We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Flavonoids and Pectins

Zhiping Zhang, Yanzhi He and Xinyue Zhang

Abstract

Pectins and flavonoids are two related groups of important secondary metabolites derived from plants. The interaction between pectins and flavonoids can affect their shelf-life stability, functionality, bioavailability, and bioaccessibility. In this chapter, we will concentrate on the current opinions on the flavonoids to understand how to classify this group of secondary metabolites, what biological and pharmacological activities they possess, and how to biosynthesize them in plants. We will then discuss the general strategies for the derivation of these small secondary compounds. The strategies comprise traditional plant extraction, chemical synthesis, and biosynthesis. We will also discuss the advantages and disadvantages of these three production strategies in the derivation of flavonoids and the future research directions in generating health-beneficial flavonoids using the biosynthetic strategy.

Keywords: flavonoids, pectins, secondary metabolites, interaction, pharmacological activity, biological activity, biosynthetic pathway, extraction, characterization, chemical synthesis, microbial cell factory, enzymatic synthesis, multienzyme synthetic system

1. Introduction

Peels represent a large percentage of the total weight of fruits, for example, 50–65% of *Citrus* fruits (lemon, lime, orange, and grapefruit) [1]. During processing of fruits for juice and oil extractions, the peels remain as the primary byproducts and become waste if not processed further, which can lead to serious environmental pollution [1]. Therefore, the fruit-processing industries are also interested in making use of these wastes.

The peels are also a good commercial source of pectins (polygalacturonic acid) and flavonoids [1]. The pectins are polysaccharide macromolecules contained in the primary cell wall of plants and involved in controlling cell wall ionic status, cell expansion, and separation [1]. Usually, the pectins are commercially extracted and isolated from *Citrus* peels and apple pomace. They are not only used as a gelling agent, dessert filling, or juice and milk stabilizer in food industry but also as a source of dietary fiber. Flavonoids are a large group of small secondary metabolites contained in the vacuoles and possess a wide range of biological activities, especially those with human health benefits [2, 3]. In the *Citrus* peels, flavonoids mainly include flavones (e.g., rhoifolin, isorhoifolin, diosmin, and neodiosmin), flavanones (e.g., eriocitrin, neoeriocitrin, narirutin, naringin, hesperidin, neohesperidin, poncirin, and neoponcirin), and flavonols (e.g., rutin) [4]. It has been reported that the highest concentrations of *Citrus* flavonoids occur in the peels [1]. Due to the importance of pectins and flavonoids in food, cosmetic, and medicinal industries,

quite a number of studies have been focused on these two groups of compounds. Accordingly, a variety of approaches have been developed for efficient isolation of pectins and flavonoids from fruit peels and pomace. For example, to make a better use of yellow passion fruit rind, de Souza and colleagues have developed a strategy for sequential extraction of flavonoids and pectin [5].

As we know, pectins are abundant in the middle lamella of the plant cell walls with a gradual decrease in the content toward the plasma membrane, whereas flavonoids are naturally located within the cells [6]. Generally, flavonoids within the cells do not come into contact with the cell wall materials, such as pectins, celluloses, and hemicelluloses, prior to food processing. When fruits are processed and eaten, intracellular flavonoids can be released from the cells, leading to their interaction with substances like metal ions and plant cell wall materials [7, 8]. For example, procyanidins and anthocyanins can spontaneously bind to water-, chelator-, and sodium carbonate-soluble pectins. It is believed that the binding of flavonoids to cell wall materials results from noncovalent, hydrophobic, hydrogen bonding, and ionic interactions [9–11]. Recently, Chirug and colleagues have presented a novel possible mechanism that iron ions mediate the interaction between pectins and quercetin [6]. Such interaction might affect their shelf-life stability and functionality, as well as their bioavailability and bioaccessibility [6, 12]. Therefore, it could be of high importance to study their interaction. Since there are several reviews on the interaction [8, 13], we will not discuss it in this chapter. Instead, we will concentrate on understanding the current opinions on flavonoids, including the classification, biological activities, and biosynthetic pathway of these secondary compounds. We will then review the general strategies for derivation of these compounds, including the traditional plant extraction, chemical synthesis, and biosynthesis of these important small bioactive molecules in a microbial cell factory or an *in vitro* multienzyme synthetic platform. We will also discuss the advantages and disadvantages of these strategies and the future research directions in the field of flavonoid biosynthesis.

2. Classification and biological activities of flavonoids

Flavonoids belong to a class of secondary metabolites and comprise a large group of natural products that are widespread in higher plants but also found in mosses and liverworts [14, 15]. Chemically, flavonoid compounds have the basic structure of 15-carbon atoms with two phenolic rings connected by a 3-carbon chain [16], forming a C₆-C₃-C₆ carbon framework (**Figure 1**). Generally, these small molecules can be divided into six major subclasses on the basis of the variations on the

Figure 1.
Structure and atom numbering of flavonoid backbone.

heterocyclic C-ring and the degree of oxidation: the flavanols, flavones, flavonols, flavanones, anthocyanidins, and isoflavones [2, 16–18]. The flavonoids can exist in a free aglycone form but are often glycosylated (most commonly glucose), and the glycosylation in turn increases their water solubility [19].

The flavonoids are involved in the formation of plant pigments [20] and protect plants against pathogens, herbivores, and UV radiation [21]. However, the study of flavonoids, like that of most natural products, has emerged from the search of new compounds with promising pharmacological properties. After decades of endeavors, scientists have found that flavonoids possess a wide variety of biological and pharmacological properties, which leads to numerous studies on these secondary metabolites. These health-beneficial properties include antiangiogenic [22], antibacterial [23–27], anti-cancer [24, 28], anti-inflammatory [28–33], antiglycating [34], antimalarial [35], antimicrobial [36–42], anti-oxidant [26, 36, 38, 42–51], anti-platelet [48], anti-proliferation [52], agonistic/antagonistic [53], ammonia-lowering and regulation of urea cycle [54], anxiolytic [55], atheroprotective [56], cardioprotective and hypouricemic [57], cytotoxic [51, 58], endocrine disrupting [59], free radical-scavenging [31–33, 39, 40, 46, 51, 52, 58, 60–66], hepatoprotective [67], leishmanicidal [68], neuroprotective [69], photoprotective [43], and trypanocidal activities [68, 70]. In addition, the flavonoids can inhibit eukaryotic protein synthesis [71] and a variety of important enzymes such as aggrecanase [72], aldose reductase [30, 73], alpha-glucosidase [60], cholinesterase [26, 74], protein tyrosine phosphatase and acetylcholinesterase [75], and tyrosinase [44, 64].

3. Biosynthetic pathway of flavonoids

After several decades of efforts, the pathway for flavonoid biosynthesis has been largely deciphered even though quite a number of details remain unknown (**Figure 2**). The flavonoids and their derivatives are biosynthesized by a variety of enzymes. These enzymes belong to different families [76], mainly including 2-oxoglutarate-dependent dioxygenase (2-ODD), cytochrome P450 hydroxylase, short-chain dehydrogenase/reductase (SDR), *O*-methyltransferase (OMT), and *O*-glycosyltransferase (GT). The 2-ODD, cytochrome P450, and SDR enzymes constitute the major pathway for flavonoid biosynthesis [76], and the OMT and GT enzymes are involved in modification of flavonoids. The involved 2-ODD enzymes mainly comprise flavanone 3-hydroxylase (F3H), flavonol synthase (FLS), flavone synthase I (FSI), anthocyanidin synthase (ANS), and flavonol 6-hydroxylase (F6H) [17, 76–81]. The related cytochrome P450 enzymes contain cinnamate 4-hydroxylase (C4H), isoflavone synthase (IFS), flavanone 2-hydroxylase (F2H), flavone synthase II (FSII), flavonol 6-hydroxylase (F6H), flavonoid 3'-hydroxylase (F3'H), flavonoid 3',5'-hydroxylase (F3'5'H), isoflavone 2'-hydroxylase (I2'H), and isoflavone 3'-hydroxylase (I3'H) [17, 18, 76, 80, 82, 83]. The SDR enzymes participating in flavonoid biosynthesis include dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANR) [76]. Interestingly, the flavone synthase (FS) activity is specified either by a 2-ODD (FSI) or a P450 (FSII) enzyme in a plant species-dependent manner [84, 85]. Similarly, the flavonol 6-hydroxylase (F6H) activity is also endowed either by a 2-ODD [81, 86] or P450 [87, 88] enzyme in different plant species. These findings further increase the complexity of flavonoid biosynthesis.

Basically, biosynthesis of flavonoids can be arbitrarily divided into three major stages. The first stage (a.k.a the phenylpropanoid pathway) includes three successive chemical reactions catalyzed by phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-coumaroyl:CoA ligase (4CL), respectively, to convert L-phenylalanine to 4-coumaroyl-CoA. In addition, L-tyrosine can also

Figure 2.

Schematic of the biosynthetic pathway leading to the major subclasses of flavonoids. Adapted from [10, 12, 68]. 4CL, 4-coumaroyl:CoA ligase; ACC, acetyl CoA carboxylase; ANR, anthocyanidin reductase; ANS, anthocyanidin synthase; AS, aureusidin synthase; C4H, cinnamate 4-hydroxylase; CE: condensing enzyme; CHI, chalcone isomerase; CHS, chalcone synthase; DFR, dihydroflavonol 4-reductase; DMID, 7,2'-dihydroxy-4'-methoxyisoflavanol dehydratase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; F3'5'H, flavonoid 3',5'-hydroxylase; FLS, flavonol synthase; FSI/FSII, flavone synthase I/II; I2'H, isoflavone 2'-hydroxylase; IFR, isoflavone reductase; IFS, isoflavone synthase; IOMT, isoflavone O-methyltransferase; LAR, leucoanthocyanidin reductase; LDOX, leucoanthocyanidin dioxygenase; OMT, O-methyltransferase; PAL, phenylalanine ammonia-lyase; PPO, polyphenol oxidase; RT, rhamnosyltransferase; TAL, tyrosine ammonia-lyase; UFGT, UDP flavonoid glucosyltransferase; VR, vestitone reductase.

participate in the flavonoid biosynthesis via two successive enzymatic reactions catalyzed by tyrosine ammonia lyase (TAL) and 4CL, respectively. The second stage is crucial for the biosynthesis of flavonoids, in which the backbones of major subclasses of flavonoids are formed. This stage begins from the formation of chalcone by conversion of the 4-coumroyl-CoA from the first stage and the malonyl-CoA from carboxylation of acetyl-CoA. Chalcone synthase (CHS), an entry point enzyme into the pathway, catalyzes this chemical reaction by conversion of one molecule of 4-coumroyl-CoA and three molecules of malonyl-CoA to one molecule of chalcone (e.g., tetrahydroxychalcone). Then, the chalcone molecule is cyclized to form a flavanone (e.g., naringenin) by chalcone isomerase (CHI) and an aurone (e.g., aureusidin) by aureusidin synthase (AS). The flavanone can be further converted to dihydroflavonol by F3H and then flavonol by FLS. Alternatively, the flavanone molecule can also be converted to a flavone by FS, a flavanol by DFR, an isoflavone by IFS, and an anthocyanidin by a series of successive enzymatic reactions catalyzed by F3H, DFR, and leucoanthocyanidin dioxygenase (LDOX), respectively. The resulting anthocyanidin molecule can be further modified to form anthocyanins by a series of chemical modifications by OMT, UDP flavonoid glucosyltransferase (UGT), and rhamnosyltransferase (RT). The third stage is mainly involved in various chemical decorations of flavonoids. Generally, natural flavonoids are often extensively modified by chemical reactions, including glycosylation and methylation [76], acylation [89], sulfonation [90, 91], prenylation [92, 93], and galloylation [94], which further contribute to the structural and functional diversity of flavonoids.

4. Derivation of flavonoids

Due to the intrinsic health benefits possessed by flavonoids, numerous approaches have been developed during the past decades for the derivation of a wide range of flavonoids. Basically, these approaches can be divided into three major categories: traditional plant extraction, chemical synthesis, and biosynthesis.

4.1 Traditional plant extraction via organic solvents

Traditionally, flavonoids are extracted from various plant species, which currently remains the most commonly used methods. During the past decades, researchers have developed plenty of methods to improve the yield and purity of flavonoids derived from plants. Generally, the plant tissues are air-dried and ground into powder for extraction via organic solvents (most commonly methanol and ethanol), and the extracts are then subjected to successive fractionation with other organic solvents (most commonly petroleum ether, chloroform, ethyl acetate, and n-butyl alcohol), followed by repeated silica gel and Sephadex LH-20 column chromatographies [44, 95]. The yield of plant-derived flavonoids can be improved by ultrasonic wave- [96], microwave- [97], and enzyme-assisted extraction [98]; aqueous two-phase extraction [99]; and a combination of these modifications [100]. The isolated flavonoids are then subjected to polyamide thin plate chromatography (TLC), high performance liquid chromatography (HPLC), electrospray ionization mass spectrometry (ESI-MS), and nuclear magnetic resonance (NMR) analyses to determine their identity and purity [2, 3]. Due to the high solubility of most flavonoids in organic solvents, this strategy often demonstrates a high efficiency in the derivation of flavonoids from plant tissues. However, the disadvantage of the plant extraction is obvious. Due to the very low content of most flavonoids in plant tissues, the extraction and isolation of flavonoids often requires multiple

steps and plenty of time, labor, and organic solvents, which greatly increase the production cost. Moreover, different plant tissues often need to develop different approaches for processing, which makes the extraction more complicated and further increase the cost for the production of flavonoids. Therefore, this approach is not cost-effective, and it is crucial to develop alternative strategies to reduce the cost for producing flavonoids.

4.2 Chemical synthesis of flavonoids

Another approach for producing flavonoids is chemical synthesis. Basically, there are two strategies for chemical synthesis of flavones, that is, the chalcone route and the Baker-Venkataraman method [101]. Even though there are a few successful examples, chemical synthesis of flavonoids is often very complicated and involved in many steps [2]. It requires toxic reagents and extreme reaction conditions [3, 102]. Chiral synthesis and subsequent modifications further increase the difficulty of this approach in the production of flavonoids [3]. Moreover, the multi-step chemical reactions often produce quite a number of intermediate products with a high similarity in structure, which further increases the difficulty in purification of the desired products. Therefore, chemical synthesis is not economically feasible for the mass production of flavonoids [3].

4.3 Biosynthesis of flavonoids

Since the biosynthetic pathway of flavonoids is largely elucidated in plants [20], other promising alternative strategies have been developed to produce these secondary compounds [2, 103–106]. One of these alternative strategies is to produce flavonoids in a microbial cell factory. It has been well known that *Escherichia coli* and *Saccharomyces cerevisiae* are the two most commonly used model organisms for the construction of a microbial cell factory. There are quite a few paradigms for the production of flavonoids using this strategy. For example, eriodictyol has been produced using L-tyrosine as a substrate in *E. coli* BL21(DE3) genetically modified by *TAL*, *4CL*, *CHS*, *CHI*, *F3H*, and *F3'H* genes and the production can reach up to 107 mg/L by further introducing three other genes *acs*, *accBC*, and *dtsR1* to enhance the availability of malonyl-CoA [103]. Kaempferol has been produced in a microbial cell factory by introducing a *de novo* biosynthetic pathway into *S. cerevisiae*, and the biosynthesis has been further improved by introducing two more pathways to enhance the generation of acetyl-CoA and malonyl-CoA [107]. Obviously, this strategy circumvents some inherent disadvantages of traditional plant extraction and chemical synthesis. However, not all genetically modified microbes can produce desired products due to the well-known complexity of a microbial cell system, the incompatibility of artificially synthesized genetic elements in host cells, the growth inhibition of host cells by desired and intermediate products, and the instability of an engineered biosystem itself [2, 108].

Recently, we have developed an *in vitro* platform to produce flavonoids by constructing a multienzyme synthetic system to convert naringenin into kaempferol in one pot [2]. After optimizing a series of reaction parameters, including the components and pH value of the buffer system, reaction temperature and time, and total amount and ratio of the enzymes, the production yield can reach up to 37.55 ± 1.62 mg/L within 40–50 min with a conversion rate of $55.89\% \pm 2.74\%$ [2]. The advantages of this strategy are obvious. It is time- and labor-saving. The reaction conditions are easy to control accurately. Due to the clearness in the buffer components and the lack of complex physiological regulation as occurred in the microbial cell factory, it is possible to easily make further optimization in the future.

It is also much easier to purify desired products from this *in vitro* synthetic system than from the cell factory because of the simplicity of the components in the system. In addition, the strategy is highly cost-effective because of the cheap chemicals and recombinant proteins used in this system. More importantly, the system is easy to scale up and therefore possesses a huge industrialization potential. It also provides a guide for other secondary metabolites to produce economically. However, problems still exist in this production strategy. For example, due to the lack of P450-reductase function, prokaryotically expressed cytochrome P450 enzymes lose their enzymatic activities [109]. To achieve a functional expression, Leonard and colleagues fused a plant P450 enzyme gene *F3'5'H* with its redox partner cytochrome P450 reductase gene *cpr* from *Catharanthus roseus* and successfully produced a hydroxylated flavonol quercetin from *p*-coumaric acid in *E. coli* by simultaneous coexpression of the fusion protein with 4CL, CHS, CHI, F3H, and FLS [110], which provides a guide to solve this kind of problem. To further improve the efficiency of the biosynthetic system, future research should be focused on screening key enzymes with high activities from various plants, mutation of genes encoding key enzymes to enhance their activities, and immobilization of the highly active enzymes to inert carriers.

5. Conclusions

Pectins and flavonoids are two distinctive classes of bioactive secondary metabolites presented in the fruit peels and used in food industry. The flavonoids can be divided into six major subclasses, including the flavanols, flavones, flavonols, flavanones, anthocyanidins, and isoflavones, and their flavonoid biosynthetic pathway has been largely elucidated. These natural small compounds possess a wide range of health-beneficial properties and can be derived by traditional plant extraction via organic solvents, chemical synthesis, and biosynthesis by constructing a microbial cell factory or an *in vitro* multienzyme synthetic system.

Conflict of interest

The authors declare that they have no competing financial interests.

IntechOpen

Author details

Zhiping Zhang¹, Yanzhi He¹ and Xinyue Zhang^{1,2,3,4*}

1 College of Bioscience and Biotechnology, Yangzhou University, Yangzhou City, Jiangsu, China

2 Key Laboratory of Prevention and Control of Biological Hazard Factors (Animal Origin) for Agrifood Safety and Quality, Ministry of Agriculture of China, Yangzhou University, Yangzhou City, Jiangsu, China

3 Joint International Research Laboratory of Agriculture & Agri-Product Safety, Yangzhou University, Yangzhou City, Jiangsu, China

4 Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou City, Jiangsu, China

*Address all correspondence to: zhangxinyue@yzu.edu.cn

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Mandalari G, Bennett RN, Bisignano G, Saija A, Dugo G, Lo Curto RB, et al. Characterization of flavonoids and pectins from bergamot (*Citrus bergamia* Risso) peel, a major byproduct of essential oil extraction. *Journal of Agricultural and Food Chemistry*. 2006;**54**(1):197-203. DOI: 10.1021/jf051847n
- [2] Zhang Z, He Y, Huang Y, Ding L, Chen L, Liu Y, et al. Development and optimization of an *in vitro* multienzyme synthetic system for production of kaempferol from naringenin. *Journal of Agricultural and Food Chemistry*. 2018;**66**(31):8272-8279. DOI: 10.1021/acs.jafc.8b01299
- [3] Malla S, Pandey RP, Kim BG, Sohng JK. Regiospecific modifications of naringenin for astragalin production in *Escherichia coli*. *Biotechnology and Bioengineering*. 2013;**110**(9):2525-2535. DOI: 10.1002/bit.24919
- [4] Nogata Y, Sakamoto K, Shiratsuchi H, Ishii T, Yano M, Ohta H. Flavonoid composition of fruit tissues of citrus species. *Bioscience, Biotechnology, and Biochemistry*. 2006;**70**(1):178-192. DOI: 10.1271/bbb.70.178
- [5] de Souza CG, Rodrigues TH, LM ES, Ribeiro PR, de Brito ES. Sequential extraction of flavonoids and pectin from yellow passion fruit rind using pressurized solvent or ultrasound. *Journal of the Science of Food and Agriculture*. 2018;**98**(4):1362-1368. DOI: 10.1002/jsfa.8601
- [6] Chirug L, Okun Z, Ramon O, Shpigelman A. Iron ions as mediators in pectin-flavonols interactions. *Food Hydrocolloids*. 2018;**84**:441-449. DOI: 10.1016/j.foodhyd.2018.06.039
- [7] El Hajji H, Nkhili E, Tomao V, Dangles O. Interactions of quercetin with iron and copper ions: Complexation and autoxidation. *Free Radical Research*. 2006;**40**(3):303-320. DOI: 10.1080/10715760500484351
- [8] Zhu F. Interactions between cell wall polysaccharides and polyphenols. *Critical Reviews in Food Science and Nutrition*. 2018;**58**(11):1808-1831. DOI: 10.1080/10408398.2017.1287659
- [9] Lin Z, Fischer J, Wicker L. Intermolecular binding of blueberry pectin-rich fractions and anthocyanin. *Food Chemistry*. 2016;**194**:986-993. DOI: 10.1016/j.foodchem.2015.08.113
- [10] Le Bourvellec C, Bouchet B, Renard CM. Non-covalent interaction between procyanidins and apple cell wall material. Part III: Study on model polysaccharides. *Biochimica et Biophysica Acta*. 2005;**1725**(1):10-18. DOI: 10.1016/j.bbagen.2005.06.004
- [11] Le Bourvellec C, Guyot S, Renard C. Non-covalent interaction between procyanidins and apple cell wall material: Part I. Effect of some environmental parameters. *Biochimica et Biophysica Acta*. 2004;**1672**:192-202. DOI: 10.1016/j.bbagen.2004.04.001
- [12] Phan AD, Netzel G, Wang D, Flanagan BM, D'Arcy BR, Gidley MJ. Binding of dietary polyphenols to cellulose: Structural and nutritional aspects. *Food Chemistry*. 2015;**171**:388-396. DOI: 10.1016/j.foodchem.2014.08.118
- [13] Le Bourvellec C, Renard CMGC. Interactions between polyphenols and macromolecules: Quantification methods and mechanisms. *Critical Reviews in Food Science and Nutrition*. 2012;**52**(1-3):213-248. DOI: 10.1080/10408398.2010.499808
- [14] Buckingham J, Munasinghe VRN. *Dictionary of Flavonoids: With*

CD-ROM. Boca Raton: CRC Press, Taylor & Francis Group; 2015. 1169 p

[15] Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. *Journal of Nutritional Science*. 2016;**5**:e47. DOI: 10.1017/jns.2016.41

[16] Mulvihill EE, Burke AC, Huff MW. Citrus flavonoids as regulators of lipoprotein metabolism and atherosclerosis. *Annual Review of Nutrition*. 2016;**36**:275-299. DOI: 10.1146/annurev-nutr-071715-050718

[17] Tohge T, de Souza LP, Fernie AR. Current understanding of the pathways of flavonoid biosynthesis in model and crop plants. *Journal of Experimental Botany*. 2017;**68**(15): 4013-4028. DOI: 10.1093/jxb/erx177

[18] Lepiniec L, Debeaujon I, Routaboul JM, Baudry A, Pourcel L, Nesi N, et al. Genetics and biochemistry of seed flavonoids. *Annual Review of Plant Biology*. 2006;**57**:405-430. DOI: 10.1146/annurev.arplant.57.032905.105252

[19] Croft KD. The chemistry and biological effects of flavonoids and phenolic acids. *Annals of the New York Academy of Sciences*. 1998;**854**:435-442. DOI: 10.1111/j.1749-6632.1998.tb09922.x

[20] Winkel-Shirley B. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiology*. 2001;**126**(2):485-493. DOI: 10.1104/pp.126.2.485

[21] Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry*. 2002;**13**(10):572-584. DOI: 10.1016/S0955-2863(02)00208-5

[22] Hassan LE, Ahamed MB, Majid AS, Baharetha HM, Muslim NS, Nassar ZD, et al. Correlation of antiangiogenic, antioxidant and cytotoxic activities

of some Sudanese medicinal plants with phenolic and flavonoid contents. *BMC Complementary and Alternative Medicine*. 2014;**14**:406. DOI: 10.1186/1472-6882-14-406

[23] Kurnia D, Apriyanti E, Soraya C, Satari MH. Antibacterial flavonoids against oral bacteria of *Enterococcus faecalis* ATCC 29212 from Sarang Semut (*Myrmecodia pendans*) and its inhibitor activity against enzyme MurA. *Current Drug Discovery Technologies*. 2019;**16**:1-7. DOI: 10.2174/1570163815666180828113920

[24] Albadawi DA, Mothana RA, Khaled JM, Ashour AE, Kumar A, Ahmad SF, et al. Antimicrobial, anticancer, and antioxidant compounds from *Premna resinosa* growing in Saudi Arabia. *Pharmaceutical Biology*. 2017;**55**(1):1759-1766. DOI: 10.1080/13880209.2017.1322617

[25] Chakotiya AS, Tanwar A, Narula A, Sharma RK. *Zingiber officinale*: Its antibacterial activity on *Pseudomonas aeruginosa* and mode of action evaluated by flow cytometry. *Microbial Pathogenesis*. 2017;**107**:254-260. DOI: 10.1016/j.micpath.2017.03.029

[26] Bensouici C, Kabouche A, Karioti A, Ozturk M, Duru ME, Bilia AR, et al. Compounds from *Sedum caeruleum* with antioxidant, anticholinesterase, and antibacterial activities. *Pharmaceutical Biology*. 2016;**54**(1):174-179. DOI: 10.3109/13880209.2015.1028078

[27] Biva IJ, Ndi CP, Griesser HJ, Semple SJ. Antibacterial constituents of *Eremophila alternifolia*: An Australian aboriginal traditional medicinal plant. *Journal of Ethnopharmacology*. 2016;**182**:1-9. DOI: 10.1016/j.jep.2016.02.011

[28] Zhou H, Lutterodt H, Cheng Z, Yu LL. Anti-inflammatory and antiproliferative activities of trifolirhizin, a flavonoid from *Sophora*

flavescens roots. Journal of Agricultural and Food Chemistry. 2009;**57**(11): 4580-4585. DOI: 10.1021/jf900340b

[29] Zhang L, Wu T, Xiao W, Wang Z, Ding G, Zhao L. Enrichment and purification of total ginkgo flavonoid O-glycosides from *Ginkgo biloba* extract with macroporous resin and evaluation of anti-inflammation activities *in vitro*. Molecules. 2018;**23**(5):1167. DOI: 10.3390/molecules23051167

[30] Han QT, Ren Y, Li GS, Xiang KL, Dai SJ. Flavonoid alkaloids from *Scutellaria moniliorrhiza* with anti-inflammatory activities and inhibitory activities against aldose reductase. Phytochemistry. 2018;**152**:91-96. DOI: 10.1016/j.phytochem.2018.05.001

[31] Chen XM, Tait AR, Kitts DD. Flavonoid composition of orange peel and its association with antioxidant and anti-inflammatory activities. Food Chemistry. 2017;**218**:15-21. DOI: 10.1016/j.foodchem.2016.09.016

[32] Diaz P, Jeong SC, Lee S, Khoo C, Koyyalamudi SR. Antioxidant and anti-inflammatory activities of selected medicinal plants and fungi containing phenolic and flavonoid compounds. Chinese Medicine. 2012;**7**(1):26. DOI: 10.1186/1749-8546-7-26

[33] Zhang L, Ravipati AS, Koyyalamudi SR, Jeong SC, Reddy N, Smith PT, et al. Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. Journal of Agricultural and Food Chemistry. 2011;**59**(23): 12361-12367. DOI: 10.1021/jf203146e

[34] Ahmad R, Ahmad N, Naqvi AA, Exarchou V, Upadhyay A, Tuenter E, et al. Antioxidant and antiglycating constituents from leaves of *Ziziphus oxyphylla* and *Cedrela serrata*. Antioxidants (Basel). 2016;**5**(1):9. DOI: 10.3390/antiox5010009

[35] Chander MP, Pillai CR, Sunish IP, Vijayachari P. Antimicrobial and antimalarial properties of medicinal plants used by the indigenous tribes of Andaman and Nicobar Islands, India. Microbial Pathogenesis. 2016;**96**:85-88. DOI: 10.1016/j.micpath.2016.04.017

[36] Ng KR, Lyu X, Mark R, Chen WN. Antimicrobial and antioxidant activities of phenolic metabolites from flavonoid-producing yeast: Potential as natural food preservatives. Food Chemistry. 2019;**270**:123-129. DOI: 10.1016/j.foodchem.2018.07.077

[37] Tagousop CN, Tamokou JD, Ekom SE, Ngnokam D, Voutquenne-Nazabadioko L. Antimicrobial activities of flavonoid glycosides from *Graptophyllum grandulosum* and their mechanism of antibacterial action. BMC Complementary and Alternative Medicine. 2018;**18**(1):252. DOI: 10.1186/s12906-018-2321-7

[38] Pandey G, Khatoon S, Pandey MM, Rawat AKS. Altitudinal variation of berberine, total phenolics and flavonoid content in *Thalictrum foliolosum* and their correlation with antimicrobial and antioxidant activities. Journal of Ayurveda and Integrative Medicine. 2017;**9**(3):169-176. DOI: 10.1016/j.jaim.2017.02.010

[39] Silva-Beltran NP, Ruiz-Cruz S, Cira-Chavez LA, Estrada-Alvarado MI, Ornelas-Paz Jde J, Lopez-Mata MA, et al. Total phenolic, flavonoid, tomatine, and tomatidine contents and antioxidant and antimicrobial activities of extracts of tomato plant. International Journal of Analytical Chemistry. 2015;**2015**:284071. DOI: 10.1155/2015/284071

[40] Jalal TK, Ahmed IA, Mikail M, Momand L, Draman S, Isa ML, et al. Evaluation of antioxidant, total phenol and flavonoid content and antimicrobial activities of *Artocarpus altilis* (breadfruit) of underutilized tropical

- fruit extracts. *Applied Biochemistry and Biotechnology*. 2015;**175**(7):3231-3243. DOI: 10.1007/s12010-015-1499-0
- [41] Abdel Ghani SB, Weaver L, Zidan ZH, Ali HM, Keevil CW, Brown RC. Microwave-assisted synthesis and antimicrobial activities of flavonoid derivatives. *Bioorganic & Medicinal Chemistry Letters*. 2008;**18**(2):518-522. DOI: 10.1016/j.bmcl.2007.11.081
- [42] Ayub MA, Hussain AI, Hanif MA, Chatha SAS, Kamal GM, Shahid M, et al. Variation in phenolic profile, beta-carotene and flavonoid contents, biological activities of two *Tagetes* species from Pakistani Flora. *Chemistry & Biodiversity*. 2017;**14**(6):e1600463. DOI: 10.1002/cbdv.201600463
- [43] Lefahal M, Zaabat N, Ayad R, Makhloufi EH, Djarri L, Benahmed M, et al. In vitro assessment of total phenolic and flavonoid contents, antioxidant and photoprotective activities of crude methanolic extract of aerial parts of *Capnophyllum peregrinum* (L.) Lange (Apiaceae) growing in Algeria. *Medicine*. 2018;**5**(2):26. DOI: 10.3390/medicines5020026
- [44] Kishore N, Twilley D, Blom van Staden A, Verma P, Singh B, Cardinali G, et al. Isolation of flavonoids and flavonoid glycosides from *Myrsine africana* and their inhibitory activities against mushroom tyrosinase. *Journal of Natural Products*. 2018;**81**(1):49-56. DOI: 10.1021/acs.jnatprod.7b00564
- [45] Wang L, Wu Y, Bei Q, Shi K, Wu Z. Fingerprint profiles of flavonoid compounds from different *Psidium guajava* leaves and their antioxidant activities. *Journal of Separation Science*. 2017;**40**(19):3817-3829. DOI: 10.1002/jssc.201700477
- [46] Badral D, Odonbayar B, Murata T, Munkhjargal T, Tuvshintulga B, Igarashi I, et al. Flavonoid and galloyl glycosides isolated from *Saxifraga spinulosa* and their antioxidative and inhibitory activities against species that cause piroplasmiasis. *Journal of Natural Products*. 2017;**80**(9):2416-2423. DOI: 10.1021/acs.jnatprod.7b00142
- [47] Abarikwu SO, Olufemi PD, Lawrence CJ, Wekere FC, Ocholor AC, Barikuma AM. Rutin, an antioxidant flavonoid, induces glutathione and glutathione peroxidase activities to protect against ethanol effects in cadmium-induced oxidative stress in the testis of adult rats. *Andrologia*. 2017;**49**(7):e12696. DOI: 10.1111/and.12696
- [48] Assefa AD, Ko EY, Moon SH, Keum YS. Antioxidant and antiplatelet activities of flavonoid-rich fractions of three citrus fruits from Korea. *3 Biotech*. 2016;**6**(1):109. DOI: 10.1007/s13205-016-0424-8
- [49] Wang M, Xing S, Luu T, Fan M, Li X. The gastrointestinal tract metabolism and pharmacological activities of grosvenorine, a major and characteristic flavonoid in the fruits of *Siraitia grosvenorii*. *Chemistry & Biodiversity*. 2015;**12**(11):1652-1664. DOI: 10.1002/cbdv.201400397
- [50] Vukics V, Kery A, Bonn GK, Guttman A. Major flavonoid components of heartsease (*Viola tricolor* L.) and their antioxidant activities. *Analytical and Bioanalytical Chemistry*. 2008;**390**(7):1917-1925. DOI: 10.1007/s00216-008-1885-3
- [51] Mohammed RS, Abou Zeid AH, El Hawary SS, Sleem AA, Ashour WE. Flavonoid constituents, cytotoxic and antioxidant activities of *Gleditsia triacanthos* L. leaves. *Saudi Journal of Biological Sciences*. 2014;**21**(6):547-553. DOI: 10.1016/j.sjbs.2014.02.002
- [52] Kim H, Choi HK, Moon JY, Kim YS, Mosaddik A, Cho SK. Comparative antioxidant and antiproliferative activities of red and white pitayas and

their correlation with flavonoid and polyphenol content. *Journal of Food Science*. 2011;**76**(1):C38-C45. DOI: 10.1111/j.1750-3841.2010.01908.x

[53] Van der Heiden E, Bechoux N, Muller M, Sergent T, Schneider YJ, Larondelle Y, et al. Food flavonoid aryl hydrocarbon receptor-mediated agonistic/antagonistic/synergic activities in human and rat reporter gene assays. *Analytica Chimica Acta*. 2009;**637**(1-2):337-345. DOI: 10.1016/j.aca.2008.09.054

[54] Nohara K, Shin Y, Park N, Jeong K, He B, Koike N, et al. Ammonia-lowering activities and carbamoyl phosphate synthetase 1 (Cps1) induction mechanism of a natural flavonoid. *Nutrition & Metabolism (London)*. 2015;**12**:23. DOI: 10.1186/s12986-015-0020-7

[55] Li H, Zhou P, Yang Q, Shen Y, Deng J, Li L, et al. Comparative studies on anxiolytic activities and flavonoid compositions of *Passiflora edulis* 'edulis' and *Passiflora edulis* 'flavicarpa'. *Journal of Ethnopharmacology*. 2011;**133**(3):1085-1090. DOI: 10.1016/j.jep.2010.11.039

[56] Lara-Guzman OJ, Tabares-Guevara JH, Leon-Varela YM, Alvarez RM, Roldan M, Sierra JA, et al. Proatherogenic macrophage activities are targeted by the flavonoid quercetin. *The Journal of Pharmacology and Experimental Therapeutics*. 2012;**343**(2):296-306. DOI: 10.1124/jpet.112.196147

[57] Zhu JX, Wang Y, Kong LD, Yang C, Zhang X. Effects of *Biota orientalis* extract and its flavonoid constituents, quercetin and rutin on serum uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. *Journal of Ethnopharmacology*. 2004;**93**(1):133-140. DOI: 10.1016/j.jep.2004.03.037

[58] El-Alfy TS, El-Gohary HM, Sokkar NM, Hosny M, Al-Mahdy DA. A new flavonoid C-glycoside from *Celtis australis* L. and *Celtis occidentalis* L. leaves and potential antioxidant and cytotoxic activities. *Scientia Pharmaceutica*. 2011;**79**(4):963-975. DOI: 10.3797/scipharm.1108-19

[59] Nordeen SK, Bona BJ, Jones DN, Lambert JR, Jackson TA. Endocrine disrupting activities of the flavonoid nutraceuticals luteolin and quercetin. *Hormones and Cancer*. 2013;**4**(5):293-300. DOI: 10.1007/s12672-013-0150-1

[60] Xiao X, Xu L, Hu H, Yang Y, Zhang X, Peng Y, et al. DPPH radical scavenging and postprandial hyperglycemia inhibition activities and flavonoid composition analysis of hawk tea by UPLC-DAD and UPLC-Q/TOF MS(E). *Molecules*. 2017;**22**(10):1622. DOI: 10.3390/molecules22101622

[61] Ismail HF, Hashim Z, Soon WT, Rahman NSA, Zainudin AN, Majid FAA. Comparative study of herbal plants on the phenolic and flavonoid content, antioxidant activities and toxicity on cells and zebrafish embryo. *Journal of Traditional and Complementary Medicine*. 2017;**7**(4):452-465. DOI: 10.1016/j.jtcme.2016.12.006

[62] Chakraborty S, Basu S. Multi-functional activities of citrus flavonoid narirutin in Alzheimer's disease therapeutics: An integrated screening approach and in vitro validation. *International Journal of Biological Macromolecules*. 2017;**103**:733-743. DOI: 10.1016/j.ijbiomac.2017.05.110

[63] Xi W, Fang B, Zhao Q, Jiao B, Zhou Z. Flavonoid composition and antioxidant activities of Chinese local pummelo (*Citrus grandis* Osbeck.) varieties. *Food Chemistry*. 2014;**161**:230-238. DOI: 10.1016/j.foodchem.2014.04.001

- [64] Liang CP, Chang CH, Liang CC, Hung KY, Hsieh CW. In vitro antioxidant activities, free radical scavenging capacity, and tyrosinase inhibitory of flavonoid compounds and ferulic acid from *Spiranthes sinensis* (Pers.) Ames. *Molecules*. 2014;**19**(4):4681-4694. DOI: 10.3390/molecules19044681
- [65] Li P, Jia J, Zhang D, Xie J, Xu X, Wei D. In vitro and in vivo antioxidant activities of a flavonoid isolated from celery (*Apium graveolens* L. var. dulce). *Food & Function*. 2014;**5**(1):50-56. DOI: 10.1039/c3fo60273g
- [66] Ekuadzi E, Dickson R, Fleischer T, Annan K, Pistorius D, Oberer L, et al. Flavonoid glycosides from the stem bark of *Margaritaria discoidea* demonstrate antibacterial and free radical scavenging activities. *Phytotherapy Research*. 2014;**28**(5):784-787. DOI: 10.1002/ptr.5053
- [67] Fernando CD, Soysa P. Total phenolic, flavonoid contents, in-vitro antioxidant activities and hepatoprotective effect of aqueous leaf extract of *Atalantia ceylanica*. *BMC Complementary and Alternative Medicine*. 2014;**14**:395. DOI: 10.1186/1472-6882-14-395
- [68] Beer MF, Frank FM, German Elso O, Ernesto Bivona A, Cerny N, Giberti G, et al. Trypanocidal and leishmanicidal activities of flavonoids isolated from *Stevia satureiifolia* var. *satireiifolia*. *Pharmaceutical Biology*. 2016;**54**(10):2188-2195. DOI: 10.3109/13880209.2016.1150304
- [69] Zhang YC, Gan FF, Shelar SB, Ng KY, Chew EH. Antioxidant and Nrf2 inducing activities of luteolin, a flavonoid constituent in *Ixeris sonchifolia* Hance, provide neuroprotective effects against ischemia-induced cellular injury. *Food and Chemical Toxicology*. 2013;**59**: 272-280. DOI: 10.1016/j.fct.2013.05.058
- [70] Mergia E, Shibeshi W, Terefe G, Teklehaymanot T. Antitrypanosomal activity of *Verbascum sinaiticum* Benth. (Scrophulariaceae) against *Trypanosoma congolense* isolates. *BMC Complementary and Alternative Medicine*. 2016;**16**:362. DOI: 10.1186/s12906-016-1346-z
- [71] Itoh A, Tanahashi T, Nagakura N, Takenaka Y, Chen CC, Pelletier J. Flavonoid glycosides from *Adina racemosa* and their inhibitory activities on eukaryotic protein synthesis. *Journal of Natural Products*. 2004;**67**(3): 427-431. DOI: 10.1021/np030440e
- [72] Moncada-Pazos A, Obaya AJ, Vilorio CG, Lopez-Otin C, Cal S. The nutraceutical flavonoid luteolin inhibits ADAMTS-4 and ADAMTS-5 aggrecanase activities. *Journal of Molecular Medicine (Berlin, Germany)*. 2011;**89**(6):611-619. DOI: 10.1007/s00109-011-0741-7
- [73] Mok SY, Lee S. Identification of flavonoids and flavonoid rhamnosides from *Rhododendron mucronulatum* for albiflorum and their inhibitory activities against aldose reductase. *Food Chemistry*. 2013;**136**(2):969-974. DOI: 10.1016/j.foodchem.2012.08.091
- [74] Khan MT, Orhan I, Senol FS, Kartal M, Sener B, Dvorska M, et al. Cholinesterase inhibitory activities of some flavonoid derivatives and chosen xanthone and their molecular docking studies. *Chemico-Biological Interactions*. 2009;**181**(3):383-389. DOI: 10.1016/j.cbi.2009.06.024
- [75] Nguyen DH, Seo UM, Zhao BT, Le DD, Seong SH, Choi JS, et al. Ellagitannin and flavonoid constituents from *Agrimonia pilosa* Ledeb. with their protein tyrosine phosphatase and acetylcholinesterase inhibitory activities. *Bioorganic Chemistry*. 2017;**72**:293-300. DOI: 10.1016/j.bioorg.2017.04.017
- [76] Winkel BSJ. The biosynthesis of flavonoids. In: Grotewold E, editor. *The*

Science of Flavonoids. New York, NY: Springer New York; 2006. pp. 71-95

[77] Tohge T, Watanabe M, Hoefgen R, Fernie AR. The evolution of phenylpropanoid metabolism in the green lineage. *Critical Reviews in Biochemistry and Molecular Biology*. 2013;**48**(2):123-152. DOI: 10.3109/10409238.2012.758083

[78] Araujo WL, Tohge T, Nunes-Nesi A, Daloso DM, Nimick M, Krahnert I, et al. Phosphonate analogs of 2-oxoglutarate perturb metabolism and gene expression in illuminated Arabidopsis leaves. *Frontiers in Plant Science*. 2012;**3**:114. DOI: 10.3389/fpls.2012.00114

[79] Bredebach M, Matern U, Martens S. Three 2-oxoglutarate-dependent dioxygenase activities of *Equisetum arvense* L. forming flavone and flavonol from (2S)-naringenin. *Phytochemistry*. 2011;**72**(7):557-563. DOI: 10.1016/j.phytochem.2011.01.036

[80] Lee YJ, Kim JH, Kim BG, Lim Y, Ahn JH. Characterization of flavone synthase I from rice. *BMB Reports*. 2008;**41**(1):68-71. DOI: <https://www.ncbi.nlm.nih.gov/pubmed/18304453>

[81] Anzellotti D, Ibrahim RK. Molecular characterization and functional expression of flavonol 6-hydroxylase. *BMC Plant Biology*. 2004;**4**:20. DOI: 10.1186/1471-2229-4-20

[82] Barz W, Welle R. Biosynthesis and metabolism of isoflavones and pterocarpan phytoalexins in chickpea, soybean and phytopathogenic fungi. In: Stafford HA, Ibrahim RK, editors. *Phenolic Metabolism in Plants*. Boston, MA: Springer US; 1992. pp. 139-164

[83] Du YG, Chu H, Chu IK, Lo C. CYP93G2 is a flavanone 2-hydroxylase required for C-glycosylflavone biosynthesis in rice. *Plant Physiology*. 2010;**154**(1):324-333. DOI: 10.1104/pp.110.161042

[84] Britsch L. Purification and characterization of flavone synthase I, a 2-oxoglutarate-dependent desaturase. *Archives of Biochemistry and Biophysics*. 1990;**282**(1):152-160. DOI: 10.1016/0003-9861(90)90099-K

[85] Martens S, Forkmann G. Cloning and expression of flavone synthase II from *Gerbera* hybrids. *The Plant Journal*. 1999;**20**(5):611-618. DOI: 10.1046/j.1365-313X.1999.00636.x

[86] Anzellotti D, Ibrahim RK. Novel flavonol 2-oxoglutarate dependent dioxygenase: Affinity purification, characterization, and kinetic properties. *Archives of Biochemistry and Biophysics*. 2000;**382**(2):161-172. DOI: 10.1006/abbi.2000.2002

[87] Latunde-Dada AO, Cabello-Hurtado F, Czittrich N, Didierjean L, Schopfer C, Hertkorn N, et al. Flavonoid 6-hydroxylase from soybean (*Glycine max* L.), a novel plant P-450 monooxygenase. *The Journal of Biological Chemistry*. 2001;**276**(3):1688-1695. DOI: 10.1074/jbc.M006277200

[88] Artigot MP, Baes M, Dayde J, Berger M. Expression of flavonoid 6-hydroxylase candidate genes in normal and mutant soybean genotypes for glycitein content. *Molecular Biology Reports*. 2013;**40**(7):4361-4369. DOI: 10.1007/s11033-013-2526-2

[89] Chebil L, Anthoni J, Humeau C, Gerardin C, Engasser JM, Ghoul M. Enzymatic acylation of flavonoids: Effect of the nature of the substrate, origin of lipase, and operating conditions on conversion yield and regioselectivity. *Journal of Agricultural and Food Chemistry*. 2007;**55**(23):9496-9502. DOI: 10.1021/jf071943j

[90] Hirschmann F, Krause F, Papenbrock J. The multi-protein family of sulfotransferases in plants: Composition, occurrence, substrate

- specificity, and functions. *Frontiers in Plant Science*. 2014;5:556. DOI: 10.3389/fpls.2014.00556
- [91] Gidda SK, Varin L. Biochemical and molecular characterization of flavonoid 7-sulfotransferase from *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*. 2006;44(11-12):628-636. DOI: 10.1016/j.plaphy.2006.10.004
- [92] Sasaki K, Tsurumaru Y, Yamamoto H, Yazaki K. Molecular characterization of a membrane-bound prenyltransferase specific for isoflavone from *Sophora flavescens*. *The Journal of Biological Chemistry*. 2011;286(27):24125-24134. DOI: 10.1074/jbc.M111.244426
- [93] Sasaki K, Mito K, Ohara K, Yamamoto H, Yazaki K. Cloning and characterization of naringenin 8-prenyltransferase, a flavonoid-specific prenyltransferase of *Sophora flavescens*. *Plant Physiology*. 2008;146(3):1075-1084. DOI: 10.1104/pp.107.110544
- [94] Liu Y, Gao L, Liu L, Yang Q, Lu Z, Nie Z, et al. Purification and characterization of a novel galloyltransferase involved in catechin galloylation in the tea plant (*Camellia sinensis*). *The Journal of Biological Chemistry*. 2012;287(53):44406-44417. DOI: 10.1074/jbc.M112.403071
- [95] Zhang HH, Yu WY, Li L, Wu F, Chen Q, Yang Y, et al. Protective effects of diketopiperazines from *Moslae Herba* against influenza A virus-induced pulmonary inflammation via inhibition of viral replication and platelets aggregation. *Journal of Ethnopharmacology*. 2018;215:156-166. DOI: 10.1016/j.jep.2018.01.005
- [96] Jeong EJ, Yang H, Kim SH, Kang SY, Sung SH, Kim YC. Inhibitory constituents of *Euonymus alatus* leaves and twigs on nitric oxide production in BV2 microglia cells. *Food and Chemical Toxicology*. 2011;49(6):1394-1398. DOI: 10.1016/j.fct.2011.03.028
- [97] Chen L, Ding L, Yu A, Yang R, Wang X, Li J, et al. Continuous determination of total flavonoids in *Platycladus orientalis* (L.) Franco by dynamic microwave-assisted extraction coupled with on-line derivatization and ultraviolet-visible detection. *Analytica Chimica Acta*. 2007;596(1):164-170. DOI: 10.1016/j.aca.2007.05.063
- [98] Chen S, Xing XH, Huang JJ, Xu MS. Enzyme-assisted extraction of flavonoids from *Ginkgo biloba* leaves: Improvement effect of flavonol transglycosylation catalyzed by *Penicillium decumbens* cellulase. *Enzyme and Microbial Technology*. 2011;48(1):100-105. DOI: 10.1016/j.enzmictec.2010.09.017
- [99] Peng S, Peng MJ, Huang ME, Bu XY, Wu G. Extraction and separation of flavonoids from leave of *Eucommia ulmoides* in aqueous two-phase system. *Zhong Yao Cai*. 2009;32(11):1754-1757. DOI: 10.13863/j.issn1001-4454.2009.11.039
- [100] Fu XQ, Ma N, Sun WP, Dang YY. Microwave and enzyme co-assisted aqueous two-phase extraction of polyphenol and lutein from marigold (*Tagetes erecta* L.) flower. *Industrial Crop Production*. 2018;123:296-302. DOI: 10.1016/j.indcrop.2018.06.087
- [101] Tang LJ, Zhang SF, Yang JZ, Gao WT. New synthetic methods of flavones. *Chinese Journal of Organic Chemistry*. 2004;24(8):882-889
- [102] Lu YH, Ji Z, Qi JX, Du CP, Chen RC, Wu SC. Synthesis of luteolin and kaempferol (author's transl). *Yao Xue Xue Bao*. 1980;15(8):477-481. DOI: 10.16438/j.0513-4870.1980.08.005
- [103] Zhu S, Wu J, Du G, Zhou J, Chen J. Efficient synthesis of eriodictyol from L-tyrosine in *Escherichia coli*. *Applied and Environmental Microbiology*. 2014;80(10):3072-3080. DOI: 10.1128/AEM.03986-13

- [104] Trantas E, Panopoulos N, Ververidis F. Metabolic engineering of the complete pathway leading to heterologous biosynthesis of various flavonoids and stilbenoids in *Saccharomyces cerevisiae*. *Metabolic Engineering*. 2009;**11**(6):355-366. DOI: 10.1016/j.ymben.2009.07.004
- [105] Miyahisa I, Funa N, Ohnishi Y, Martens S, Moriguchi T, Horinouchi S. Combinatorial biosynthesis of flavones and flavonols in *Escherichia coli*. *Applied Microbiology and Biotechnology*. 2006;**71**(1):53-58. DOI: 10.1007/s00253-005-0116-5
- [106] Koopman F, Beekwilder J, Crimi B, van Houwelingen A, Hall RD, Bosch D, et al. De novo production of the flavonoid naringenin in engineered *Saccharomyces cerevisiae*. *Microbial Cell Factories*. 2012;**11**:155. DOI: 10.1186/1475-2859-11-155
- [107] Duan L, Ding W, Liu X, Cheng X, Cai J, Hua E, et al. Biosynthesis and engineering of kaempferol in *Saccharomyces cerevisiae*. *Microbial Cell Factories*. 2017;**16**(1):165. DOI: 10.1186/s12934-017-0774-x
- [108] Xu K, Lv B, Li C. Cell-free synthetic biotechnology—Multi-enzyme catalysis and biosynthesis. *Scientia Sinica Chimica*. 2015;**45**(5):429-437. DOI: 10.1360/N032014-00263
- [109] Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, et al. The P450 superfamily: Update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA and Cell Biology*. 1993;**12**(1):1-51. DOI: 10.1089/dna.1993.12.1
- [110] Leonard E, Yan Y, Koffas MA. Functional expression of a P450 flavonoid hydroxylase for the biosynthesis of plant-specific hydroxylated flavonols in *Escherichia coli*. *Metabolic Engineering*. 2006;**8**(2):172-181. DOI: 10.1016/j.ymben.2005.11.001