest quality lettuce. The results indicate that BB500 performed agronomically better than the rest of the amendments and is thus recommended as an effective BSG recycling measure.

**Rui Wang, Yu-ming You, Xiong Liu. Effect of *Zanthoxylum* alkylamides on glucose metabolism in streptozotocin-induced diabetic Sprague–Dawley rats. (2021) Appl. Biol. Chem. 64: 11**

This research aimed at investigating the hypoglycemic effect of *Zanthoxylum* alkylamides and whether TRPV1 receptor could participate in the glucose metabolism by using streptozotocin-induced diabetic rat model. The results showed that the blood glucose measured in the *Zanthoxylum* alkylamides treated group (ALK) showed significantly lower values than that in the model group (Model). Significant improvements in the oral glucose tolerance as well as plasma insulin and hepatic glycogen were also observed in the ALK group, when compared to the model group. However, the improving effects of *Zanthoxylum* alkylamides on glucose metabolism disorder in diabetic rats were markedly inhibited by capsazepine as the TRPV1 receptor antagonist. In addition, there were significant differences in the levels of mRNA and protein of phosphoenolpyruvate carboxylase (PEPCK), gucose-6-phosphatase (G6Pase), glucokinase (GK) and cannabinoid receptor l (CB1) in the livers of the ALK group compared to model group. Meanwhile, ALK group also exhibited a remarkable increase in the pancreatic-duodenal homeobox 1 (PDX-1), glucose transporter 2 (GLUT 2), GK levels and a significant decrease in the expression levels of CB1 in the pancreas, while the presence of capsazepine would affected the expression of these genes. These findings indicate that *Zanthoxylum* alkylamides could ameliorate the glucose metabolism disorder in diabetic rats. Furthermore, the TRPV1 receptor could participate in regulating the expressions of genes and proteins related to glucose metabolism and insulin secretion in the liver and pancreas, and takes a role in the hypoglycemic process of *Zanthoxylum* alkylamides.

**Wanggang Xu, Yingmin Kuang, Dan Wang, Zhen Li, Renpin Xia. Irigenin exerts anticancer effects on human liver cancer cells via induction of mitochondrial apoptosis and cell cycle arrest. (2021) Appl. Biol. Chem. 64: 12**

Irigenin has been reported to exhibit remarkable anticancer effects against several human cancers. Nonetheless, the anticancer effects of irigenin against the human liver cancer cells are still largely unexplored. Consistently, this study was designed to evaluate the anticancer effects of irigenin against human liver cancer cells and to unveil the underlying molecular mechanisms. The results showed that irigenin significantly (*p* < 0.05) inhibited the growth of the human HepG2 and SNU-182 liver cancer cells with an IC50 value of 14 µM. Nonetheless, the cytotoxic effects of irigenin against the normal THLE-2 cells were comparatively lower as evident from the IC50 of 120 μM. The AO/EB and DAPI staining showed that irigenin induces apoptosis in the human liver cancer cells. Annexin V/PI staining assay revealed a significant (*p* < 0.05) increase in the percentage of apoptotic HepG2 and SNU-182 liver cancer cells upon treatment with irigenin. It was found that the number of apoptotic HepG2 and SNU-182 cells enhanced from 2.3 to 41.75% and 1.16 to 51.9% at IC50, respectively. Western blot showed a considerable increase in Bax and decrease in the Bcl-2 expression upon irigenin treatment further confirming the induction of apoptosis. Flow cytometric analysis revealed that irigenin also induces G2/M cell cycle arrest of HepG2 and SNU-182 cells. The percentage of G2/M phase HepG2 and SNU-182 cells increased from 17.92 to 34.35% and 23.97 to 38.23% at IC50, respectively This was also accompanied by decrease in the expression of CDK1 and Cyclin-B in HepG2 and SNU-182 cells. Taken together, the results of the present study suggest that irigenin inhibits the growth of the human liver cancer cells via induction of apoptosis and cell cycle arrest. These results point towards the potential of irigenin as a lead for the development of chemotherapy for liver cancer.

**Junghak Lee, Heeju Han, Xiu Yuan, Eunyoung Park, Jonghwa Lee, Jeong-Han Kim. A rapid, simultaneous and quantitative analysis of 26 ginsenosides in white and red *Panax ginseng* using LC–MS/MS. (2021) Appl. Biol. Chem. 64: 13**

A quantitative analysis of ginsenoside is very important for ginseng studies because each ginsenoside shows different medical activity and metabolic pathway. In this study, a rapid, simultaneous, and quantitative analysis of 26 ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2(R), Rg2(S), Rg3(S), Rg3(R), Rg5, Rg6, Rh1(R), Rh1(S), Rh2(R), Rh2(S), F1, F2, F3, F4, K, Mc, PPT(S), XVII, and Y) in white, and red *Panax ginseng* was established using multiple reaction monitoring (MRM) mode on ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS). The mobile phase of water and methanol containing 0.1% formic acid and HSS T3 C18 analytical column was used for the chromatographic separation. The four sets of stereoisomers were successfully separated within a 26-min run time, eluting the S-isomer faster than the R-isomer with higher concentration. The ginseng extract was diluted by 100, 400 and 8000 times to fit in the calibration range and quantitated by the standard addition method. Matrix matched calibration by mixing 64 µL of the ginseng extract with 16 µL of the standard solution was used for compensating the matrix effect. Such quantitation methodology using dilution, standard addition and matrix matching resulted in precise and unambiguous quantitation of 26 ginsenosides in ginseng products. Major ginsenosides were observed at relatively higher concentrations in red *Panax ginseng* and the Mc was detected and quantitated for the first time in this study. The comprehensive quantitation system established in this study will contribute to quality evaluation, breeding and culturing, and quantitative metabolomics study of ginseng.

**Yeong-Ju Park, Unsik Hwang, Suyeon Park, Sol Sim, Soyeon Jeong, Misun Park, Minji Kang, Youngsoo Lee, Youngju Song, Hoon Park, Hee-Jae Suh. Optimal bioconversion for compound K production from red ginseng root (C.A. Mayer) by sequential enzymatic hydrolysis and its characteristics. (2021) Appl. Biol. Chem. 64: 14**

Compound K (CK; 20-*O*-β-(D-glucopyranosyl)-20(S)-protopanaxadiol) is one of the metabolites of ginsenosides contained in red ginseng (RG) and is known to have high bioavailability. This study aimed to establish the optimal conditions for enzyme treatment to convert ginsenosides from RG extract to CK, and to prove the characteristics of bioconverted red ginseng (BRG) extract. CK was not detected in unenzyme-treated RG extract, and in the single-step enzyme treatment, it was produced at less than 4.58 mg/g only in treatment group with Pyr-flo or Sumizyme AC (at 50 °C for 48 h). The highest yield of CK (14.32 mg/g) was obtained by Ultimase MFC treatment at 50 °C for 48 h after treatment with a mixture of Pyr-flo and Rapidase at 50 °C for 24 h. Total polyphenol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) (ABTS) radical scavenging activity were higher in BRG than in RG (*p*< 0.5). High-fat diet (HD) rat fed 1% BRG had significantly lower body weight, heart weight, fat pads (periosteal fat, epididymal fat), serum glucose levels, and hepatic triglyceride levels than those HD rat fed 1% RG (*p*< 0.05). In conclusion, the sequential enzymatic bioconversion was produces higher CK in RG root extract than single-step enzyme treatment.

**Bowei Yu, Guozhi Fan, Sijiu Zhao, Yuchan Lu, Qiao He, Qunpeng Cheng, Juntao Yan, Bo Chai, Guangsen Song. Simultaneous isolation of cellulose and lignin from wheat straw and catalytic conversion to valuable chemical products. (2021) Appl. Biol. Chem. 64: 15**

Convertible cellulose and lignin were simultaneously isolated from wheat straw using a two-stage process via simply varying temperature and H2SO4 concentration. At the first-stage, cellulose was obtained by pretreating wheat straw at lower temperature and acid concentration using an organosolv process. The purity, yield and recovery rate of cellulose reached 86.8 wt%, 55.2 and 92.8% at 150 °C with 1 wt% H2SO4. At the second stage, the residual liquid was further treated at higher temperature and acid concentration, giving 17.4% lignin yield with 86.6% recovery rate and 93.2 wt% purity at 180 °C with 1.5 wt% H2SO4. The conversion of the as-isolated cellulose and lignin into chemicals was further investigated. The total yield of 5-hydroxymethylfurfural and glucose derived from wheat straw cellulose reached 82.5%, and 18.3% yield of monophenolic compounds from lignin were obtained, respectively. These results indicated that the two-stage process was effective for obtaining high-quality cellulose and lignin from wheat straw. Both of them displayed excellent convertible property.

**Dorsilla Anono Katimbwa, Jinsung Ma, Chang-Kil Kim, Dongyup Hahn, Jinkyu Lim. Potato extract inhibits lipase activity and ameliorates gut microbiome dysbiosis and weight gain in mice fed a high-fat diet. (2021) Appl. Biol. Chem. 64: 17**

Curtailing the absorption of triglycerides (TGs) is a preferred pathway for treating obesity. Our previous study demonstrated that the water-soluble fraction from potato could inhibit the lipase activity of patatin, one of the major proteins in potato. This aqueous fraction was purified and concentrated by deproteination and reversed-phase chromatography to investigate the effectiveness against obesity. Biochemical analyses indicated that the fraction non-competitively inhibited pancreatic lipase (PLase) with a half-maximal inhibitory concentration of 10.17 µg/mL, and was named as potato-derived lipase inhibitory fraction (PI). Animal studies on C57BL/6 mice showed that in mice fed a high-fat diet (HFD), PI treatment resulted in reductions in body weight gain, adipose fat deposition, and liver TGs, and ameliorated the gut microbiome dysbiosis caused by HFD feeding; meanwhile, orlistat, a well-known lipase inhibitor, diverged the gut microbiome profile in mice fed a HFD. High resolution electronspray ionization-Orbitrap tandem mass spectrometry identified gallic acid, 4-hydroxybenzoic acid, and protocatechuic acid, which are known to have lipase inhibitory activities, in PI. However, these compounds could not reconstitute comparable specific inhibitory activity of PI inferring the existence of another inhibitory compound(s) to be identified in PI.

**Sung Un Kim, Hyun Ho Lee, Sung Min Moon, Hae Ri Han, Chang Oh Hong. Nitrous oxide emissions and maize yield as influenced by nitrogen fertilization and tillage operations in upland soil. (2021) Appl. Biol. Chem. 64: 18**

Previous studies simply focused on determining nitrous oxide (N2O) emissions from the soil under different tillage operations and nitrogen (N) fertilizations without considering crop yield. Therefore, the objective of this study was to determine the effects of different tillage operations and N fertilizations on N2O emissions and crop yield from upland soil. Two different tillage operations [conventional tillage (CT) and no-tillage (NT)] and N fertilizations [without urea (WOU) and with 186 kg N ha−1 of urea (WU)] were established in a randomized block design with three replications on upland soil. Maize (*Zea mays*) was cultivated from 6th July to 4th October, 2018 (year 1), and from 15th April to 26th July, 2019 (year 2). The daily N2O flux did not peak soon after tillage operation and N fertilization, but it was more related to the change in water-filled pore space (WFPS). The mean value of WFPS across N fertilizations and seasons (years) was higher in CT than in NT. The changes of nitrification and denitrification rates could be attributed to the differences in WFPS between CT and NT. Nitrification was the predominant process producing N2O with CT, but denitrification was with NT. The application of urea increased cumulative N2O emissions, while CT also increased it compared with NT. The order of the mean values of cumulative N2O emissions across seasons from the highest to the lowest was as follows: CT + WU (7.12 kg N2O ha−1 year−1) > NT + WU (5.69 kg N2O ha−1 year−1) ≥ CT + WOU (5.02 kg N2O ha−1 year−1) > NT + WOU (4.24 kg N2O ha−1 year−1). Tillage operation did not affect the grain yield of maize or yield-scaled N2O emissions (YSNE). However, the application of urea increased the grain yield of maize and decreased YSNE, implying it could reduce N2O emission per unit of maize grain production. No-tillage management did not decrease YSNE value compared to CT operation, but N fertilization significantly decreased YSNE in the current study.

**Truc Trung Nguyen, Apiradee Uthairatanakij, Varit Srilaong, Natta Laohakunjit, Masaya Kato, Pongphen Jitareerat. Impact of electron beam irradiation on the chlorophyll degradation and antioxidant capacity of mango fruit. (2021) Appl. Biol. Chem. 64: 19**

At the present, the mechanism of chlorophyll degradation in response to ionizing irradiation in harvested fruits have not been examined. To understand the effect of electron beam (E-beam) irradiation on the chlorophyll degrading pathway in relation to chlorophyll degrading enzymes activity, reactive oxygen species (ROS) and antioxidant capacities of harvested mangoes stored at 13 °C for 16 days were studied. E-beam-treated fruit significantly suppressed the activities of chlorophyll degrading enzymes especially pheophytinase (PPH) and chlorophyll degrading peroxidase (Chl-POX) in the late stage of storage. This resulted in the chlorophyll content being maintained. However, E-beam irradiation did not affect the activities of chlorophyllase (Chlase) and magnesium de-chelatase (MD). The respiration rate, ethylene production, ROS accumulation (hydrogen peroxide [H2O2] and superoxide radical [O−**.**2]) immediately increased after E-beam treatment, following which they significantly decreased in comparison to the control. E-beam treatment enhanced the fruit’s antioxidant capacity by activating the activities of catalase (CAT) and ascorbate peroxidase (APX) and glutathione (GSH) content, and inactivated the activity of superoxide dismutase (SOD). Further, it did not affect the activity of glutathione reductase (GR) and glutathione disulfide (GSSG), vitamin C content, or total phenolic content. These results imply that E-beam treatment has the potential to delay chlorophyll degradation by suppressing the Chl-POX and PPH activities as well as reduce ROS production via CAT, APX, and SOD activities and GSH content.

**Jin Yeong Park, Jeong-Hyeon Lim, Joong-Hoon Ahn, Bong-Gyu Kim. Biosynthesis of resveratrol using metabolically engineered *Escherichia coli*. (2021) Appl. Biol. Chem. 64: 20**

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a phenolic compound widely used in pharmaceutics and nutraceutics. Although resveratrol is produced by some plant species, including grapes, peanuts, and berries, the content of resveratrol and its derivatives are very low. Therefore, an alternative biosynthetic method using microorganisms, such as *Escherichia coli*, has been developed over the past two decades. In the present study, a resveratrol-over-producing *E. coli* strain was developed using three strategies. First, we increased the synthesis of *p*-coumaric acid, a precursor of resveratrol, by manipulating genes in the shikimate pathway of *E. coli*. Second, three genes involved in resveratrol biosynthesis, such as tyrosine ammonia lyase (*TAL*), 4-coumaroyl CoA ligase (*4CL*), and stilbene synthase (*STS*), were cloned from diverse sources, such as plants and microorganisms, and the best combination was selected to maximize resveratrol production in *E. coli*. Finally, culture conditions, such as cell concentration, culture temperature, and carbon sources, were established for optimal resveratrol production. Through these strategies, approximately 80.4 mg/L of resveratrol was biosynthesized after 48 h of culture using glycerol as a carbon source.

**Soon Young Shin, Junho Lee, Seunghyun Ahn, Miri Yoo, Young Han Lee, Dongsoo Koh, Yoongho Lim. Design, synthesis, and evaluation of 4-chromenone derivatives combined with *N*-acylhydrazone for aurora kinase A inhibitor. (2021) Appl. Biol. Chem. 64: 21**

There is accumulating evidence that compounds containing *N*-acylhydrazone or 4-chromenone moieties can be active against multiple cancer cell types, yet the combined effect of these chemical groups is unclear. This study aimed to develop more effective anti-cancer compounds by combining 4-chromenone and *N*-acylhydrazone. Thirteen derivatives were designed, synthesized, and characterized, and their structures were identified using nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry. Most of the derivatives exhibited moderate to high efficacy in inhibiting the clonogenicity of HCT116 colon cancer cells. In particular, derivative **12**, (*E*)-*N*'-((6-methoxy-4-oxo-4*H*-chromen-3-yl)methylene)isonicotinohydrazide, strongly inhibited clonogenicity (GI50 = 34.8 μM) of HCT116 cells and aurora kinase A (aurA) activity in vitro (IC50 = 1.4 μM). In silico docking experiment predicted that derivative **12** interacts with aurA based on computational docking and calculations of binding free energy. When derivative **12** was exposed to HCT116 cells, the phosphorylation of aurA at Thr288 was dose-dependently decreased within 60 min. Further analysis showed that derivative **12** destroyed the mitotic spindle in HCT116 cells. These results suggest that derivatives of 4-chromenone combined with *N*-acylhydrazone are feasible in the development of selective aurA inhibitor and could be considered potential chemotherapeutic agents.

**Il Kyu Cho, Jae Ung Seol, Md. Musfiqur Rahman, Dong-Gi Lee, Hyeongkyu Son, Hoon Cho. Laboratory studies of the algaecide GreenTD: stability, algaecidal activity and reduction of microcystin production. (2021) Appl. Biol. Chem. 64: 22**

GreenTD (Dichlorobenzyl derivative, MW 362.17) is a new dichlorobenzyl derivative algaecide. It is effective and selective against harmful algal blooms (HABs). HABs cause serious problems for public health and fishery industries. Algae that cause HABs include *Microcystis* spp., *Anabaena* spp., and *Aphanizomenon* spp. Blooms of toxin-producing *Microcystis aeruginosa* occur regularly in fresh water where is rich in nitrogen and phosphorus nutrients. Environmental fate studies are needed to investigate the degradation of GreenTD. In the present study, we studied the persistence of GreenTD (90% aqueous solution) in water and toxic effects GreenTD on *M. aeruginosa* and reduction of microcystin production in the culture media. GreenTD was added in the water pots and microcystis media tubes at levels of 25 g/0.1 ha (0.5 mg/kg) and 50 g/0.1 ha (1.0 mg/kg). Samples were collected after 1, 3, 5, 7 and 14 days. The residues of GreenTD and microcystins in water and the media were determined using high performance liquid chromatography-diode array detection and ultra-performance liquid chromatography-tandem mass spectrometry, respectively. The half-life of GreenTD at concentrations of 0.5 and 1.0 mg/kg was 4.5 and 3.6 days, respectively. This result presents a safety level suitable for the acceptable guideline of water residue. The average recoveries of microcystins RR, YR, LR, and LA were 106–115%, 103–110%, 96–105% and 89–113% in the microcystis media, respectively. The limit of detection of (LOD) the microcystins was 0.1 µg/kg. No microcystins in the media were detected at the LOD (0.1 µg/kg). GreenTD at concentrations of 0.2 and 0.5 µg/kg had a 100% of control efficacy of *M. aeruginosa*. No growth of the blue-green algae was observed after 14 days of GreenTD application.

**Byoung Hee Park, Oh Wook Kwon, In Sung Kim, Hae Min Lee, Yeon Jin Roh, Minseo Kim, Youngho Jo, Hwayeon Cho, Jung Kuk Park, Zheng Zhi, Byung Cheon Lee. *Physalis alkekengi* L. var. francheti alleviates neuronal cell death caused by activated microglia in vitro. (2021) Appl. Biol. Chem. 64: 23**

Microglia are the macrophages that reside in the brain. Activated microglia induces further activation of astrocytes and neuronal cells for mounting an immune response. However, activated microglia release neurotoxic mediators causing neuroinflammation, which is associated with chronic etiology of neurodegenerative diseases. We investigated the effect of ethanol extract of *Physalis alkekengi* L. var. francheti fruit (PAFE) on neuronal cell death mediated by activated microglia. PAFE decreased NO production and IL-6 secretion in LPS-stimulated BV-2 and primary microglial cells without reducing cell viability. Consistently, treatment with PAFE decreased iNOS and COX-2 expression and ERK phosphorylation in LPS-stimulated BV-2 cells. Finally, apoptosis of N2a cells grown in conditioned media prepared from LPS-stimulated BV-2 cells containing PAFE was inhibited via downregulation of the Bax/Bcl-2 ratio. Taken together, PAFE alleviates neuronal cell death by reducing neurotoxic mediators such as NO and IL-6 from activated microglia. Therefore, it could be a potential candidate to treat neurodegenerative diseases caused by chronic neuroinflammation.

**Eun-Ho Lee, Young-Je Cho. Responses of inflammation signaling pathway by saucerneol D from elicitor-treated *Saururus chinensis* on pro-inflammatory responses in LPS-stimulated Raw 264.7 cell. (2021) Appl. Biol. Chem. 64: 24**

This study confirmed the association with inflammation-related proteins, mediators, and cytokines using saucerneol D from *Saururus chinensis* leaf, a useful ingredient increased through elicitor treatment. To confirm the anti-inflammatory effect, saucerneol D were treated with lipopolysaccharide, which induces pro-inflammatory factors in Raw 264.7 cell. The pro-inflammatory influences were measured by dint of chemical assay and western blotting as well as ELISA. As a result, the content of saucerneol D was changed when eicitor was treated by various concentration (1.5, and 3 mg/mL) in *S. chinensis* leaves. In addition, the expression levels of hyaluronidase and pro-inflammatory-related factors [nitric oxide (NO), inducible NO synthase (iNOS), and cyclooxygenase-2 (COX-2)] were regulated according to the saucerneol D content in the elicitor-treated and non-treated groups. Therefore, after confirming that saucerneol D has an inhibitory effect on pro-inflammatory-related factors, saucerneol D was adjusted by concentration and compared with the control substance to verify the efficacy. Saucerneol D was adjusted to a concentration that did not toxic to macrophages through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Saucerneol D controlled at various concentrations inhibited iNOS and COX-2 proteins. NO produced by iNOS activity, prostaglandin E2 (PGE2), an inflammatory mediator produced by COX-2 activity, and pro-inflammatory cytokines [interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α)] were significantly suppressed. Therefore, it was confirmed that saucerneol D, an active ingredient increased by the elicitor treatment, could be used as a functional material that controls inflammatory factors.

**Minjoo Kim, Jeong-Ah Seo. Fermentation profiling of rice wine produced by *Aspergillus oryzae* KSS2 and *Rhizopus oryzae* KJJ39 newly isolated from Korean fermentation starter. (2021) Appl. Biol. Chem. 64: 25**

The objective of this study was to determine the fermentation characteristics of rice wine produced by *koji* inoculated with *Aspergillus oryzae* KSS2 and *Rhizopus oryzae* KJJ39 on moisturized wheat-bran and rice grain. We also compared rice wine samples produced in this study and three commercial *Makgeolli*. The alcohol content was about 12% higher in the rice wine samples fermented by wheat-bran *koji* than in the other samples. In all of the samples, the range of pH value was 3.8–4.6 and the total acid was below 0.5. The soluble solid content was highest in the rice wine sample prepared by the wheat-bran *R*. *oryzae* KJJ39 *koji* (15.5°Brix) and overall relatively higher in the samples with wheat-bran *koji* than rice *koji*. The content of reducing sugar was twofold higher in rice wine prepared by *koji* inoculated with *R*. *oryzae* KJJ39 than *A*. *oryzae* KSS2. The volatile acid content was higher in rice wine produced by the wheat-bran *A*. *oryzae* KSS2 *koji* than the others. In the analyses of five organic acids, *A*. *oryzae* KSS2 was found to produce more malic acid and fumaric acid while *R*. *oryzae* KJJ39 to do more citric acid, lactic acid and acetic acid. The rice wine sample prepared with the wheat-bran *A*. *oryzae* KSS2 *koji* contained much higher concentration of sucrose, maltose and amino acids. Comprehensively, the results of fermentation profiling suggest that both *A*. *oryzae* KSS2 and *R*. *oryzae* KJJ39 can be applied to the production of rice wine as a valuable fungal isolate for fermentation start.

**Dae-Yong Yun, Young-Gyu Kang, Eun Jung Lee, Donghyun Kim, Eun-Hee Kim, Young-Shick Hong. Metabolomics study for exploring metabolic perturbations in soybean adventitious roots by fluorescent light irradiation. (2021) Appl. Biol. Chem. 64: 26**

Soybeans are the most popular cultivated crops worldwide. They contain abundant functional components. As part of the research to enhance functional components in soybean plants, soybean adventitious roots were cultured under dark and fluorescent light irradiation conditions and difference in their metabolome was explored using 1H NMR-based metabolomics approach. Results revealed that fluorescent light irradiation increased the biomass of soybean adventitious roots and caused considerable metabolic perturbations. In particular, health-beneficial secondary metabolites such as soyasaponin (3.4-fold), isoflavones (3.9-fold), and coumestrol derivatives (1.3-fold) were accumulated more in soybean adventitious roots grown under fluorescent light irradiation than in those grown under a dark condition due to increased photosynthesis that was evidenced by increased levels of glucose. The present study provides useful information on global metabolite compositions of soybean adventitious roots and their quality improvement by controlling growth conditions to enhance functional potentials of soybeans.

**Xin Wang, Qian Liu, Yunbao Wan, Chenxi Cao, Jiuyuan Bai, Rui Wang, Yun Zhao. Effect of passivator DHJ-C on the growth and cadmium accumulation of *Brassica napus* in Cd-contaminated soil. (2021) Appl. Biol. Chem. 64: 27**

The farmland polluted by cadmium is increasing drastically, which seriously threatened agricultural production and food safety. Nowadays, efficient and convenient way to solve the problem is urgently needed. In this experiment, a particular compound passivator DHJ-C was applied for soil remediation by pot experiment and the effect on both soil and plant was evaluated. The DHJ-C reduced the toxicity of Cd on soil enzyme activity and growth inhibition on *Brassica napus*. The soil urease and sucrase activity were significantly increased. The dry weight of mature oilseed rape increased by 14.6–36.0% and the yield of seeds increased by 14.1–52% per plant, which suggested that the passivator effectively reduced the detrimental effects on rape. Similarly, the results of physiology and biochemistry also indicated that DHJ-C can distinctly alleviate the inhibitory effect of Cd on plant growth. Such as the MDA content in plant was reduced by 52.1% in 10 mg/kg Cd treatment. Compared with control, Cd accumulation in seedling stage and mature period was significantly reduced as the concentration of Cd in aboveground part even decreased by 18.4 and 32.0% respectively. Overall, DHJ-C hold sufficient ability to be applied as an excellent passivator to reduce Cd toxicity in contaminated soil and significantly increase the yield of rapeseed.

**Tebogo Mokgehle, Ntakadzeni Madala, Wilson Gitari, Nikita Tavengwa. Deciphering the effects of kosmotrope and chaotrope salts during aqueous two phase extraction (ATPE) of polyphenolic compounds and glycoalkaloids from the leaves of a nutraceutical plant, *Solanum retroflexum,* with the aid of UPLC-QTOF-MS. (2021) Appl. Biol. Chem. 64: 28**

*Solanum* plants (Solanaceae) are renowned source of nutraceuticals and have widely been explored for their phytochemical constituents. This work investigated the effects of kosmotropic and chaotropic salts on the number of phytochemicals extracted from the leaves of a nutraceutical plant, *Solanum retroflexum,* and analyzed on the ultra-performance liquid chromatography hyphenated to a quadrupole time of flight mass spectrometer (UPLC-QTOF-MS) detector. Here, a total of 20 different compounds were putatively characterized. The majority of the identified compounds were polyphenols and glycoalkaloids. Another compound, caffeoyl malate was identified for the first time in this plant. Glycoalkaloids such as solanelagnin, solamargine, solasonine, β-solanine (I) and β-solanine (II) were found to be extracted by almost all the salts used herein. Kosmotrope salts, overall, were more efficient in extracting polar compounds with 4 more polyphenolic compounds extracted compared to the chaotropes. Chaotropes were generally more selective for the extraction of less polar compounds (glycoalkaloids) with 3 more extracted than the kosmotropes. The chaotrope and the kosmotrope that extracted the most metabolites were NaCl and Na2SO4, respectively, with 12 metabolites extracted for each salt. This work demonstrated that a comprehensive metabolome of *S. retroflexum,* more than what was previously reported on the same plant, can be achieved by application of kosmotropes and chaotropes as extractants with the aid of the Aqueous Two Phase Extraction approach. The best-performing salts, Na2SO4 or NaCl, could potentially be applied on a commercial scale, to meet the ever-growing demand of the studied metabolites. The Aqueous Two Phase Extraction technique was found to be efficient in simultaneous extraction of multiple metabolites which can be applied in metabolomics.

**Nagendran Rajalingam, Jae-Hyun Yoon, Bohyun Yoon, Nguyen Bao Hung, Won-Il Kim, Hyunju Kim, Byeong Yong Park, Se-Ri Kim. Prevalence and molecular characterization of *Escherichia coli* isolates during radish sprout production in the Republic of Korea. (2021) Appl. Biol. Chem. 64: 29**

This study was conducted to investigate the prevalence of coliforms and *Escherichia coli* across radish sprout production facilities in the Republic of Korea and to characterize isolated *E. coli*. A total of 324 samples were collected from three different radish sprout production units in spring and summer. Overall, the levels of coliforms were significantly higher (> 3.75 log CFU/g) in radish sprouts during summer season than in spring. *E. coli* was detected in the pre-harvest (50%) and post-harvest (39%) sprouts and water samples (33%) from farms B and C. Of the 48 *E. coli* isolates, five *E. coli* isolates were positive for the *eaeA* gene, indicative of EPEC O110, two isolates were *eaeA*-positive (EPEC ONT) and one isolate was *ipaH*-positive (ETEC O71). These three *E. coli* strains were identified as novel sequence types by the multilocus sequence-typing assay based on the combination of allelic profiles. In addition, the EPEC O110 isolates obtained from the stream water stored in container of farm C were toxic to *Caenorhabditis elegans.* It is necessary to implement safety measures during the production of radish sprouts to prevent *E. coli* outbreaks.

**Sun Il Kim, Wan Heo, So Jung Lee, Bok Kyung Han, Hong Gu Lee, Young Jun Kim. Characterisitcs of *Saccharomyces boulardii* for reducing ammonia emission from livestock manure. (2021) Appl. Biol. Chem. 64: 30**

Ammonia from livestock manure acts as a precursor to produce particulate matter (PM) by reacting with atmospheric chemical components volatilized from various sources. Ammonia itself acts as a toxic substance to human health, and thus has direct or indirect adverse effects on human health. This study aimed to verify the effectiveness and mechanism of action of *Saccharomyces boulardii* (SB) in reducing the ammonia emission from livestock manure. The specific ability of SB was confirmed through comparative verification with *S. cerevisiae* (SC) belonging to the same genus. SB and SC could use 50% of ammonia–nitrogen as inorganic nitrogen source in minimal medium. In the control group, the pH level of manure was significantly increased compared to the pH level at 0 h, and the DNA concentration of *Proteus mirabilis*, which increase the manure pH through ammonia production, was found to increase by 2.7-fold. Significant decrease in pH and proliferation of *P. mirabilis* was found in SB group compared to control (*p* < 0.05). The SB group also reduced the amount of ammonia emitted from manure by 25% for 35 days. These results suggested that SB contributed to reducing ammonia emission from manure by reducing pH and inhibiting HAB as well as removing ammonia–nitrogen. Accordingly, SB as a microbiological agent is expected to contribute not only to reduce ammonia emission but also to improve manure quality as a fertilizer.

**Moon Ho Do, Mina Kim, Sang-Yoon Choi, Pyeongjae Lee, Yoonsook Kim, Jinyoung Hur. Wild radish (*Raphanus sativus* var. *hortensis f. raphanistroides*) root extract protects neuronal cells by inhibiting microglial activation. (2021) Appl. Biol. Chem. 64: 31**

External stimulus-induced activation of microglia plays an important role in the protection of neurons in the central nervous system; however, over-activation of microglia could cause neuronal damage, and it is implicated in the pathogenesis of neurodegenerative diseases. The aim of the present study was to investigate the effects of wild radish (*Raphanus sativus* var. *hortensis f. raphanistroides*) root extract (WRE) on microglial over-activation. Mouse microglia BV-2 cells and rat primary microglia were stimulated with lipopolysaccharide (LPS), treated with WRE, and analyzed for nitric oxide (NO) production, pro-inflammatory cytokine secretion, inducible NO synthase (iNOS) expression, and p38 kinase phosphorylation. Human neuroblastoma SH-SY5Y cells were treated with microglia-conditioned medium and analyzed for cell viability. Stimulation with LPS increased NO production and iNOS expression in BV-2 cells and primary microglia, but the treatment with WRE decreased both. Furthermore, WRE downregulated the mRNA expression and secretion of inflammatory cytokines interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α), and inhibited the phosphorylation of p38 in LPS-activated microglia. Treatment with the conditioned medium of LPS-induced BV-2 cells decreased the viability of SH-SY5Y cells, but the damaging effect was significantly alleviated in cells treated with the conditioned medium of LPS plus WRE-cultured microglia. This indicated that the WRE treatment of microglia could protect neuronal cells from microglial activation-induced neurotoxicity. WRE may be a potential food product to attenuate neuroinflammation via the inhibition of microglial over-activation, which can slow down the neurodegenerative processes in the brain.

**Jingnan zhang, Feng Wang, Gengbao Qu, Baokai Wang. Murrayanine suppresses the proliferation and metastasis of human breast cancer cells via induction of apoptosis and inhibition of RANK/RANKL signaling pathway. (2021) Appl. Biol. Chem. 64: 32**

Murrayanine and its derivatives have been shown to exhibit anticancer activities against different types of human cancer cells. However, the effects of murrayanine on the proliferation and metastasis of breast cancer cells are yet to be studied. The present study was designed to evaluate the effects of murrayanine on the proliferation and metastasis of human breast cancer via regulation of RANK/RANKL pathway. The results showed RANK/RANKL pathway to be highly activated in human breast cancer tissues and cell lines. However, treatment of the CAMA-1 breast cancer cells with murrayanine (0, 9, 18 and 36 μM) caused a significant (P < 0.05) decline in the expression of RANK, RANKL and OPG in CAMA-1 cells. Additionally, murrayanine inhibited the growth of CAMA-1 cells with an IC50 of 18 μM. The antiproliferative of murrayanine were found be due to its ability to inhibit the expression of RANK, RANKL and OPG in CAMA-1 cells. To unveil if murrayanine exerted its effects via inhibition of RANK/RANKL pathway, the expression of RNAK was knocked down in CAMA-1 cells. It was found that murrayanine and RANK silencing both inhibited the growth CAMA-1 cells via induction of apoptosis. Additionally, murrayanine and RANK silencing both inhibited the migration, invasion and epithelial to mesenchymal transition of the CAMA-1 cells. Taken together, murrayanine exhibits significant anticancer activity against the breast cancer cells via induction of apoptosis and inhibition of RANK/RANKL signaling pathway. These findings suggest that murrayanine may prove to be a beneficial lead molecule for the development of breast cancer chemotherapy.

**Yoojin Chong, Hye Lim Lee, Jihyeon Song, Youngshim Lee, Bong-Gyu Kim, Hyejung Mok, Joong-Hoon Ahn. Biosynthesis of resveratrol derivatives and evaluation of their anti-inflammatory activity. (2021) Appl. Biol. Chem. 64: 33**

Resveratrol is a typical plant phenolic compound whose derivatives are synthesized through hydroxylation, *O*-methylation, prenylation, and oligomerization. Resveratrol and its derivatives exhibit anti-neurodegenerative, anti-rheumatoid, and anti-inflammatory effects. Owing to the diverse biological activities of these compounds and their importance in human health, this study attempted to synthesize five resveratrol derivatives (isorhapontigenin, pterostilbene, 4-methoxyresveratrol, piceatannol, and rhapontigenin) using *Escherichia coli*. Two-culture system was used to improve the final yield of resveratrol derivatives. Resveratrol was synthesized in the first *E. coli* cell that harbored genes for resveratrol biosynthesis including *TAL* (tyrosine ammonia lyase), *4CL* (4-coumaroyl CoA ligase), *STS* (stilbene synthase) and genes for tyrosine biosynthesis such as *aroG* (deoxyphosphoheptonate aldolase) and *tyrA* (prephenate dehydrogenase). Thereafter, culture filtrate from the first cell was used for the modification reaction carried out using the second *E. coli* harboring hydroxylase and/or *O*-methyltransferase. Approximately, 89.8 mg/L of resveratrol was synthesized and using the same, five derivatives were prepared with a conversion rate of 88.2% to 22.9%. Using these synthesized resveratrol derivatives, we evaluated their anti-inflammatory activity. 4-Methoxyresveratrol, pterostilbene and isorhapontigenin showed the anti-inflammatory effects without any toxicity. In addition, pterostilbene exhibited the enhanced anti-inflammatory effects for macrophages compared to resveratrol.

**Hyeong-Wook Jo, Min-Kyu Park, Hyo-min Heo, Hwang-Ju Jeon, Sung-Deuk Choi, Sung-Eun Lee, Joon-Kwan Moon. Simultaneous determination of 13 mycotoxins in feedstuffs using QuEChERS extraction. (2021) Appl. Biol. Chem. 64: 34**

Mycotoxins are secondary metabolites produced by various fungi and are known to have a significant negative impact on human and animal health. When feedstuffs are contaminated with mycotoxins, their toxicities may be caused a variety of diseases. In this study, the residual mycotoxins in feedstuffs were analyzed using LC–MS/MS incorporated with QuEChERS extraction. Analytical method validation was performed for LOD, LOQ, linearity, and recoveries with consideration of matrix effects prior to the residual analysis. They were all reached to the accepted range of validation level. Using 39 feedstuff samples (5 g) for mycotoxin analysis, nine samples were contaminated by four major mycotoxins such as fumonisin B1 (FB1), deoxynivalenol, fumonisin B2, and zearalenone. Among them, FB1 was detected at the highest concentration as 18.0943 mg/kg. The total sum of fumonisins in 39 samples did not exceed the maximum residual level (MRL) criterion set by Korean Food and Drug Administration. Altogether, intensive management of mycotoxins in Korean feedstuffs should be implemented with proper and routine monitoring, even their residual concentrations are not exceeded over the MRL levels because of high frequent detection found in this study.

**Nurlelasari, Intan Rahmayanti, Supriatno Salam, Agus Safari, Desi Harneti, Rani Maharani, Ace Tatang Hidayat, Mulyadi Tanjung, Rurini Retnowati, Yoshihito Shiono, Unang Supratman. A new havanensin-type limonoid from *Chisocheton macrophyllus*. (2021) Appl. Biol. Chem. 64: 35**

A new havanensin-type limonoid, 16β-hydroxydysobinin (**1**), along with four known limonoids (**2–5**), have been isolated from the seeds of *Chisocheton macrophyllus*. The chemical structure of the new compound was determined by referencing spectroscopic data, and by comparison to those related spectra previously reported. Each compound was evaluated for their cytotoxic effects against Michigan Cancer Foundation-7 (MCF-7) breast cancer cells and display no significant activity.

**Kaleigh E. Beane, Mersady C. Redding, Xiaofan Wang, Jeong Hoon Pan, Brandy Le, Cara Cicalo, Suwon Jeon, Young Jun Kim, Jin Hyup Lee, Eui-Cheol Shin, Ying Li, Jiangchao Zhao, Jae Kyeom Kim. Effects of dietary fibers, micronutrients, and phytonutrients on gut microbiome: a review. (2021) Appl. Biol. Chem. 64: 36**

The human gastrointestinal tract harbors a magnitude of bacteria, which are collectively known as the gut microbiome. Research has demonstrated that the gut microbiome significantly impacts the health of its host and alters the host’s risk for various chronic diseases. Many factors, such as diet, could potentially be manipulated to alter the host gut microbiome and induce subsequent preventative and/or therapeutic effects. It has been established that diet partakes in the regulation and maintenance of the gut microbiome; however, specific crosstalk between the microbiome, gut, and host has not been clearly elucidated in relation to diet. In this review of the scientific literature, we outline current knowledge of the differential effects of major plant-derived dietary constituents (fiber, phytochemicals, vitamins, and minerals) on the diversity and composition of the gut microbiome.

**Aslı Özkök, Merve Keskin, Aslı Elif Tanuğur Samancı, Elif Yorulmaz Önder, Çiğdem Takma. Determination of antioxidant activity and phenolic compounds for basic standardization of Turkish propolis. (2021) Appl. Biol. Chem. 64: 37**

This study aimed to determine the standard amount of antioxidant content and compounds of the propolis for the standardization of propolis. For this purpose, the total flavonoids, total phenolic, CUPRAC antioxidant capacity content and the diversity of phenolic and flavonoid components of these propolis samples were found by HPLC determined at the 23 propolis samples which were collected different regions of Turkey. Beside that, the similarities and differences of these 23 provinces to each other according to their antioxidant capacities were investigated by multidimensional scaling analysis. The total flavonoid content in the propolis samples were determined between 21.28 and 152.56 mg CE/g. The total phenolic content in the propolis samples was found between 34.53 mg and 259.4 mg GAE/g. CUPRAC antioxidant capacity of the propolis samples and antioxidant range was found from 95.35 to 710.43 mg TE/g. Also, 4 flavonoid [Quercetin (min.1.12–max.4.14 mg/g), Galangin (min.0.72–max.40.79 mg/g), Apigenin (min.1.07–max.17.35 mg/g), Pinocembrin (min.1.32–max.39.92 mg/g] and 6 phenolic acid [Caffeic acid (min.1.20–max.7.6 mg/g), p-Coumaric acid (min.1.26–max.4.47 mg/g), trans-Ferulic acid (min.1.28–max.4.92 mg/g), Protocatechuic acid (1.78 mg/g), trans-Cinnamic acid (min.1.05–max.3.83 mg/g), Caffeic Acid Phenethyl Ester (CAPE) (min.1.41–max.30.15 mg/g)] components were detected as mg/g, in different ratios in propolis samples collected from different regions. The feature of this study, so far, is to have the maximum number of samples representing the Turkish propolis, and so is thought to help to national and international propolis standard workings.

**Philipp Rudolf Michael Peter Eyring, Susan Strange Herrmann, Mette Erecius Poulsen. Multiresidue analysis of 184 pesticides in high-fat fish feed using a new generic extraction method coupled with gas and liquid chromatography-tandem mass spectrometry. (2021) Appl. Biol. Chem. 64: 38**

Animal feed is typically plant-based and can contain pesticide residues. Methods for testing food and feed samples, such as the Quick Easy Cheap Effective Rugged Safe (QuEChERS) method or the Swedish Ethyl Acetate (SweEt) method, successfully extract many pesticide residues. However, nonpolar pesticides, such as organochlorine pesticides, show poor recovery when extracted from lipid-rich samples. The previously developed water-acetonitrile-heptane-solid-phase-extraction (WAHSPE) method shows better recoveries for the nonpolar pesticides but requires two injections per sample and per instrument. Here, we present a modified version of the WAHSPE method for pesticides in fish feed using one injection per sample and per instrument. Of the 184 pesticides tested, 179 met the European Union Legislation’s validation criteria at a spike level of 50 μg/kg, showing recoveries between 70 and 120% and a relative standard deviation (RSD) below 20%. Organochlorine pesticides accounted for 14 of the tested compounds.

**Eun Ko, Min Young Um, Taewon Han, Sooim Shin, Moonsung Choi. Emodin and rhein in *Cassia tora* ameliorates activity of mitochondrial enzymes involved in oxidative phosphorylation in the retina of diabetic mice. (2021) Appl. Biol. Chem. 64: 39**

*Cassia tora* is an annual herb, which has pharmacological effects such as antioxidant, hypolipidemic, and antidiabetic effects. Accordingly, its effect on diabetes has been well-studied. However, it is unclear whether it has an effect on mitochondrial dysfunction associated with diabetes. In this study, the effects of emodin and rhein in *C. tora* seed ethanolic extract (ER/CSEE) on retinal mitochondrial function were examined in high-fat diet (HFD)-fed mice. HFD-fed mice exhibited decreased mitochondrial function followed by compensatory increase in the expression levels of mitochondrial enzymes. However, ER/CSEE treatment for 12 weeks ameliorated the activity of retinal mitochondrial complexes and reduced the expression level of enzymes involved in oxidative phosphorylation, except that of complex II and citrate synthase in citric acid cycle. This suggests that repairing capacity of enzymes in electron transport chain and citric acid cycle of mitochondria are different in response to the metabolic state. Therefore, it concluded that emodin and rhein play a pharmacological role in fat metabolism by influencing activities of enzymes in citric acid cycle linked with beta-oxidation in retina.

**Eunjung Kim, Hyunjin Park, Sihyun Park, Jangduck Choi, Hae Jung Yoon, Jeong-Han Kim. Simultaneous determination of multi-class veterinary drugs in fishery products with liquid chromatography–tandem mass spectrometry. (2021) Appl. Biol. Chem. 64: 40**

The objective of this study was to optimize the analytical method for multi-class veterinary drug residues of 64 compounds in fishery products. Several compounds from veterinary drugs are banned or unauthorized in fishery products according to the Korean Food Code. Samples were extracted using acetonitrile/water (4:1, v/v) and the clean-up step was carried out by adding octadecylsilane and acetonitrile-saturated hexane. The target compounds were confirmed and quantified using liquid chromatography–tandem mass spectrometry (LC–MS/MS). The proposed method was validated according to the CODEX guidelines (CAC/GL-71), and most target compounds were found to be in acceptable quantities under the requirements of the validation guidelines. The recovery of analytes was typically in the 60–120% range, and precision, expressed as the coefficient of variation was less than 31% at all levels of concentration. The limit of quantification ranged from 0.03 to 3 μg kg−1 in the fishery products. Moreover, the application of the proposed method to 96 real samples demonstrated that no drug residues exceeded the Korean maximum residue limits (MRLs). This evaluation method provides reliable identification and quantification of multi-class veterinary drugs in fishery products and can be an efficient means to inspect drugs currently banned or not approved for aquaculture in Korea.

**Fengjuan Liu, Wenshu Huang, Zuoshan Feng, Yongxia Tao, Yingying Fan, Weizhong He, XiaoLi Li, Xiaotong Fang, Cheng Wang, Yujia Bai. Proteomic analyses on the browning of shade-dried Thompson seedless grape. (2021) Appl. Biol. Chem. 64: 41**

China is one of the main producers in the worldwide raisin market. Most China’s raisins are produced in Xinjiang where the Thompson seedless grape (*Vitis vinifera* L.cv.Thompson seedless) is the main variety of green raisin. However, the browning of Thompson seedless grape during drying has been well-acknowledged as the primary factor affecting the development of the raisin industry. Data independent acquisition (DIA)-based protein profiling was performed on fresh and shade-dried Thompson seedless grapes. As a result, 5431 proteins were identified, among which the amounts of 739 proteins in fresh grape were found to be significantly different with those in dried grape. The functional annotation based on the Blast2GO showed that the ‘organic substance metabolic process’, ‘regulation of molecular function’, ‘enzyme regulator activity’, and ‘isomerase activity’ related proteins became very active during browning. Further analyses revealed that the browning-related proteins, which with significant different amounts in fresh and in dried grapes, are primarily involved in the phenylpropanoid biosynthesis, tyrosine metabolism, phenylalanine metabolism, oxidative phosphorylation metabolism, plutathione metabolism, peroxisome pathway, and fatty acid degradation. And five random differential proteins were verified with parallel reaction monitoring (PRM). The PRM results were in agreement with the DIA data. The main browning-related proteins of Thompson seedless grape were identified in this study. Their properties were tested, and their roles in the browning mechanism were indicated. This will lay base to a better understanding on the enzymatic browning of Thompson seedless grape, and it will also provide guidance for controlling the quality of Thompson seedless grapes in industry.

**Kathyleen Nogrado, Tatsuya Unno, Hor-Gil Hur, Ji-Hoon Lee. Tetracycline-resistant bacteria and ribosomal protection protein genes in soils from selected agricultural fields and livestock farms. (2021) Appl. Biol. Chem. 64: 42**

Antibiotic resistance in soil environment has eminently been compared and studied between agricultural and pristine soils, and the role of concentrated animal feeding operations has markedly been recognized as one of the major sources of antibiotic resistance. This study described the tetracycline resistance in small-scale farms in pursuit of presenting its possible role and contribution to the persistence of antibiotic resistance in the environment. Results of the study would render additional information on the occurrence of the ribosomal protection protein (RPP) *tet* genes among the isolated bacteria from the selected agricultural soils. Four tetracycline resistance and RPP genes were determined in two different agricultural soil settings. Both the culture and molecular method were used to determine and measure tetracycline resistance in soils from arable land and animal house. Results revealed a significantly higher number of culturable antibiotic-resistant bacteria in animal houses than arable lands which was suggestive of higher antibiotic resistance in areas where there was direct administration of the antibiotics. However, quantification of the gene copy numbers in the agricultural soils indicated a different result. Higher gene copy number of *tet*O was determined in one animal house (IAH-3), while the two other *tet* genes *tet*Q and *tet*W were found to be higher in arable lands. Of the total 110 bacterial isolates, *tet*W gene was frequently detected, while *tet*O gene was absent in any of the culturable bacterial isolates. Principal component analysis of occurrence and gene copy number of RPP *tet* genes *tet*O, *tet*Q, and *tet*W also revealed highest abundance of RPP *tet* genes in the manure and arable soils. Another important highlight of this study was the similarity of the RPP *tet* genes detected in the isolated bacteria from the agricultural soils to the identified RPP *tet* genes among pathogenic bacteria. Some of the tetracycline-resistant bacterial isolates were also multidrug resistant as it displayed resistance to tetracycline, erythromycin, and streptomycin using disk diffusion testing.

**Jong-Hwan Park, Se-Wook Hwang, Su-Lim Lee, Jae-Hoon Lee, Dong-Cheol Seo. Sorption behavior of phosphate by fly ash discharged from biomass thermal power plant. (2021) Appl. Biol. Chem. 64: 43**

This study evaluated the characteristics and mechanism of phosphate adsorption by fly ash discharged from a biomass thermal power plant (BTP-FA) under various environmental conditions in order to increase the recyclability of BTP-FA. The phosphate adsorption properties of BTP-FA and fly ash derived from coal thermal power plant (CTP-FA) were better matched by those predicted by the Langmuir isothermal model and the pseudo-second-order model, and their maximum adsorption capacities were 62.1 and 4.1 mg/g, respectively. It was found that the adsorption of phosphate by BTP-FA was predominantly influenced by the outer boundary layer rather than the inner diffusion in the pores. The phosphate adsorption process by BTP-FA was greatly influenced by the initial pH and the BTP-FA dose. Therefore, to effectively treat phosphate using BTP-FA, the concentration and flow rate of phosphate in the incoming wastewater must be considered. The concentration of dissolved calcium from BTP-FA decreased sharply during the phosphate adsorption process compared to that in the phosphate-free solution. This was thought to be due to surface adsorption/reaction between calcium and phosphate. The SEM–EDS and FTIR results also supported the surface adsorption/precipitation reaction of Ca-P. Recycling fly ash discharged from biomass power plants as phosphate adsorbents is expected to contribute not only to waste reduction, but also to wastewater purification.

**Sora Shin, Eun Hea Jho, Han Sol Park. Effect of Triton X-100 on the wheat and lettuce growth and contaminant absorption. (2021) Appl. Biol. Chem. 64: 44**

This study was set to study the effects of surfactants on crops using Triton X-100, one of widely used surfactants for various purposes including agricultural uses, as a target surfactant. The effects of Triton X-100 on the growth of wheat and lettuce were studied and the germination and shoot growth of wheat were not significantly affected by Triton X-100. With lettuce, the increasing Triton X-100 concentrations tend to negatively affect the growth, possibly due to the absorption of Triton X-100 by lettuce. The average lettuce fresh mass was reduced by 31% when Triton X-100 concentration increased from 0 to 240 mg L−1. This may mean that chemicals dissolved or mobilized by Triton X-100 can be absorbed by lettuce. The Cd mobilization was facilitated with Triton X-100, and the absorption of procymidone in soil by lettuce was greater when Triton X-100 was applied (i.e., 0.18 mg kg−1) than when water was applied (i.e., 0.15 mg kg−1), although they were statistically not different (*p*-value > 0.05). The average lettuce masses in the presence of residual procymidone in soil and Triton X-100 (16 g) were lower than that of the control soils (20 g), although they were statistically not different (*p*-value > 0.05). The results suggest that surfactants contained in pesticide formulations can potentially affect crop growth and absorption of other contaminants. Therefore, the residual surfactants and active ingredients in pesticide formulations need to be properly managed to protect the environment and to produce crops free of contaminants.

**Senthil Nagarajan, Jae Kwon Lee. Therapeutic effects of sesamolin on leukemia induced by WEHI-3B in model mice. (2021) Appl. Biol. Chem. 64: 45**

Sesamolin is one of the lignans derived from sesame oil. It has demonstrated significant antioxidant, anti-aging, and anti-mutagenic properties. It also reportedly augments natural killer (NK) cell lysis activity. We previously reported that sesamolin also exerts anticancer effects in vitro and induces enhanced NK cell cytolytic activity against tumor cells. Herein, we aimed to determine the mechanism by which sesamolin prevents and retards tumorigenesis in BALB/c mouse models of leukemia induced by murine (BALB/c) myelomonocytic leukemia WEHI-3B cells. Banded neutrophils, myeloblasts, and monocytic leukemic cells were more abundant in the leukemia model than in normal mice. Sesamolin decreased the number of leukemic cells by almost 60% in the leukemia model mice in vivo; additionally, sesamolin and the positive control drug, vinblastine, similarly hindered neoplastic cell proliferation. Spleen samples were ~ 4.5-fold heavier in leukemic mice than those obtained from normal mice, whereas spleen samples obtained from leukemic mice treated with sesamolin had a similar weight to those of normal mice. Moreover, sesamolin induced a twofold increase in the cytotoxic activity of leukemic mouse NK cells against WEHI-3B cells. These results indicated that sesamolin exerts anti-leukemic effects in vivo.

**Trong Nguyen Nguyen, Yeong-Geun Lee, Hyoung-Geun Kim, Dahye Yoon, Jin Tae Jeong, Dae Young Lee, Nam-In Baek. New dibenzocyclooctadiene lignan from *Schisandra chinensis* (Turcz.) Baill. fruits. (2021) Appl. Biol. Chem. 64: 46**

Repeated column chromatography using Sephadex LH-20, silica gel (SiO2), and octadecyl SiO2 (ODS) as well as preparative HPLC column chromatography led to isolation of a new dibenzocyclooctadiene lignan along with four known ones, gomisin L2 (**1**), L1 (**2**), M1 (**3**), and M2 (**4**). Their chemical structures were fixed based on MS, IR, and NMR data analyses. In addition, the stereochemistry of atropisomers, the absolute configuration of the axial chirality in a biphenyl structure, was confirmed by a CD experiment. The new lignan was named gomisin M3 (**5**).

**Xiu Yuan, Junghak Lee, Heeju Han, Boeun Ju, Eunyoung Park, Yongho Shin, Jonghwa Lee, Jeong-Han Kim. Translocation of residual ethoprophos and tricyclazole from soil to spinach. (2021) Appl. Biol. Chem. 64: 47**

The dissipation of ethoprophos and tricyclazole in soil and their translocation tendency to spinach were investigated. Prior to field trials, the analytical method for the determination of these pesticide residues was optimized and validated on soil and spinach. The field trial was conducted under greenhouse conditions for two different pretreatment periods with the pesticides. After treating with pesticides 30 (PBI-30) and 60 days (PBI-60) before seeding, soil samples were collected on different days for the dissipation study of soil. Spinach samples were harvested from the soil, and 50% and 100% mature spinach samples were collected. The initial amounts of ethoprophos residue in the PBI-60 and PBI-30 soils were 0.21 and 2.74 mg/kg, respectively, and these both decreased to less than 0.01 mg/kg on the day of spinach harvest. Similar initial residues of tricyclazole were observed in the PBI-60 (0.87 mg/kg) and PBI-30 soils (0.84 mg/kg), and these decreased to 0.44 and 0.34 mg/kg, respectively. The half-lives of ethoprophos in the soils were calculated as 7.6 and 4.8 days, respectively, while relatively long half-lives of 36.5 and 77.0 days were calculated for tricyclazole. According to the pesticide residue amounts in the spinach, the translocation rate from the soil to the spinach was determined. In the case of ethoprophos, the residual amount was already rapidly degraded in the soil, and the translocation rate could not be confirmed. On the other hand, for tricyclazole, it was confirmed that 1.19 to 1.61% of the residual amount in soil was transferred to spinach. According to these results, safe management guidelines for tricyclazole in soil were suggested considering the maximum residue limit on spinach.

**Junghyun Park, Dongyeop Jang, Hung Manh Phung, Tae Joon Choi, Chang-Eop Kim, Sanghyun Lee, Ki Sung Kang, Seo-Hyung Choi. The potential of pharmacological activities of the multi-compound treatment for GERD: literature review and a network pharmacology-based analysis. (2021) Appl. Biol. Chem. 64: 48**

The prevalence of gastroesophageal reflux disease (GERD) is rapidly increasing due to the adoption of a Westernized lifestyle; at the same time, safe and efficient treatment is required due to the side effects and refractoriness of proton pump inhibitors (PPIs). The frequently used multi-compound treatment for GERD in the current traditional Korean medicine (TKM) clinical field comprises *Crassostrea gigas* Thunberg shell (CGTS), Bambusae Caulis in Taeniam (BCT), Ponciri Fructus Immaturus (PFI), *Scutellaria baicalensis* Georgi (SBG), medicated leaven (ML) and Glycyrrhizae Radix et Rhizoma (GRR). The current review was based on “Kun-Shin-Choa-Sa” theory and network analysis was conducted to explore the potential pharmacological activities, including efficacy and mechanisms of action of multi-compound treatment against GERD. Hypergeometric test results showed that the targets of multi-compound treatment are significantly associated with GERD gene sets, consistent with the literature review findings. In particular, the enrichment analysis indicated that the SBG targets are related to the IL-17 signaling pathway, bile secretion, small-cell lung cancer, and non-small cell lung cancer, corroborating the literature review, particularly concerning anti-inflammatory effect. In the literature review, CGTS and BCT, classified as “Kun,” play a role in anti-acid, anti-inflammatory, and anti-oxidative effects. The complementary “Shin” herbs, PFI and SBG, showed functions related to improving the prolonged gastric emptying rate, peristalsis, and a gastric cytoprotective effect. With the role of “Choa,” ML was suggested to inhibit *H. pylori* growth and diminish gastric acid secretion, consistent with the gastric acid secretion pathway in the enrichment analysis. However, the enrichment analysis did not show any significantly related pathways for CGTS and PFI, which may reflect the lack of information in the KEGG database in terms of the link between GERD, its mechanisms, and the abundance of minerals in CGTS. Despite the pharmacological potential of multi-compound treatment, this study should be corroborated by well-designed future experimental studies.

**Hak-Dong Lee, Leo Adrianne Paje, Sullim Lee, Ki Sung Kang, Kyungki Hong, Hyukjin Kwon, Sanghyun Lee. Optimization of an analytical HPLC-DAD method for detecting hydroxycinnamic acid derivatives from mixtures of *Saussurea grandifolia* and *Taraxacum coreanum*. (2021) Appl. Biol. Chem. 64: 49**

An analytical method was established to identify and quantify hydroxycinnamic acids, such as 1,5-dicaffeoylquinic acid (DCQA) and chicoric acid (CA), in mixtures of *Saussurea grandifolia* and *Taraxacum coreanum* (MST) by using reverse-phase high-performance liquid chromatography coupled with diode array detector (HPLC-DAD). Analyses were carried out by using an INNO C18 column with a gradient elution system, and different parameters were used to validate our optimized method. Results demonstrated limits of detection and quantification of 5.46 × 10–3 and 16.54 × 10–3 mg/mL for DCQA and 0.37 × 10–3 and 1.14 × 10–3 mg/mL for CA, respectively. The calibration curves for DCQA and CA showed good linearity over the concentration ranges of 0.025–0.4 and 0.00625–0.1 mg/mL, respectively, and both exhibited *r*2 = 1.0000. In the accuracy test, high recovery rates were obtained ranging from 101.16–104.18% for DCQA and 97.55–108.49% for CA, while the precision values were ≤ 1.00% for DCQA and ≤ 1.21% for CA. The values obtained from our analyses support the use of this analytical method for the accurate identification and quantification of DCQA and CA from MST. Our methodology could be used further to determine the content of hydroxycinnamic acid derivatives in routine analyses and large-scale extraction processes.

**Myun Soo Kim, Minjee Lee, Hanna Oh, Wonsang Seo, Gwang-Seob Kim, O-Hyun Ban, Minhye Shin, Young Hoon Jung, Jungwoo Yang. Enhanced ceramides production by *Lactobacillus rhamnosus* IDCC 3201 and its proposed mechanism. (2021) Appl. Biol. Chem. 64: 50**

The use of probiotics has been applied for a variety of fields (e.g., immune system, mental health, and heart). In this study, the feasibility of lysates from *L. rhamnosus* IDCC 3201 for cosmetic ingredients was evaluated. More specifically, enhanced ceramides production in human epidermal keratinocytes by the lysates and its proposed machanism were investigated through in vitro and genome analysis. In results, enhanced spingomyelinase activity and thereby increased ceramides production by the lysates from *L. rhamnosus* IDCC 3201 was observed. Furthermore, it was found that the existence of glucosylceramdase in *L. rhamonsus* IDCC 3201 was attributed to enhanced ceramides production. Finally, it was verified that the lysates from *L. rhamonsus* IDCC 3201 was regarded as safe for its use as cosmetic materials. Thus, these findings have significant implications that might lead to the development of functional and safe cosmetic products from probiotics.

**Sehee Park, Igojo Kang, Chanseok Shin. MicroRNA clustering on the biogenesis of suboptimal microRNAs. (2021) Appl. Biol. Chem. 64: 51**

Most microRNAs (miRNAs) are processed by two ribonuclease III enzymes. The first cleavage is performed by Microprocessor that is composed of RNase III enzyme Drosha and DGCR8, and the second by another RNase III enzyme Dicer. There are many examples of miRNAs that are poor substrates for Drosha and Dicer, owing to their suboptimal structures. However, a number of these suboptimal miRNAs are known to be expressed at the same or higher level as their neighboring structurally-optimal miRNAs. Recent studies suggest that the clustered orientation of these suboptimal miRNAs is the explanation for this phenomenon. It has been observed that the biogenesis of these suboptimal miRNAs can be affected by the expression of their neighboring optimal miRNAs. This principle is expected to apply more broadly, as it has been shown that a large percentage of suboptimal miRNAs reside within operons.

**Xiu Yuan, Junghak Lee, Eunyoung Park, Hwa-Kyung Lee, Jeong-Han Kim. Toxicometabolomics of lindane in adult zebrafish (*Danio rerio*) using GC-MS/MS and LC-Orbitrap-MS/MS. (2021) Appl. Biol. Chem. 64: 52**

Lindane is a broad-spectrum persistent organochlorine pesticide that has been used to control pests for many years. In this study, its toxic mechanisms in adult zebrafish were investigated using targeted metabolomics with GC-MS/MS and non-targeted metabolomics with LC-Orbitrap-MS/MS. Zebrafish was exposed to lindane in water for 48 h in three groups: control, low exposure (1/10 LC50) and high exposure (LC50). In the zebrafish exposed to low concentration of lindane, 2.24–3.98 mg/kg of lindane were determined, while 35.67–56.46 mg/kg were observed in the zebrafish exposed to high concentration. A total of 118 metabolites were identified from 394 metabolites on GC-MS/MS and 45 metabolites were selected as biomarkers. A total of 62 metabolites were identified on LC-Orbitrap-MS/MS and 7 metabolites were selected as biomarkers. Three groups were well separated on partial least squares-discriminant analysis (PLS-DA), and a total of 52 metabolites in both the targeted and non-targeted metabolites were selected as biomarkers through VIP and ANOVA tests to construct a heatmap. Five metabolic pathways such as the pentose phosphate pathway (PPP), histidine metabolism, phenylalanine metabolism, alanine/aspartate/glutamate metabolism, and phenylalanine/tyrosine/tryptophan biosynthesis, were observed to show toxicologically significant alterations. Oxidative stress was also confirmed through MDA and ROS assays. Such perturbations of the metabolic pathways of zebrafish caused by the exposure to lindane resulted in significant toxicological effects.

**Kyung Yuk Ko, Se Hee Jeong, Eun Young Choi, Kyungjin Lee, Yongmin Hong, Il hyun Kang, Sooyeul Cho, Chulhyun Lee. A LC–ESI–MS/MS analysis procedure coupled with solid phase extraction and MeOH extraction method for determination of pyrrolizidine alkaloids in *Tussilago farfara* and *Lithospermi erythrorhzion*. (2021) Appl. Biol. Chem. 64: 53**

Pyrrolizidine alkaloids (PAs) that are plant toxin naturally produced for protection against herbivores in some plant families. They are associated with the potential hepatotoxic and carcinogenic diseases serious hepatic disease in humans and animals. As the concern of human health risk posed by exposure to PAs has been gradually increased, precise and reliable analysis is required for monitoring PAs. The present study developed a new and simple pretreatment using 50% MeOH (methanol) for quantification analysis of the PAs contained with high content in the herbal medicines. Another pretreatment method using cation-ion exchange solid-phase extraction (MCX-SPE) was employed for determining most of the PAs that are not contained in the herbal medicines. That is, the proposed LC–MS/MS method coupled with MCX-SPE extraction and 50% MeOH extraction method was developed. And to evaluate the reliability of its application for Farfarae Flos and Lithospermi Radix, a validation study was conducted. In addition, monitory study was performed with ten samples in each herbal medicine. As a result, the proposed method had good linearity with r2 ≥ 0.997. Also, the recoveries indicated to be in the ranges of 70.4–118.0% for the Farfarae Flos, 70.2–119.7% for the Lithospermi Radix. In two herbal medicines, the intra-day precision was revealed to satisfy the reference criteria in most of the PAs. In monitoring results, most of the PAs were not contained in two herbal medicines, whereas a part of PAs revealed to have high concentration in Farfarae Flos and Lithospermi Radix. The proposed method is considered as a simple and reliable method to quantify 28 PAs present in two herbal medicines. Especially, the simple MeOH extraction method seems to be available for quantification analysis of certain PAs in herbal medicines with high content.

**Dayoung Kim, Eun Ko, Moonsung Choi, Sooim Shin. Steady-state kinetic analysis of mitochondrial respiratory enzymes from bovine heart mitochondria. (2021) Appl. Biol. Chem. 64: 54**

Mitochondria is a decisive organelle of cells that produces adenosine triphosphate (ATP) by the process of oxidative phosphorylation of the Krebs cycle and the electron transport chain. The electron transport chain system of mitochondria embodies multiple enzyme supercomplexes including complex I to V which located in the inner membrane. Although the simple enzyme activity of some as-isolated complex has been studied so far, the steady-state kinetic analysis of each complex within the form of mitochondrial supercomplex has not been studied in depth. To this end, kinetic parameters of mitochondrial complex I–IV were determined using steady-kinetic analysis using corresponding substrates of them. Catalytic activity and binding affinity between substrates and enzymes were obtained by fitting the data to the Michaelis–Menten equation. Acquired kinetic parameters represented distinctive values depending on the complexes that can be interpreted by the characteristics of the enzymes including the distinction of substrates or the ratio of the enzyme itself under the supercomplex form. The indirect *kcat* of the mitochondrial enzymes were varied from 0.0609 to 0.334 s−1 in order of complex III, II, I, and IV and *Km* of substrates were also diverse from 5.1 μM to 12.14 mM. This is the first attempt to get exact kinetic values that should provide profound information to evaluate the mitochondrial function practically in advance.

**Hyeon Jeong Seong & Yu-Sin Jang. Effect of deregulation of repressor-specific carbon catabolite repression on carbon source consumption in *Escherichia coli*. (2021) Appl. Biol. Chem. 64: 55**

*Escherichia coli* has been used as a host to construct the cell factory for biobased production of chemicals from renewable feedstocks. Because galactose is found in marine biomass as a major component, the strategy for galactose utilization in *E. coli* has been gained more attention. Although galactose and glucose co-fermentation has been reported using the engineered *E. coli* strain, few reports have covered fermentation supplemented with galactose as a sole carbon source in the mutant lacking the repressor-specific carbon catabolite repression (CCR). Here, we report the effects of the deregulation of the repressor-specific CCR (*galR−* and *galS−*) in fermentation supplemented with galactose as a sole carbon source, using the engineered *E. coli* strains. In the fermentation using the *galR−* and *galS−* double mutant (GR2 strain), an increase of rates in sugar consumption and cell growth was observed compared to the parent strain. In the glucose fermentation, wild-type W3110 and its mutant GR2 and GR2PZ (*galR−*, *galS−*, *pfkA−*, and *zwf−*) consumed sugar at a higher rate than those values obtained from galactose fermentation. However, the GR2P strain (*galR−*, *galS−*, and *pfkA−*) showed no difference between fermentations using glucose and galactose as a sole carbon source. This study provides essential information for galactose fermentation using the CCR-deregulated *E. coli* strains.

**Hwan-Hee Bae, Gibum Yi, Young Sam Go, Jun Young Ha, Yuchan Choi, Jae-Han Son, Seonghyu Shin, Tae-Wook Jung, Sungwoo Lee. Measuring antioxidant activity in yellow corn (*Zea mays* L.) inbreds from three different geographic regions. (2021) Appl. Biol. Chem. 64: 56**

Corn (*Zea mays* L.) provides a major source of calories for human consumption and therefore, the nutritional components of corn have a large impact on human health. For example, corn kernels contain antioxidants, such as polyphenols (including anthocyanins and other flavonoids) and carotenoids. Such compounds represent useful targets for biofortification breeding. In this study, we used 34 corn inbred lines from three different regions (East Asia, Southern Asia, and subtropical regions) and 11 F1 hybrids derived from the inbreds to investigate antioxidant activity in yellow corn. We compared different methods for measuring antioxidant activity to test their consistency and to determine whether color could be used as an indicator of antioxidant activity. We also measured carotenoid levels in yellow corn. No difference in antioxidant activity was detected between inbred corn lines from temperate vs. tropical regions. We determined that carotenoid is a major contributor to antioxidant activity in yellow corn and that kernel color, especially yellowness, could be used as an indicator of antioxidant activity in yellow corn. These findings lay the foundation for the biofortification of yellow corn by providing information about the correlations among kernel color, carotenoid contents, and antioxidant activity and by identifying an easy method to assess antioxidant activity in yellow corn.

**Ju Sung Lee, Leo Adrianne Paje, Min Jeong Kim, Seung Hee Jang, Jong Tae Kim, Sanghyun Lee. Validation of an optimized HPLC–UV method for the quantification of formononetin and biochanin A in *Trifolium pratense* extract. (2021) Appl. Biol. Chem. 64: 57**

Here, two important isoflavones present in *Trifolium pratense,* formononetin and biochanin A, were analyzed by high performance liquid chromatography coupled with UV (HPLC–UV). These isoflavones are marker compounds with anti-inflammatory, anti-cancer, and anti-oxidant properties, and are also potent phytoestrogens that can be used in hormonal therapies. Till date, no study regarding rapid identification and quantification of *T. pratense* extract has been conducted. This study developed and validated an optimized method for quantifying formononetin and biochanin A using HPLC–UV. The results showed excellent linearity of the calibration curve (r2 ≥ 0.999), and good resolutions of chromatographic peaks were obtained. Other validation parameters such as specificity, accuracy, and precision demonstrated that our method had good reliability and sensitivity. Furthermore, our method for quantifying formononetin and biochanin A in *T. pratense* extract is convenient.

**Hee-Weon Lee, Min ji Gu, Jinyoung Hur, Ho-Young Park, Yoonsook Kim, Sang Keun Ha. Glycolaldehyde disrupts insulin signaling and glucose uptake through adipogenesis. (2021) Appl. Biol. Chem. 64: 58**

The accumulation of advanced glycation end products (AGEs) plays critical roles in exacerbating obesity, arteriosclerosis, cardiovascular disease, diabetes, and their associated complications. Glycolaldehyde (GA) is the metabolic precursor of several AGEs, and its effects vary based on food and cooking methods. Here, 3T3-L1 adipocytes were used to examine the effects of GA on obesity and insulin resistance. We found that GA treatment did not increase lipid accumulation but increased the distribution of adipocyte differentiation. We also investigated the production of receptor for AGEs (RAGE) and reactive oxygen species (ROS) upon GA treatment, as well as the expression levels of peroxisome proliferator-activated receptors γ (PPARγ), CCAAT enhancer binding protein α (c/EBPα), and CCAAT enhancer binding protein β (c/EBPβ), which are transcription factors for adipogenesis, were significantly increased upon GA treatment in a concentration-dependent manner. GA arrested the cell cycle at the G0/G1 stage during the early phase of adipogenesis and suppressed the expression of p21 and p27. GA increased the expression of CDK2, phosphorylation of mitogen-activated protein kinases, and secretion of pro-inflammatory cytokines. Overall, these results suggest that GA can stimulate lipid metabolism, hence, we suggest that the stimulation of adipogenesis and insulin resistance by GA may be associated with the interaction between RAGE and adipogenic factors in adipocytes.

**Fang Dong, Hu-Zhe Zheng, Woo-Sik Jeong, Shin-Kyo Chung, Zhong-Yuan Qu, Xiang Zou, Chen Liu, Qiong Xiang, Feng Feng. Synthesis, characterization, and antioxidant activity in vitro of selenium-*Euryale ferox* Salisb. polysaccharide. (2021) Appl. Biol. Chem. 64: 59**

In order to obtain and explore selenide composed of selenium and polysaccharide, three parameters were selected to optimize the synthesis process of selenium-*Euryale ferox* Salisb. polysaccharide (Se-ESPS) by Box-Behnken design. Furthermore, ESPS-B1 separated from ESPS was selenitized to Se-ESPS-B1 by the optimal synthesis process, then the characterization, and antioxidant activity in vitro of Se-ESPS-B1 were explored. The result manifested that the Se content of Se-ESPS was (2.915  ±  0.03) mg/g according to the optimal synthesis process of Se-ESPS (reaction time at 5 h, reaction temperature at 81 ℃, weight ratio of Na2SeO3 to ESPS at 0.9 g/g). A series of detection results indicated that the characterizations of Se-ESPS-B1 were apparently distinguished from that of ESPS-B1. Moreover, the antioxidant experiments in vitro demonstrated that Se-ESPS-B1 could exert antioxidant activity by scavenging DPPH, ABTS,·OH, and increasing reduction ability. In conclusion, the synthesis process is an effective approach to harvest selenium-polysaccharide, and Se-ESPS-B1 may be utilized as a potential antioxidant or selenium nutritional supplement.

**Hye Yun Jeong, Oran Kwon. Dietary phytochemicals as a promising nutritional strategy for sarcopenia: a systematic review and meta-analysis of randomized controlled trials. (2021) Appl. Biol. Chem. 64: 60**

The decline in skeletal muscle mass and strength, also called sarcopenia, accelerates with age, leading to negative health outcomes and poor quality of life. Diet is important to promote health and plays a key role in muscle aging. Plant-based foods have recently received attention as sources of phytochemical components to attenuate loss of muscle mass and strength in older adults. This systematic review and meta-analysis evaluated the benefits of botanical extracts and their phytochemical compounds for muscle health in older adults. Randomized controlled trials were identified via systematic searches of four electronic databases (PubMed, Cochrane Library, Web of Science, and KoreaMed) up to June 2021 and were quality assessed. The results of muscle strength, mass, and physical performance were pooled using a random-effects model. Fourteen studies involving 528 subjects aged between 50 and 80 years met the inclusion criteria. Dietary phytochemicals significantly increased handgrip strength [0.90 kg; 95% confidence interval (CI) 0.26–1.53, *p* =  0.01] and physical performance (timed up-and-go test: − 0.5 s, 2.73 times; 95% CI − 0.84 to − 0.15, *p* <  0.01; 30-s chair stand test: 95% CI 0.88–4.59, *p* <  0.01; 6-min walk test: 29.36 m; 95% CI 14.58–44.13, *p* <  0.0001) but had no effect on improvement in muscle mass. Publication bias evaluated by funnel plots and Egger’s regression test demonstrated no evidence of substantial publication bias (*p* >  0.05). The findings of this systematic review and meta-analysis suggest that phytochemicals are a potential nutritional strategy to improve muscle health in older adults.

**Nabi Shariatifar, Maryam Moazzen, Majid Arabameri , Mojtaba Moazzen, Gholamreza Jehed Khaniki, Parisa Sadighara. Measurement of polycyclic aromatic hydrocarbons (PAHs) in edible mushrooms (raw, grilled and fried) using MSPE-GC/MS method: a risk assessment study. (2021) Appl. Biol. Chem. 64: 61**

The purpose of this study was to evaluate the concentration of PAHs and health risk in edible mushrooms using magnetic solid phase extraction (MSPE) and gas chromatography/mass spectrometry (GC/MS) method. Based on the results, the concentration of total PAHs was in the range from 0.82 to 6.25 µg/kg (with the means of 3.85  ±  0.24 µg/kg). In this study, acenaphthylene (Ace) had the maximum mean level in mushroom samples (0.84  ±  0.19 μg/kg) and benzo(a)pyrene (BaP), acenaphthene (Ac), phenanthrene (Pa), dibenzo[a,h]anthracene (DahA), benzo(a)anthracene (BaA), fluoranthene (Fl), indeno[1,2,3-cd]pyrene (IP) and benzo[g,h,i]perylene (BgP) had minimum mean level (non-detected). The highest mean values of total PAHs (4.88  ±  0.30 μg/kg) was detected in grilled mushroom samples and lowest mean of total PAHs (2.77  ±  0.04 μg/kg) was detected in raw mushroom samples. The concentration of contamination in edible mushrooms was raw  <  fried  >  grilled, respectively. Furthermore, heat map visualization was applied to evaluate the relationship between the quantity and type of 16 PAHs in edible mushroom samples. Ultimately, the incremental lifetime cancer risk in edible mushrooms for adults and children was 6.85E-08 and 3.47E-07, respectively, which was lower than the acceptable risk limit (10–6) and all of the edible mushrooms sold in Tehran were considered safe for consumers.

**Jin Hwan Lee, Hee Yul Lee, Du Yong Cho, Min Ju Kim, Jea Gack Jung, Eun Hye Jeong, Md. Azizul Haque, Kye Man Cho. Biodegradable properties of organophosphorus insecticides by the potential probiotic *Lactobacillus plantarum* WCP931 with a degrading gene (*opd*C). (2021) Appl. Biol. Chem. 64: 62**

An organophosphorus (OP) insecticide-mineralizing strain, *Lactobacillus plantarum* WCP931, harboring a new OP hydrolase (opdC) gene, was isolated during *kimchi* (Korean traditional food) fermentation. Strain WCP931 exhibited a significant survival rate of 51 to 96% under artificial gastric acid conditions at pH 2 to 3 after 3 h. The opdC gene, consisting of 831 bp encoding 276 amino acids, was cloned from strain WCP907. Recombinant Escherischia coli harboring the opdC gene depleted 77% chlorpyrifos (CP) in M9 minimal medium after 6 days of incubation. The OpdC enzyme represents a novel member of the GHSQG family of esterolytic enzymes or a new Opd group. The OpdC molecular mass was estimated to be approximately 31 kDa by SDS-PAGE and showed maximum activity at pH 6 and 35 °C. The mutated OpdC (Ser116 → Ala116) enzyme had no activity towards OP insecticides and *ρ*-nitrophenol-β-butyrate. Importantly, the relative activity of OpdC protein against chlorpyrifos, coumafos, diazinon, fenamifos, methyl parathion, and parathion was higher than that against cadosafos, dyfonate, and ethoprofos insecticides. These results suggested the involvement of OpdC in the biodegradation of OP insecticide-contaminated cabbage during fermentation. The new OpdC enzyme expands the heterogeneity of the lactic acid bacterial Opd enzyme group in nature.

**Tae hyung Kwon, Dong bin Kim, Kyung won Kim, Min goo Park, Gwang hyun Roh, Byung ho Lee. Scaled-up ethyl formate fumigation to replace methyl bromide on traded mushroom to disinfest mushroom fly (*Lycoriella mali*). (2021) Appl. Biol. Chem. 64: 64**

Mushroom fly, *Lycoriella mali* (Diptera: Sciaridae), is the primary pest in imported mushrooms. The amount of *Tricholoma matsutake* imported from China increases every fall when it is harvested. When importing *T. matsutake,* disinfestation using methyl bromide (MB) or phosphine (PH3) is performed to prevent the introduction of *L. mali*. However, MB will be phased out due to ozone-depletion, chronic toxicity to workers, and residual issues. PH3 fumigation in mushroom disinfestation requires a long exposure time (24 h). In this study, we used ethyl formate (EF), which can replace MB and reduce exposure time. The efficacy of EF, PH3 and EF + PH3 on *L. mali* was evaluated. Using 4-h EF fumigation at 5 °C, the 3rd and 4th instar was the most tolerant stage in terms of 99% killed lethal concentration × time products (LCt99%). When 4-h EF fumigation at 5 °C was applied on all stages of *L. mali*, the LCt99% values of EF were 73.1 g h/m3 to the 1st and 2nd instar, 112.9 g h/m3 to the 3rd and 4th instar, 68.9 g h/m3 to pupae, and 20.1 g h/m3 to adult. It was confirmed that combination treatment with EF + PH3 had a synergistic effect on *L. mali*. The LCt99% of EF + 0.5 g/m3 of PH3 to the 3rd and 4th instar was 48.3 g h/m3. When only 140 g/m3 of EF was applied for 4 h at > 5 °C and 35 g/m3 of EF + 0.5 g/m3 of PH3 for 4 h at > 5 °C in commercial trials containing *T. matsutake*, proven efficacy (100%) on *L. mali* was confirmed. In the case of EF treatment only, phytotoxic damage occurred due to high Ct products, and there was no phytotoxic damage in combination treatment with EF + PH3. This study provides a new guideline for EF + PH3 combination treatment within a shorter exposure time (4 h) than existing PH3 treatment (24 h) and replacement of MB use.

**Seung-A. Baek, So Yeon Kim, Young Jin Park, Tae Jin Kim, Sun-Hyung Lim, Sang Un Park, Jae Kwang Kim. Improved annotation and quantification of metabolites in rice (*Oryza sativa* L.) seeds using two-dimensional gas chromatography–time-of-flight mass spectrometry**

**. (2021) Appl. Biol. Chem. 64: 65**

Two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC  ×  GC-TOFMS) is a powerful tool for identification of compounds in complex samples. Herein, we compared the GC  ×  GC-TOFMS and GC-TOFMS systems for polar metabolite profiling of rice seeds. Forty-seven and thirty-six metabolites were detected in a Korean rice cultivar, Dongjin, using GC  ×  GC-TOFMS and GC-TOFMS, respectively. The limits of detection of shikimic, *p*-coumaric, and sinapinic acids were 30.0-, 1.6-, and 2.5-times lower, respectively, with GC  ×  GC-TOFMS than with GC-TOFMS. The overlapped peaks of glycerol and phosphoric acid in GC-TOFMS were separated in GC  ×  GC-TOFMS. Polar metabolite profiling of two white and six red rice cultivars was performed using GC  ×  GC-TOFMS and the obtained data were subjected to principal component analysis. Remarkably, principal component 1 separated Heugdaegu from other cultivars, indicating that Heugdaegu has high levels of caffeic, sinapinic, and vanillic acids. Findings from this work may aid breeding programs aimed at improving the quality of rice seeds.

**Do Huu Nghi, Harald Kellner, Enrico Büttner, Le Mai Huong, Le Xuan Duy, Vu Dinh Giap, Dang Thu Quynh, Tran Thi Nhu Hang, An Verberckmoes, Ludo Diels, Christiane Liers, Martin Hofrichter. Cellobiose dehydrogenase from the agaricomycete *Coprinellus aureogranulatus* and its application for the synergistic conversion of rice straw. (2021) Appl. Biol. Chem. 64: 66**

From the biotechnological viewpoint, the enzymatic disintegration of plant lignocellulosic biomass is a promising goal since it would deliver fermentable sugars for the chemical sector. Cellobiose dehydrogenase (CDH) is a vital component of the extracellular lignocellulose-degrading enzyme system of fungi and has a great potential to improve catalyst efficiency for biomass processing. In the present study, a CDH from a newly isolated strain of the agaricomycete *Coprinellus aureogranulatus* (*Cau*CDH) was successfully purified with a specific activity of 28.9 U mg−1. This pure enzyme (MW = 109 kDa, *p*I = 5.4) displayed the high oxidative activity towards β-1–4-linked oligosaccharides. Not least, *Cau*CDH was used for the enzymatic degradation of rice straw without chemical pretreatment. As main metabolites, glucose (up to 165.18 ± 3.19 mg g−1), xylose (64.21 ± 1.22 mg g−1), and gluconic acid (5.17 ± 0.13 mg g−1) could be identified during the synergistic conversion of this raw material with the fungal hydrolases (e.g., esterase, cellulase, and xylanase) and further optimization by using an RSM statistical approach.

**Xianjun Liu, Xinxin Gao, Hao Li, Zhandong Li, Xiaoe Wang, Li Zhang, Bo Wang, Xinxin Chen, Xianglong Meng, Jiaao Yu. Ellagic acid exerts anti-fibrotic effects on hypertrophic scar fibroblasts via inhibition of TGF-β1/Smad2/3 pathway. (2021) Appl. Biol. Chem. 64: 67**

Hypertrophic scar (HS) is a kind of serious pathological scar with no currently effective treatment. HS fibroblasts (HSFs) are the main effector cells for HS formation. Ellagic acid (EA) exerts regulatory effects in some diseases, but its role in HS remains unclear. This study aimed to evaluate the effect of EA on the fibrotic phenotypes of HSFs and to further investigate the downstream signaling mechanism. The cell counting kit-8 (CCK-8) assay was used to perform cytotoxicity and proliferation assays. HSFs migration was assessed using wound healing and transwell assays. HSFs contraction was measured by a collagen lattice contraction assay and detection of α-smooth muscle actin (α-SMA) expression. The levels of mRNA and protein were determined by qPCR and western blotting, respectively. The results showed that EA inhibited the proliferation, migration, and contraction of HSFs and collagen expression in HSFs in a dose-dependent manner. Furthermore, EA not only suppressed the Smad2/3 pathway but also reversed TGF-β1-induced activation of the Smad2/3 pathway and up-regulation of the fibrotic cellular phenotypes in HSFs. These findings demonstrate that EA exerts anti-fibrotic effects on HSFs by blocking the TGF-β1/Smad2/3 pathway, which indicates that EA is a potential therapeutic candidate for treatment of HS.

**Shamim Ahmed, Tae-Young Heo, Aritra Roy Choudhury, Denver I. Walitang, Jeongyun Choi, Tongmin Sa. Accumulation of compatible solutes in rice (*Oryza sativa* L.) cultivars by inoculation of endophytic plant growth promoting bacteria to alleviate salt stress. (2021) Appl. Biol. Chem. 64: 68**

Salinization of agricultural lands, particularly rice paddies, results in the drastic decline of crop yields. Soil salinization impacts the plant physiology by inducing salt stress which may leads to osmotic stress, ionic stress and water-related nutrient imbalance. These imbalances necessitate the need for plants to produce osmolytes including proline and glycine betaine. This study aimed to elucidate the dynamic changes in proline and glycine betaine accumulation modulated by the inoculation of *Brevibacterium linens* RS16 in salt-sensitive and moderately salt-tolerant rice plants under salt stress conditions. This study showed the interaction of four major factors including rice genotypes with differing tolerance to salt stress, length of exposure to salt stress, level of salt stress and effects of inoculation. Salt stress resulted in significant reduction in plant growth parameters with the salt-sensitive rice genotype (IR29) having a more significant growth reduction. Both the salt-sensitive and salt-tolerant rice genotypes increased in total proline and glycine betaine accumulation at 3 days and 10 days after subjecting under 50 mM and 150 mM salt stress conditions. A significant increase in proline and glycine betaine was observed in the salt-sensitive genotype after 10 days under 50 mM and 150 mM salt stress conditions. Inoculation of the rice genotypes with *B. linens* RS16 resulted in the improvement of plant growth parameters in both rice genotypes, and total proline and glycine betaine accumulation, especially in IR29. This study showed that proline and glycine betaine are compatible osmolytes of rice under salt stress, and that inoculation of rice genotypes with *B. linens* RS16 mediated salt tolerance through improvement of plant growth parameters and proline and glycine betaine accumulation in rice plants.

**Pandu Hariyono, Jasvidianto Chriza Kotta, Christophorus Fideluno Adhipandito, Eko Aprilianto, Evan Julian Candaya, Habibah A. Wahab, Maywan Hariono. A study on catalytic and non-catalytic sites of H5N1 and H1N1 neuraminidase as the target for chalcone inhibitors. (2021) Appl. Biol. Chem. 64: 69**

The H1N1 pandemic in 2009 and the H5N1 outbreak in 2005 have shocked the world as millions of people were infected and hundreds of thousands died due to the infections by the influenza virus. Oseltamivir, the most common drug to block the viral life cycle by inhibiting neuraminidase (NA) enzyme, has been less effective in some resistant cases due to the virus mutation. Presently, the binding of 10 chalcone derivatives towards H5N1 and H1N1 NAs in the non-catalytic and catalytic sites was studied using molecular docking. The in silico study was also conducted for its drug-like likeness such as Lipinski Rule, mutagenicity, toxicity and pharmacokinetic profiles. The result demonstrates that two chalcones (**1c** and **2b**) have the potential for future NA inhibitor development. Compound **1c** inhibits H5N1 NA and H1N1 NA with IC50 of 27.63 µM and 28.11 µM, respectively, whereas compound **2b** inhibits NAs with IC50 of 87.54 µM and 73.17 µM for H5N1 and H1N1, respectively. The in silico drug-like likeness prediction reveals that **1c** is 62% better than **2b** (58%) in meeting the criteria. The results suggested that **1c** and **2b** have potencies to be developed as non-competitive inhibitors of neuraminidase for the future development of anti-influenza drugs.

**Sunhye Lee, Soyeon Jeong, Yeongju Park, Hyunji Seo, Cheongbin You, Unsik Hwang, Hoon Park, Hee-jae Suh. Supplementation of non-fermented and fermented goji berry (*Lycium barbarum*) improves hepatic function and corresponding lipid metabolism via their anti-inflammatory and antioxidant properties in high fat-fed rats. (2021) Appl. Biol. Chem. 64: 70**

Development of obesity is associated with excessive fat accumulation and oxidative stress along with chronic inflammation. Goji berries (*Lycium barbarum*) are high in polyphenolic compounds and have anti-inflammatory, anti-oxidant, and hypolipidemic properties that may alleviate the pathogenesis of obesity and related metabolic complications. Thus, the aim of this study was to investigate potential metabolic benefits of GB supplementation against high fat (HF) diet-induced obesity and its comorbidities in HF diet-fed rats (male Sprague–Dawley, n  =  8/group, 6 weeks old). We also sought to examine the potential metabolic benefits of fermented GB (FGB) with *L. plantarum* CB3 and possible distinctions in the degree and/or mechanism of action compared to GB. GB and FGB supplementation suppressed the gene expression of inflammation indices at the local (adipose tissues) and systemic (liver) levels. In addition, GB and FGB supplementation upregulated the gene expression of antioxidant enzymes compared to the HF and/or even low fat (LF) group with more remarkable antioxidant effects by GB supplementation. Also, GB and FGB supplementation protected from HF-induced damages of the liver and dyslipidemia. In conclusion, we demonstrated that GB and FGB supplementation protected from HF-induced metabolic complications primarily by improving hepatic function and corresponding lipid metabolism via their anti-inflammatory and antioxidant properties. To our knowledge, this is the first in vivo study confirming metabolic benefits of GB in a fermented form. Thus, these findings support the potential application of both GB and FGB to ameliorate obesity-associated metabolic abnormalities.

**Shin-Won Lee, Han Kim, Joong-Hoon Ahn. Biosynthesis of ethyl caffeate via caffeoyl-CoA acyltransferase expression in *Escherichia coli*. (2021) Appl. Biol. Chem. 64: 71**

Hydroxycinnamic acids (HCs) are natural compounds that form conjugates with diverse compounds in nature. Ethyl caffeate (EC) is a conjugate of caffeic acid (an HC) and ethanol. It has been found in several plants, including *Prunus yedoensis*, *Polygonum amplexicaule*, and *Ligularia fischeri*. Although it exhibits anticancer, anti-inflammatory, and antifibrotic activities, its biosynthetic pathway in plants still remains unknown. This study aimed to design an EC synthesis pathway and clone genes relevant to the same. Genes involved in the caffeic acid synthesis pathway (tyrosine ammonia-lyase (TAL) and *p*-coumaric acid hydroxylase (HpaBC)) were introduced into *Escherichia coli* along with 4-coumaroyl CoA ligase (4CL) and acyltransferases (AtCAT) cloned from *Arabidopsis thaliana*. In presence of ethanol, *E. coli* harboring the above genes successfully synthesized EC. Providing more tyrosine through the overexpression of shikimate-pathway gene-module construct and using *E. coli* mutant enhanced EC yield; approximately 116.7 mg/L EC could be synthesized in the process. Synthesis of four more alkyl caffeates was confirmed in this study; these might potentially possess novel biological properties, which would require further investigation.

**Jianyu Li, Xin Zhao, Laura S. Bailey, Manasi N. Kamat, Kari B. Basso. Identification and characterization of proteins, lipids, and metabolites in two organic fertilizer products derived from different nutrient sources. (2021) Appl. Biol. Chem. 64: 72**

The biochemical composition of organic fertilizers largely determines their nutrient supply characteristics following soil application as well as their potential impact on soil microbial communities. Yet, limited information is available regarding the biochemical composition of organic fertilizers derived from different nutrient sources. Here, we qualitatively analyzed the presence and abundance of proteins, lipids, and metabolites in a liquid fish fertilizer (LFF) product and a type of granular organic fertilizer (GOF) commonly used in organic vegetable production, using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Our results suggest that the presence and abundance of proteins, lipids, and metabolites differ greatly between GOF and LFF. The qualitative analysis shows LFF as a rich source of metabolites, while complex proteins and long-chain saturated fatty acids are dominant in GOF. The degree of biochemical composition complexity may help explain the varying impacts of different types of organic fertilizers on nutrient availability, soil health, and environmental quality.

**GahYoung Baek, Maham Saeed & Hyung-Kyoon Choi. Duckweeds: their utilization, metabolites and cultivation. (2021) Appl. Biol. Chem. 64: 73**

Duckweeds are floating plants of the family *Lemnaceae*, comprising 5 genera and 36 species. They typically live in ponds or lakes and are found worldwide, except the polar regions. There are two duckweed subfamilies*—*namely *Lemnoidea* and *Wolffioideae*, with 15 and 21 species, respectively. Additionally, they have characteristic reproduction methods. Several metabolites have also been reported in various duckweeds. Duckweeds have a wide range of adaptive capabilities and are particularly suitable for experiments requiring high productivity because of their speedy growth and reproduction rates. Duckweeds have been studied for their use as food/feed resources and pharmaceuticals, as well as for phytoremediation and industrial applications. Because there are numerous duckweed species, culture conditions should be optimized for industrial applications. Here, we review and summarize studies on duckweed species and their utilization, metabolites, and cultivation methods to support the extended application of duckweeds in future.

**Chen-Xi Ren, Xin Jin, Dan-Ping Xie, Xiao-Yu Guo, Li-Yun Yu, Yu-Dong Cui, Taeho Kwon, Hu-Nan Sun. Hispidin attenuates bleomycin-induced idiopathic pulmonary fibrosis via an anti-oxidative effect in A549 cells. (2021) Appl. Biol. Chem. 64: 74**

Idiopathic pulmonary fibrosis (IPF) is a serious and irreversible chronic lung disease. Bleomycin (BLM) is an anticancer drug, which can cause severe lung toxicity. The main target of oxidative stress-induced lung injury is alveolar epithelial cells, which lead to interstitial fibrosis. The present study investigated whether hispidin (HP), which has excellent antioxidant activity, attenuates bleomycin-induced pulmonary fibrosis via anti-oxidative effects in A549 cells. We found that hispidin reduced bleomycin-induced fibrosis of A549 cells by reducing reactive oxygen species (ROS) levels and inhibiting epithelial-mesenchymal transition. Taken together, our data suggest that hispidin has therapeutic potential in preventing bleomycin-induced pulmonary fibrosis.

**Seong-Ah Shin, Jun Seob Lee, Byeong Jun Joo, Gyoungah Ryu, Minjoo Han, Huiji Kim, Jangeun An, Man Hyung Koo, Ui Joung Youn, Jun Hyuck Lee, Hyun Ho Park, Chang Sup Lee. Anti-cancer effects of lucidadiol against malignant melanoma cells. (2021) Appl. Biol. Chem. 64: 75**

Melanoma is one of the most aggressive and lethal skin cancers. Lucidadiol is a triterpenoid isolated from *Ganoderma lucidum* and is known to have various biological functions, including antibacterial effects. However, the anti-cancer effects and mechanism of action of lucidadiol in malignant melanoma are unknown. In this study, lucidadiol significantly reduced B16 melanoma cell viability in a dose- and time-dependent manner. In addition, lucidadiol induced apoptosis and suppressed cell mobility in B16 melanoma cells. Moreover, our findings revealed that lucidadiol remarkably downregulated phospho-Akt/ERK/JNK, but not p38. Taken together, our results suggest that lucidadiol could exerts its anti-cancer effects by inducing apoptosis via modulation of the Akt/MAPK pathway. Therefore, lucidadiol may be a potential cancer therapeutic agent for malignant melanoma.

**Bin Dong, Guowen Zhou, Yanjun Lin, Cailing Yu, Jun Wang, Chunlong Sun, Tao Wu. Antimicrobial property of recombinant Lactolisterin BU in vitro and its initial application in pork refrigerated storage. (2021) Appl. Biol. Chem. 64: 76**

Lactolisterin BU is a novel bacteriocin identified from *Lactococcus lactis* in 2017. It exhibits antimicrobial activity against food spoilage and foodborne pathogens. In this study, Lactolisterin BU was expressed in *Pichia pastoris* (*P.pastoris*) and isolated from the supernatant of yeast culture for the first time. It was found to exhibit a broad antimicrobial spectrum and rapid bactericidal activity against foodborne bacterial pathogens, both gram-positive and gram-negative ones, with minimum inhibition concentrations ranging within 10–60 μg/mL. The recombinant Lactolisterin BU **(**rLactolisterin BU) also had an antioxidant effect and was resistant to heating, acid–base, and high-dose-saline treatments and barely had any hemolytic activity or cytotoxicity. Moreover, rLactolisterin BU effectively suppressed the growth of bacterial pathogens; suppressed the increases in pH, total volatile basic nitrogen (TVB-N), and thiobarbituric acid reactive substances (TBARS) of pork samples; and maintained a high quality of fresh pork during storage at 4 ℃. Furthermore, rLactolisterin BU effectively inhibited the growth of three kinds of bacteria in a pork-spoilage model. Taken together, rLactolisterin BU could be a promising preservative for food storage.

**Xingyao Long, Yong-kyu Kim, Ting Yu, Ruokun Yi, Xin Zhao, Kun-young Park. The protective effect of Jangkanghwan (Korean traditional food) on lipopolysaccharide-induced disruption of the colonic epithelial barrier. (2021) Appl. Biol. Chem. 64: 77**

Jangkanghwan (JKH) is a Korean traditional food that is a mixture of food ingredients and traditional Korean medicine ingredients, and it has been observed to produce satisfactory anti-inflammatory, antioxidant, and antibacterial effects. In the current study, JKH was administered by gavage to BALB/C mice with lipopolysaccharide (LPS)-induced colonic epithelial dysfunction, and mouse body weight and food intake were recorded. Indexes such as colonic paracellular permeability, serum inflammatory cytokines, and bacterial translocation were used to comprehensively evaluate the regulatory effect of JKH on mouse colonic epithelial function, and qPCR and Western blot were also used to analyze the expression of tight junction (TJ)-related genes, such as occludin, claudin, zonula occludens (ZOs) proteins, and junction adhesion molecules (JAM) in the colonic epithelial tissue. The experimental results indicated that JKH relieved the edema of the liver, spleen, and mesenteric lymph node tissues, and reduced the loss of appetite and diarrhea caused by LPS injection in mice. It increased the amount of mice food intake from 3.7 g/day in the LPS group to 4.7 g per day; the water content in the feces of mice in the JKH group was 13.86% less than that in the LPS group. JKH reduced the inflammatory response in mice caused by LPS, protected the integrity of the colon, the permeability of fluorescent macromolecules was one-fourth of the LPS group, and enhanced the mRNA and protein expression of TJ-related proteins in colon tissue. Our findings highlight that JKH has benefits in intestinal health and relieving systemic inflammation, relevant aspects of its use as a functional food.

**Haonan Liu, Han Wu, Ying Wang, Fan Wang, Xiaoli Liu, Jianzhong Zhou. Enhancement on antioxidant and antibacterial activities of Brightwell blueberry by extraction and purification. (2021) Appl. Biol. Chem. 64: 78**

A blueberry anthocyanin extract was obtained from Brightwell blueberry fruits cultivated in eastern China and the extraction and purification conditions were optimized. The components of the anthocyanin extract were identified using ultra-performance liquid chromatography-electrospray ionization interface-mass spectrometer. The antioxidant and antibacterial activities of the blueberry fruit supernatant (BFS), blueberry anthocyanin crude extract (BCE), and blueberry anthocyanin rich extract (BRE) were evaluated. The extraction yield was 1.79 ± 0.0014 mg/g under the following optimal conditions: 1:20 solid-to-liquid ratio (v/w), 24 h, 34 °C, and 90% ethanol containing 0.21% (v/v) hydrochloric acid. With regard to purification, anthocyanin purity increased 19.1-fold. Nine fractions were identified as the glycosides of delphinidin, cyanidin, petunidin, and malvidin. The biological activities of the blueberry anthocyanin extract were improved through extraction and purification. Compared with BFS and BCE, BRE had a higher DPPH radical scavenging activity (EC50 = 0.51 mg/mL), ABTS antioxidant capacity (EC50 = 0.32 mg/mL), and oxygen radical absorbance capacity (0.43 mmol Trolox/g). Furthermore, BRE (2 mg/mL) showed a maximum of 84.64 ± 0.35% reduction in the biofilm biomass of *Listeria monocytogenes* and the inhibition zone given by BRE against *Escherichia coli* was 16.04 ± 0.38 mm. BRE showed the highest antioxidant capacities and obvious antibacterial effects against food-related microorganisms than the other samples. Therefore, BRE can be used as a natural antioxidant and antibacterial agent and has potential health advantages and food industry applications.

**Hyoung-Geun Kim, Trong Nguyen Nguyen, Yeong-Geun Lee, Min-Ho Lee, Dae Young Lee, Youn-Hyung Lee, Nam-In Baek. New phenylalkanoids from the rhizome of *Cnidium officinalis* Makino. (2021) Appl. Biol. Chem. 64: 79**

*Cnidium officinalis* rhizomes were immersed in 80% MeOH. The extract was fractionated to water, *n*-butanol, and ethyl acetate fractions (Fr). Open column chromatography was repeatedly carried out on n-butanol and ethyl acetate Fr using silica gel, octadecyl silica gel, and Sephadex LH-20 as the stationary phase affording five phenyl alkanoids **1**–**5** including two new ones. The molecular structures including stereochemistry were decided based on spectroscopic interpretation of nuclear magnetic resonance, mass spectrometry, and infrared spectroscopy as well as chemical reaction. Three known compounds, coniferyl alcohol methyl ether (**1**), vanillin (**2)**, and coniferyl aldehyde (**3**), were reported in the beginning for this plant by authors. Two new phenyl alkanoids were named, 7-methoxyeugenol and cnidiumoside.

**So Hyun Jeon, Cha-Gyun Shin. Effect of a novel piperazine compound on cancer cells. (2021) Appl. Biol. Chem. 64: 80**

*Many drugs have been developed for anticancer chemotherapy. However, more anti-cancer drugs should be developed from potential chemicals to circumvent the disadvantages of existing drugs. Most anti-cancer chemicals induce apoptosis in cancer cells. This study tested the efficiency of a new chemical, the piperazine derivative 1-[2-(Allylthio) benzoyl]-4-(4-methoxyphenyl) piperazine (CB01), on glioblastoma (U87) and cervix cancer (HeLa) cells. CB01 was highly cytotoxic to these cells (IC50S  < 50 nM) and induced the traditional apoptotic symptoms of DNA fragmentation and nuclear condensation at 40 nM. Western-blot analysis of the cell lysates revealed that the intracellular apoptotic marker proteins, such as cleaved caspase-3, cytochrome c, and Bax, were highly upregulated in the CB01-treated cells. Furthermore, increased activities of caspase-3 and -9, but not caspase-8, were observed. Therefore, these results suggest that CB01 can act as an anticancer chemotherapeutic by stimulating the intrinsic mitochondrial signaling pathway to induce cytotoxicity and apoptosis in cancer cells.*

**Jong Eun Park, Geum Seok Jeong, Hyun Woo Lee, Hoon Kim. Biochemical characterization of a family IV esterase with *R*-form enantioselectivity from a compost metagenomic library. (2021) Appl. Biol. Chem. 64: 81**

A novel family IV esterase (hormone-sensitive lipase, HSL) gene, *est15L,* was isolated from a compost metagenomic library. Encoded Est15L comprised 328 amino acids with a molecular weight of 34,770 kDa and was an intracellular esterase without a signal peptide. The multiple sequence alignment (MSA) of Est15L with other family IV esterases showed conserved regions such as HGG, DYR, GXSXG, DPL, and GXIH. Native Est15L was a dimeric form from the results of size exclusion chromatography. It was optimally active at 50 ℃ and pH 9.0, indicating alkaline esterase. However, it showed a low thermostability with half-lives of 30.3 at 30 ℃ and 2.7 min at 40 ℃. It preferred *p*-nitrophenyl butyrate (C4) with Km and Vmax values of 0.28 mM and 270.8 U/mg, respectively. Est15L was inhibited by organic solvents such as 30% methanol, isopropanol, and acetonitrile with residual activities of 12.5, 0.9, and 0.3%, respectively. It was also inhibited by 1% SDS and 1% PMSF; however, Est15L maintained its activity at 1% Triton X-100 and EDTA. Est15L was inhibited by Cu2+, Zn2+, Mn2+, Co2+, Fe2+, and Na+. In addition, Est15L hydrolyzed glyceryl tributyrate with a residual substrate amount of 43.7% at 60 min but could not hydrolyze the oils (fish and olive) and glyceryl trioleate. Interestingly, Est15L showed significant enantioselectivity toward the *R*-form with a residual substrate amount of 44.6%, lower than that of the *S*-form (83.5%). Considering its properties, Est15L can be a potential candidate for chemical reactions, such as the synthesis of pharmaceutical compounds.

**Hyun Young Shin, Hoon Kim, Soontag Jung, Eun-Jin Jeong, Kyung-Haeng Lee, Yun-Jung Bae, Hyung Joo Suh, Keum-Il Jang, Kwang-Won Yu. Interrelationship between secondary metabolites and antioxidant capacities of *Centella asiatica* using bivariate and multivariate correlation analyses. (2021) Appl. Biol. Chem. 64: 82**

*Centella asiatica* L. *Urban* (CA) is a valuable medicinal herb that contains various bioactive secondary metabolites. In the present study, the harvested CA was divided into whole plant and leaf parts, and were heated-air-dried or freeze-dried. The dried CA was extracted under various extraction conditions to obtain 16 extracts, and their antioxidant activities were examined. Twelve types of secondary metabolites (five polyphenolic acids, four triterpenoids, and three flavonoids) were quantified in each extract. Finally, the intercorrelations between secondary metabolites and antioxidant activities were analyzed through statistical analyses, such as Pearson correlation coefficient, scatter plotting, and principal component analysis. The antioxidant capacities of CA might be primarily influenced by major triterpenoids such as madecassoside and asiaticoside, which showed high content in the ethanol extracts of freeze-dried leaf parts. The present study provides a valuable groundwork for the development of optimal extraction processes for *C. asiatica* L. as an antioxidant material.

**Yujia Bai, Zuoshan Feng, Maerhaba Paerhati, Jin Wang. Phenylpropanoid metabolism enzyme activities and gene expression in postharvest melons inoculated with *Alternaria alternata*. (2021) Appl. Biol. Chem. 64: 83**

This study explored the mechanism of melon resistance to *Alternaria alternata* (*A*. *alternata*) infection in Jiashi and 86-1 melons. Melons were inoculated with *A*.*alternata* and the change in lesion diameter was measured. The changes in cinnamic acid-4-hydroxylase (C4H), phenylalanine ammonia lyase (PAL), and 4-coumaric acid coenzyme A ligase (4CL) activity and gene expression were studied in the pericarp tissues of Jiashi and 86-1 melons. The lesion diameter was smaller in Jiashi melon than in 86-1 melon, and the pericarp lesions were smaller than pulp lesions, indicating that Jiashi melon can resist *A*. *alternata* infection better than 86-1 melon. After inoculation with *A*.*alternata*, the C4H, PAL, and 4CL activities of Jiashi and 86-1 melons peaked in the middle and late storage period, and the peak was higher in Jiashi melons. The gene expression changes were consistent with the enzyme activity. The *C4H*, *PAL*, and *4CL* gene expression was significantly higher in Jiashi melon pericarp than in 86-1 melon, and the C4H, PAL, and 4CL activities in Jiashi melon were positively correlated with their gene expression, confirming the role of phenylpropanoid metabolism enzymes in resistance to *A*.*alternata*.

**Sang Mi Lee, Ji-Yoon Jeong, Joon-Goo Lee, Young-Suk Kim. Effects of the absorbent types on changes in benzo[*a*]pyrene and volatile compounds in sesame oil. (2021) Appl. Biol. Chem. 64: 84**

The effects of different absorbent types on changes in benzo[*a*]pyrene (BaP) and volatiles in sesame oil during filtration processes were investigated using gas chromatography–mass spectrometry analysis. The BaP content was greatly reduced after filtration using powdered activated carbons (activated carbon made from peat: PP, activated carbon made from coconut shell: PC, activated carbon made from wood: PW) in comparison to granular activated carbons (activated carbon made from coconut shell: GC, activated carbon made from wood: GW). The BaP content in sesame oil was also considerably reduced when filtrated with a PW–acid clay mixture in comparison to PW–kaolin and PW–celite mixtures. Most volatile compounds were also greatly reduced after filtration using powdered activated carbons (PP, PC, and PW) in comparison to granular activated carbons (GC and GW). These results might be due to the relatively ionic structure on the surface and internal surface area of absorbent pores.

**Eun-Ha Kim, Kyeong Min Lee, So-Young Lee, Mira Kil, Oh-Hun Kwon, Sang-Gu Lee, Seong-Kon Lee, Tae-Hun Ryu, Seon-Woo Oh, Soo-Yun Park. Influence of genetic and environmental factors on the contents of carotenoids and phenolic acids in red pepper fruits (*Capsicum annuum* L.). (2021) Appl. Biol. Chem. 64: 85**

Red pepper is enriched in antioxidant components, such as carotenoids, phenolic compounds, and vitamins. In this study, we investigated the natural variability in the content of carotenoids and phenolic acids in 11 red pepper cultivars grown in two locations in South Korea during 2016, 2017, and 2018. Seven carotenoids and six phenolic acids, including soluble and insoluble forms, were detected in the red fruit pericarps. The major carotenoids were β-carotene (40%) and capsanthin (20%). The content of insoluble phenolic acids was higher than that of soluble phenolic acids because of the large amount of insoluble *p-*coumaric acid. The statistical analysis of combined data showed significant differences among varieties, locations, and years for most of the measured components. The results from variance component analysis indicated that the effects of location, year and the interaction of location and year mainly accounted for the variation in carotenoids, whereas variations in phenolic acid content were attributed to year and variety. In addition, the results of principal component analysis and orthogonal partial least-squares discriminant showed that carotenoids were well discriminated by location and year, whereas phenolic acids were distinctively separated only by year. The data from this study could explain the natural variation in the content of carotenoids and phenolic acids in red pepper fruits by genotype and environment.

**Ehsan Vafa, Reza Bazargan-Lari. Bovine serum albumin protected gold nanozymes as a novel anti-cancer nanodrug for acute T-type lymphoblastic leukemia treatment via effect on the expression of anti-apoptotic genes. (2021) Appl. Biol. Chem. 64: 86**

In this paper, the bovine serum albumin protected gold nanozymes (BSA-Au nanozymes) were utilized as a novel nanodrug for treatment of acute T-type lymphoblastic leukemia (Jurkat) by production of excessive ROS and effect on the expression of anti-apoptotic genes. The effect of BSA-Au nanozymes on the Bcl-2 expression and survivin in the Jurkat cell line was checked. The results showed that the expression of anti-apoptotic genes was significantly reduced after treatment of the Jurkat cell line with the BSA-Au nanozymes (p-value of 0.001) as the potential nanodrug while their expression in the normal PBMC was not affected by the nanodrug. Moreover, the cytotoxic effect of the developed nanodrug on the Jurkat cell line was evaluated which illustrated that survival rate in the studied cell line reaches its minimum value (100% lethality, 0.0% survival) after treatment for 48 h. The IC50 for the nanodrug was calculated at 0.05 mM of the developed nanodrug. Overall, the BSA-Au nanozymes can be used as the nanodrug for treatment of T-type lymphoblastic leukemia via reducing the expression of anti-apoptotic genes, increasing the effect of common anticancer drugs such as Adriamycin and ara-C, and consequently increasing the survival of patients with leukemia.

**Yejin Lee, Jinwoo Jang, Yangwon Jeon, Hyojin Kim, Geupil Jang, Youngdae Yoon. Assessing the effects of accumulated Cd(II) on seed germination and root development of *Arabidopsis thaliana*. (2021) Appl. Biol. Chem. 64: 87**

In this study, *Arabidopsis thaliana* was used as a model system to assess the toxic effects of cadmium on plant development and growth. The germination and growth of *A. thaliana* was inhibited by Cd(II), and the inhibitory effect was dosage-dependent. The significant decrease of germination rates and root growths of *A. thaliana* were observed from 50 mg/L and 25 mg/L of CdCl2, respectively. Although both shoot and root growths were suppressed by Cd(II), root developments were more sensitive to Cd(II) than shoot developments, as evidenced by shoot growths observed over 50 mg/L of CdCl2. In the concordance to this result, it was also observed that the expression of *DR5::VENUS*, a visual marker of auxin response, was dependent on the Cd(II) concentration and was strongly reduced from 5 mg/L of CdCl2. In addition, the *E. coli*-based biosensors were employed to quantify accumulated Cd(II) in plants to understand the correlation between toxic effects and Cd(II) in plants. As a result, it was revealed that 0.012 mg/g and 0.138 mg/g of Cd(II) in dried plants were corresponded to the concentration inhibiting root developments and root growths, respectively. Although it needs further investigations, the findings play a significant role in assessing the toxic effects of Cd(II) based on the relationship between the toxic effects and accumulated Cd(II) concentrations in plants.

**Jun-Hwan Park, Hoi-Seon Lee, Namhyun Chung. Acaricidal and repellent activities of *Litsea cubeba* (Lour.) oil and 3,7-dimethyl-2,6-octadienal against *Haemaphysalis longicornis* (Acari: Ixodidae). (2021) Appl. Biol. Chem. 64: 88**

The bioactivity of the essential oil extracted from *Litsea cubeba* fruits against tick vectors of severe fever with thrombocytopenia syndrome is unknown. In this study, *L. cubeba* oil and its main constituents, 3,7-dimethyl-2,6-octadienal and its similar structures, were evaluated for their acaricidal and repellent activities on the unfed nymphs and adults of *Haemaphysalis longicornis*. *L. cubeba* oil displayed both acaricidal and repellent activities against both life stages. Among the constituents of *L. cubeba* oil, only 3,7-dimethyl-2,6-octadienal exhibited both acaricidal and repellent activities against both life stages. In a repellent bioassay, 3,7-dimethyl-2,6-octadienal and *L. cubeba* oil at dose of 0.08 mg/cm2 provided excellent repellence (100%) against the nymphs and adults for over 60 min post-application. When the acaricidal and repellent activities of 3,7-dimethyl-2,6-octadienal and its similar structures were compared, activities of all tested derivatives were significantly less potent than those of 3,7-dimethyl-2,6-octadienal. The strong acaricidal and repellent activities of 3,7-dimethyl-2,6-octadienal in *L. cubeba* oil suggests that it is a promising natural candidate for developing new sustainable acaricidal and repellent agents.

**Dong Gun Lee, Ji Min Lee, Chang Geun Choi, Hojoung Lee, Jun Cheol Moon, Namhyun Chung. Effect of plant growth-promoting rhizobacterial treatment on growth and physiological characteristics of *Triticum aestivum* L. under salt stress. (2021) Appl. Biol. Chem. 64: 89**

Salinity stress is a serious abiotic stress that affects crop quality and production. Rhizospheric microbes have immense potential in synthesizing and releasing various compounds that regulate plant growth and soil physicochemical properties. The aim of the present study was to evaluate the efficacy of indole-3-acetic acid (IAA)-producing rhizobacteria as biofertilizers under salt stress. Among the isolated strains from various soil samples, *Bacillus megaterium* strain PN89 with multifarious plant growth-promoting traits was selected and used as a monoculture and co-culture with two other standard strains. The plant promoting activity was evaluated using the paper towel method and pot test to observe the effects on the early stage and vegetative growth of wheat (*Triticum aestivum* L.). The treatment using PGPR strain presented noticeable but varying effects on plant growth under salt stress, that is, PGPR treatment often displayed a significant increase in germination percentage, root and shoot length, and other growth parameters of wheat compared to those in the non-inoculated control. Thus, these results suggest that *B. megaterium* PN89 can be applied as a bio-fertilizer to alleviate salt stress in *T. aestivum*.

**Seung-Su Lee, Hyoung-Geun Kim, Eun-Ha Park, Kwang Joong Kim, Myun-Ho Bang, Gayoung Kim, Hyeong-Ju Jeon, Chung-Gi Lee, Min-Chul Shin, Dae-Ok Kim, Nam-In Baek. Antioxidant and anti-inflammatory effects in lipopolysaccharide-induced THP-1 cells of coumarins from the bark of *Hesperethusa crenulata* R.. (2021) Appl. Biol. Chem. 64: 90**

All parts of Thanakha (*Hesperethusa crenulata* R.) have been used as traditional skin care herbal material in Myanmar. In this study, coumarins from *H. crenulata* R. bark were isolated through solvent extraction, systematic solvent fractionation, and repeated column chromatography. Spectroscopic analyses using ESI–MS, 1D NMR (1H and 13C), 2D NMR (gHSQC and gHMBC), specific rotation, circular dichroism, and IR spectrometry revealed three coumarins 2*R*-7-hydroxy-8-(2,3-dihydroxy-3-methylbutyl)-coumarin (compound **1**), peucedanol (compound **2**), and methylpeucedanol (compound **3**), which were first isolated from Thanakha tree. Antioxidant capacities of three coumarins decreased as follows: compound **2** > compound **3** > compound **1**. Treatments of lipopolysaccharide-induced THP-1 human monocytic cells with compounds **2** and **3** at 378.8 μM and 359.7 μM inhibited tumor necrosis factor-α production by approximately 32.7% and 13.3%, respectively, compared with the negative control. In summary, these results suggest that Thanakha bark extracts can be used as a potent antioxidant and anti-inflammatory source for cosmetic ingredients.

**Meththika Vithanage, S. S. Mayakaduwage, Viraj Gunarathne, Anushka Upamali Rajapaksha, Mahtab Ahmad, Adel Abduljabbar, Adel Usman, Mohammad I. Al-Wabel, James A. Ippolito, Yong Sik Ok. Animal carcass burial management: implications for sustainable biochar use**

**. (2021) Appl. Biol. Chem. 64: 91**

This review focuses on existing technologies for carcass and corpse disposal and potential alternative treatment strategies. Furthermore, key issues related to these treatments (e.g., carcass and corpse disposal events, available methods, performances, and limitations) are addressed in conjunction with associated environmental impacts. Simultaneously, various treatment technologies have been evaluated to provide insights into the adsorptive removal of specific pollutants derived from carcass disposal and management. In this regard, it has been proposed that a low-cost pollutant sorbent may be utilized, namely, biochar. Biochar has demonstrated the ability to remove (in)organic pollutants and excess nutrients from soils and waters; thus, we identify possible biochar uses for soil and water remediation at carcass and corpse disposal sites. To date, however, little emphasis has been placed on potential biochar use to manage such disposal sites. We highlight the need for strategic efforts to accurately assess biochar effectiveness when applied towards the remediation of complex pollutants produced and circulated within carcass and corpse burial systems.

**Se-Won Kang, Jin-Ju Yun, Jae-Hyuk Park, Yong Hwa Cheong, Jong-Hwan Park, Dong-Cheol Seo, Ju-Sik Cho. Effects of biochar and barley straw application on the rice productivity and greenhouse gas emissions of paddy field. (2021) Appl. Biol. Chem. 64: 92**

To improve the agricultural environment, utilization of biochar and organic materials from paddy fields gaining importance. This is because the long-term use of inorganic fertilizers aggravates the soil environment, and also because rice paddy is a major source of CH4 and N2O emissions during rice cultivation which involves continuous flooding. Recently, the application of organic materials and biochar to the soil has received increasing attention due to their potential benefits related to soil quality, crop growth, and greenhouse gas emission. This study examines the influence of biochar and straw treatments on rice growth, soil physicochemical properties, and global warming potential in the paddy field. Five treatments were applied for the study: control (Cn), inorganic fertilizer (IF), barley straw biochar (BC), barley straw (BS), and BC + BS. Soil quality after rice harvesting improved in the BC treated group. The yield components of rice were also improved in the BC + BS, compared to other treatments. These effects resulted in increased rice yield and uptake of nutrient contents in the BC + BS treatment. Total fluxes of CH4 and N2O relative to global warming significantly decreased by 37.3% and 65.2% in the BC + BS group than in the IF treatment, respectively. Consequentially, a cropping system with BC and BS is an effective strategy to improve rice productivity and soil quality and also reduce GHG emissions from paddy fields, thereby alleviating global warming.

**Byoung Hee Park, In Sung Kim, Jung Kuk Park, Zheng Zhi, Hea Min Lee, Oh Wook Kwon, Byung Cheon Lee. Probiotic effect of *Lactococcus lactis* subsp*. cremoris* RPG-HL-0136 on intestinal mucosal immunity in mice. (2021) Appl. Biol. Chem. 64: 93**

*Lactococcus lactis* subsp*. cremoris* is a lactic acid bacterium commonly used in the cheese manufacturing industry. It is known to produce antibacterial peptides and has recently received attention for its role as a probiotic strain. Here, we report the isolation of a new strain, *Lactococcus lactis* subsp*. cremoris* RPG-HL-0136 (RPG0136) from dried compost, which exhibits strong antibacterial activity. When RPG0136 was fed to mice, it increased the intestinal population of two beneficial bacteria, *Lactobacillus* and *Bifidobacterium*, whereas it decreased the intestinal population of two harmful bacteria, *Bacteroides* and *Enterobacter*. In addition, it increased the concentration of short-chain fatty acids, including acetic acid, propionic acid, and butyric acid, with a simultaneous decrease in pH, and accelerated the catabolic degradation of proteins, lipids, and starch. Lastly, RPG0136 increased the plasma IgG and intestinal mucosal SIgA concentrations and upregulated Reg3r, MUC1, and MUC2 expression to improve the intestinal mucosal immune function. The results of this study suggest that RPG0136 is a potential probiotic strain that supports the growth of a beneficial microbiome by promoting the synthesis of organic acids and enhancing intestinal immune function.

**Seon-A Choi, Jun-Hwan Park, Hoi-Seon Lee, Ji-Hoon Lee. Acaricidal properties of 5-methylfurfural identified from *Valeriana fauriei* and its structural analogues against synanthropic mites and Asian longhorned tick with color alterations. (2021) Appl. Biol. Chem. 64: 94**

Acaricidal activities and color alterations of 5-methylfurfural derived from *Valeriana fauriei* essential oil and its structural analogues against *Dermatophagoides farinae*, *D. pteronyssinus*, *Haemaphysalis longicornis* and *Tyrophagus putrescentiae* were evaluated in the present study. Based on the LD50 values of 5-methylfurfural and its analogues, 4,5-dimethylfurfural showed the highest acaricidal activity (LD50; 9.95, 9.91, and 7.12 μg/cm2), followed by 5-methylfurfural (11.87, 11.00, and 8.59 μg/cm2), furfural (12.94, 13.25, and 10.36 μg/cm2), and *V. fauriei* essential oil (15.15, 13.64, and 10.14 μg/cm2) against *D. farinae*, *D. pteronyssinus* and *T. putrescentiae*, respectively. However, all tested compounds did not show the acaricidal activities against *H. longicornis*. Interestingly, the color alterations of the mites and ticks were observed by furfural, 5-methylfurfural, and 4,5-dimethylfurfural from colorless to red brown during the acaricidal experiments. Furthermore, 4,5-dimethylfurfural which exhibited the highest acaricidal activity was formulated as nanoemulsion. The nanoemulsion of 4,5-dimethylfurfural showed higher acaricidal activity than it was emulsified in ethanol. The nanoemulsion was also found to show color changes of the mites and ticks from colorless to red brown. The results suggest that 5-methylfurfural and its analogues could be developed as an effective and easy-to-recognize acaricides to mites and ticks.

**Ji Hwan Lee, Do Hwi Park, Sanghyun Lee, Hye Jin Seo, Shin Jung Park, Kiwon Jung, Song-Yi Kim, Ki Sung Kang. Potential and beneficial effects of *Cinnamomum cassia* on gastritis and safety: Literature review and analysis of standard extract. (2021) Appl. Biol. Chem. 64: 95**

The prevalence of gastritis in South Korea is rapidly increasing owing to the prevalence of *Helicobacter pylori* infection and fast eating habit. The usual treatment for acute gastritis following a long intake of non-steroidal anti-inflammatory drugs (NSAIDs) or alcohol is to stop the causal factors. Metronidazole and lansoprazole are recommended for the treatment of *H*. *pylori* infection gastritis. Omeprazole a proton pump inhibitor, is used to decrease gastric acid production. However, owing to the side effects and refractoriness of the drug, a safe and efficient treatment is required. Plant-derived phytochemicals have emerged as novel agents against chronic disorders. In this study, firstly, to explore the potential of pharmacological activities, including efficacy and mechanisms of *Cinnamomum cassia* against gastritis, a literature review was performed based on 20 studies out of a total of 749 records obtained using a search strategy. From the literature review, the therapeutic targets of *C. cassia* extract and cinnamaldehyde, a compound of *C. cassia,* were found to be related with NFκB activity, and their signaling pathway were verified by experiments. *C. cassia* extract plays a role in protection of gastric ulcers induced in four ways (immersion stress-induced, ethanol-induced, hydrochloric acid-induced, or NSAIDs-induced ulcer). None of the clinical studies on *C. cassia* extracts or compounds met our criteria. When the standardized extract of *C. cassia* (ECC) was orally administered repeatedly to Beagle Dog for 4 weeks, no toxicologically harmful changes were observed. Therefore, under the test condition, the no observed adverse effect level (NOAEL) of ECC was judged to be 1000 mg/kg/day for both sexes, and no toxic target organ was observed. Administration of ECC in the Sprague–Dawley rat model of acute gastric injury caused by indomethacin administration significantly increased gastric mucus volume. Administration of ECC in the acute gastric injury model caused by indomethacin administration is considered effective in improving gastric injury. However, research and efforts to develop a reliable ‘standardization of natural drugs’ by establishing the best quality evaluation system are limited. Despite the pharmacological potential of ECC, further well-designed experimental studies such as in vitro, in vivo, and clinical trials are required to validate these findings and the underlying mechanisms of ECC.

**Rongbo Wang, Sang Yong Park, Sul Woong Park, Aditi Mitra Puja, Yeon-Ju Kim. Development of a molecular marker based on chloroplast gene for specific identification of Korean Hibiscus (*Hibiscus syriacus* ‘Simbaek’). (2021) Appl. Biol. Chem. 64: 96**

Due to the rise in substitution and adulteration of herbal products, as well as the lack of genetic information on *Hibiscus* plants, more molecular markers are needed to understand the genetic diversity and avoid their misidentification. There are many allelic variants of the functional genes in *Hibiscus* and other plants, which control their respective phenotypes and other characteristics. Identifying alleles of the desired trait by determining diversification through gene-typing allele-specific markers for authentication is, therefore, a potent strategy. The purpose of this study was to use insertion/deletion (InDel) markers to identify *Hibiscus syriacus* cultivars. We developed a novel InDel marker for Korean *Hibiscus* ‘Simbaek’, based on the trnL-F region of the chloroplast gene. Through this InDel site, a modified specific primer pair and a novel multiplex polymerase chain reaction (PCR) system were developed for specific identification of the Korean *Hibiscus* Simbaek cultivar. The molecular markers developed in this study were highly specific and accurately authenticated as Simbaek from the five main cultivars of *H. syriacus*. Taken together, the described method is a potential tool for the identification and selection of germplasm resource of Simbaek cultivar.

**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.