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Natural sources, refined extraction, biosynthesis, metabolism, and bioactivities of dietary polymethoxyflavones (PMFs)

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ABSTRACT

Polymethoxyflavones (PMFs) are a type of uncommon dietary flavonoids, characterized by more than one methoxy group, which exist in limited plant species, like *Citrus* species and *Kaempferia parviflora*. In addition, different PMFs, such as nobiletin, sinensetin, tangeretin, and casticin, have been isolated from these natural sources. PMFs have received increasing attention due to their multiple bioactivities, such as antioxidant, anti-inflammatory, anti-cancer, metabolic regulatory, immunoregulatory, neuroprotective, and skin protective effects. These bioactivities of PMFs should be associated with the regulation of critical molecular targets and the interaction with gut microbiota. In order to provide a comprehensive and updated review of PMFs, their natural sources, refined extraction, biosynthesis, metabolism, and bioactivities are summarised and discussed, with the emphasis on the molecular mechanisms of PMFs on regulating different chronic diseases. Overall, PMFs may be promising flavonoids to the forefront of nutraceuticals for the prevention and/or treatment of certain human chronic diseases.

1. Introduction

Polymethoxyflavones (PMFs) are a special category of flavonoids. The difference of PMFs with other flavonoids is that the former possesses more than one methoxy group (−CH_3O), which has been suggested to significantly influence the bioactivities of PMFs. Most PMFs are hydrophobic, leading to their poor bioavailability when consumed orally. In nature, PMFs are not widely distributed, but restricted in certain plant species. *Citrus* species are the most rich dietary sources of PMFs, and recent studies suggest that several other plants, like *Kaempferia parviflora* and *Fructus viticis*, also contain abundant of PMFs. Besides, many advanced extraction, separation, purification, and identification technologies have been maturely applied to PMFs, significantly promoting their downstream research, such as studying their interaction with gut microbiota and bioactivities by using specific pure PMF compounds. As a result, increasing evidence supports that PMFs exhibit multiple bioactivities, such as antioxidant, anti-inflammatory, anti-cancer, regulation of metabolic syndrome and immune system, neuroprotective, and skin protective effects.

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Compared to common flavonoids, PMFs have received limited attention, while they may be also important for human health due to their diverse bioactivities. In order to highlight the importance of PMFs and also provide a better understanding of them, literature search was conducted in Web of Science, Scopus, and PubMed databases with the search term "polymethoxyflavone" in either titles or abstracts published up to 19th July, 2021. The relevant full-text articles were retrieved to assess their eligibility, and additional articles were retrieved by checking their references and manual searching. The included articles were mainly published in the last 5 years to reflect the recent situation. In this review paper, the main natural sources, refined extraction technologies, and metabolism of PMFs by gut microbiota are briefly summarized and discussed. Then, the main bioactivities of PMFs are emphasized, with related molecular mechanisms intensively discussed. It is believed that this review can promote the application of PMFs and their natural sources in the prevention and treatment of certain human chronic diseases.

2. Main natural sources of PMFs

PMFs are not widely distributed in the plant kingdom, but restricted in a few plants, mainly including the *Citrus* species (Rutaceae), *K. parviflora* (Zingiberaceae), *Artemisia annua* L. (Compositae), *Artemisia indica* (Compositae), *Lantana ukambensis* (Verbenaceae), *F. viticis* (Verbenaceae), *Leucosidea sericea* (Rosaceae), and *Nicotiana plumbaginifolia* (Solanaceae), and the chemical structures of representative PMFs are summarised in Fig. 1.

Citrus species are the most rich dietary sources of diverse PMFs and their representative PMFs are nobiletin and tangeretin[1]. Different *Citrus* species have been reported to contain diverse PMFs, mainly existing in the citrus peel and citrus leaves. The main PMFs in *Citrus* species are summarised in Table 1. Besides *Citrus* species, a few other plants also contain PMFs. For example, black ginger (*K. parviflora*) is rich in PMFs, and 3,5,7,3′,4′-pentamethoxyflavone, 5,7,4′-trimethoxyflavone, and 5,7-dimethoxyflavone were identified as the main PMFs in it[2]. In addition, Qinghao (*Artemisia annua* L.), a first-line antimalarial drug, also contained PMFs, such as artemetin, in its leaves and inflorescences[3,4]. Indian wormwood (*Artemisia indica*) is also a medicinal herb, and several PMFs, such as artemetin and casticin, were found in its leaves[5]. *Lantana ukambensis* is an African food and medicinal herb, and two PMFs, including 5,6,7,3,4,5-hexamethoxyflavone and 5-hydroxy-6,7,3,4,5-pentamethoxyflavone, were isolated from the whole plant[6]. Casticin, a main PMF, was found in *F. viticis*[7]. Besides, several other plant species were also reported to contain PMFs, including Eau de cologne mint (*Mentha* × *piperita citrata*) (Lamiaceae)[8], *Helichrysum cassianum* (Compositae)[9], *Laggera pterodonta* (Compositae)[10], *Leucosidea sericea* (Rosaceae)[11], *Murraya paniculate* (Rutaceae)[8], and *Nicotiana plumbaginifolia* (Solanaceae)[12], and detailed information is presented in Table 1. According to Table 1, relatively high levels of naringin (18.3 mg/g), nobiletin (8.15 mg/g), and 3,6,7,4′-tetramethoxyflavone (10.13 mg/g) were detected in *Citrus aurantium* fruits, Ougan peels, and *Kiyomi tangor* peels, respectively. As the representative PMFs, the content of tangeretin and nobiletin in *Citrus sunki* peels varied significantly due to different extraction solvents. For example, tangeretin had the yield of 9.8 and 540.4 mg/g by the *n*-butanol and *n*-hexane extraction, respectively, and nobiletin had the yield of 10.4 and 259.4 mg/g by the *n*-butanol and chloroform extraction, respectively.

Overall, *Citrus* species in Rutaceae family are the most rich dietary sources of diverse PMFs. In addition, several plants in the Compositae, Labiatae, Rosaceae, Solanaceae, Verbenaceae, and Zingiberaceae families, can also be good sources of certain PMFs. In the future, it is suggested to pay more attention to other plant species in the Compositae, Rutaceae, and Verbenaceae families to discover additional sources of PMFs.

3. Refined extraction of PMFs

Efficient extraction, separation, purification, and identification of natural products are essential for their further research and application, which is defined as "refined extraction" herein. The detailed information of the refined extraction technologies for PMFs in various plants are summarised in Table 2. In general, non-polar or low-polar organic solvents can be more appropriate for PMF extraction, and ethanol and methanol solutions are the most common solvents. Maceration extraction, reflux extraction, ultrasonic-assisted extraction, and supercritical CO_2 extraction, are commonly used to extract PMFs.

After the extraction, thin layer chromatography (TLC) coupled with silica gel, column chromatography, medium-pressure liquid chromatography (MPLC), and preparative high-performance liquid chromatography (HPLC) are widely applied to further separate and purify PMFs from crude extracts. Finally, the identification of PMFs can be carried out using different detection techniques, such as analytic HPLC coupled with diode array detector (DAD) or mass spectrometer (MS), gas chromatography (GC) coupled with MS, high-resolution electrospray ionization mass spectrometry (HRMS), and nuclear magnetic resonance (NMR).

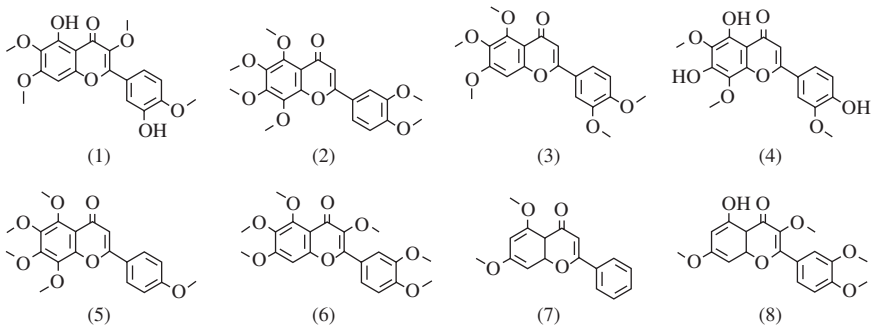

Fig. 1 Chemical structures of representative polymethoxyflavones: (1) casticin; (2) nobiletin; (3) sinensetin; (4) sudachitin; (5) tangeretin; (6) 3,5,6,7,8,3′,4′-heptamethoxyflavone; (7) 5,7-dimethoxyflavone; (8) 5-hydroxy-3,7,3′,4′-tetramethoxyflavone.

Table 1
Main natural sources of polymethoxyflavones (PMFs).

Latin name	Common name	PMF-containing part	Main PMFs	Yields	References
***Citrus* species**					
Citrus aurantium	N/A	Peels	5,6,7,8,3′,4′-Hexamethoxyflavone (nobiletin); 5,6,7,3′,4′-Pentamethoxyflavone (sinensetin); tetra-*O*-Methylscutellarein	0.12 mg/g DW; 0.07 mg/g DW; 0.01 mg/g DW	[108]
		Fruits	5,6,7,8,4′-Pentamethoxyflavone (tangeretin); Naringin	0.001 mg/g DW; 18.3 mg/g DW	[116]
*Citrus depress*a	N/A	Fruits	5,6,7,8,3′,4′-Hexamethoxyflavone (nobiletin)	ND	[117]
Citrus paradisi	Grapefruit	Whole fruit	5,6,7,3′,4′-Pentamethoxyflavone (sinensetin); 5,6,7,8,3′,4′-Hexamethoxyflavone (nobiletin); 5,6,7,8,4′-Pentamethoxyflavone (tangeretin); 3,5,6,7,8,3′,4′-Heptamethoxyflavone	ND; ND; ND; 0.02 mg/g DW	[105]
Citrus reticulata	N/A	Peels	Tangeretin	ND	[118]
Citrus reticulata B × (*Citrus reticulata* × *Citrus paradisi* Macf.)	Tangelo nova	Peels	5,6,7,3′,4′-Pentamethoxyflavone (sinensetin); 5,6,7,8,3′,4′-Hexamethoxyflavone (nobiletin); 5,6,7,8,4′-Pentamethoxyflavone (tangeretin); Heptamethoxyflavone; Quercetogetin	0.04 mg/g DW; 0.08 mg/g DW; 0.02 mg/g DW; 0.044 mg/g DW; 0.015 mg/g DW	[119]
Citrus reticulata cv. Suavissima	Ougan	Peels	5-Demethylnobiletin; 5,6,7,8,3′,4′-Hexamethoxyflavone (nobiletin); 5,6,7,8,4′-Pentamethoxyflavone (tangeretin)	1.60 mg/g DW; 8.15 mg/g DW; 1.59 mg/g DW	[46]
Citrus sinensis	Orange	Peels	3,5,6,7,8,3′,4′-Heptamethoxyflavone (3-methoxynobiletin) ($C_{22}H_{24}O_9$); 3-Methylquercetin (isorhamnetin, $C_{16}H_{12}O_7$); 5,6,7,3′,4′-Pentamethoxyflavone (sinensetin); 5,6,7,8,3′,4′-Hexamethoxyflavone (nobiletin); 5,6,7,8,4′-Pentamethoxyflavone (tangeretin); 5,7,3′-Trihydroxy-4′-methoxyflavonol (tamarixetin, $C_{16}H_{14}O_7$); 5,7-Dihydroxy-4′-methoxyflavonol ($C_{16}H_{12}O_6$); 5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavone ($C_{20}H_{20}O_8$); 7-Hydroxy-3,5-dimethoxy-3,4′-methylenedioxyflavone ($C_{18}H_{14}O_7$); 8-Hydroxy-3,4′,5,6,7-pentamethoxyflavone ($C_{20}H_{20}O_8$); Quercetogetin	ND	[110,120]
Citrus sinensis cv. Changyecheng	Long-leaf orange	Peels	5,6,7,3′,4′-Pentamethoxyflavone (sinensetin); 5,6,7,8,3′,4′-Hexamethoxyflavone (nobiletin); 5,6,7,8,4′-Pentamethoxyflavone (tangeretin); 5-Demethylnobiletin; Hexamethoxyflavone; Isosinensetin; Tetramethyl-*O*-isoscutellarein; Tetramethyl-*O*-scutellarein Total PMFs	5.1 mg/L; 47.7 mg/L; 25.6 mg/L; 0.44 mg/L; 42.2 mg/L; ND; ND; 23.30 mg/L; 164.18 mg/L	[80]
Citrus sunki	N/A	Peels	5,6,7,3′,4′-Pentamethoxyflavone (sinensetin); 5,6,7,8,3′,4′-Hexamethoxyflavone (nobiletin); 5,6,7,8,4′-Pentamethoxyflavone (tangeretin); PMFs; 5,7,4′-Trimethoxyflavone; 5-Demethylnobiletin; 5-Demethyltange etin; Isosinensetin; tetra-*O*-Methylisosutellarein	1.5−35.5 mg/g DW; 10.4−259.4 mg/g DW; 9.8−540.4 mg/g DW; 2.1%−75.8%; ND; ND; ND; ND; ND	[121]
Citrus unshiu Marc. × *C. sinensis* Osbeck × *C. reticulata* Blanco	Shiranuhi fruit	Peels	3,6,7,4′-Tetramethoxyflavone; 3,5,6,7,8,3′,4′-Hexamethoxyflavone; 3-Hydroxy-5,6,7,4′-tetramethoxyflavone; 5,6,7,3′,4′-Pentamethoxyflavone; 5,6,7,8,3′,4′-Hexamethoxyflavone (nobiletin); 5,6,7,8,4′-Pentamethoxyflavone (tangeretin); 6,7,8,3′,4′-Pentamethoxyflavone; Tetramethyl-*O*-scutellarin	15.4 mg/g DW; 0.53 mg/g DW; 1.36 mg/g DW; 0.19 mg/g DW; 0.61mg/g DW; 5.17 mg/g DW; 3.27 mg/g DW; ND	[122]
		Leaves	3,6,7,4′-Tetramethoxyflavone; 3,5,6,7,8,3′,4′-Hexamethoxyflavone; 3-Hydroxy-5,6,7,4′-tetramethoxyflavone; 5,6,7,3′,4′-Pentamethoxyflavone; 5,6,7,8,3′,4′-Hexamethoxyflavone (nobiletin); 5,6,7,8,4′-Pentamethoxyflavone (tangeretin); 6,7,8,3′,4′-Pentamethoxyflavone	7.67 mg/g DW; ND; 1.86 mg/g DW; 0.14 mg/g DW; 0.61 mg/g DW; 12.2 mg/g DW; 7.72 mg/g DW	

Table 1 (*Continued*)

Latin name	Common name	PMF-containing part	Main PMFs	Yields	References
Citrus unshiu Marc. × *C. sinensis* Osbeck × *C. reticulata* Blanco	Shiranuhi fruit	Stems	3,6,7,4′-Tetramethoxyflavone; 3,5,6,7,8,3′,4′-Hexamethoxyflavone; 3-Hydroxy-5,6,7,4′-tetramethoxyflavone; 5,6,7,3′,4′-Pentamethoxyflavone; 5,6,7,8,3′,4′-Hexamethoxyflavone; 5,6,7,8,4′-Pentamethoxyflavone (tangeretin); 6,7,8,3′,4′-Pentamethoxyflavone	2.14 mg/g DW; ND; 0.48 mg/g DW; 0.43 mg/g DW; 0.26 mg/g DW; 3.87 mg/g DW; 2.87 mg/g DW	[122]
		Peels	5,6,7,3′,4′-Pentamethoxyflavone; 6,7,8,3′,4′-Pentamethoxyflavone; 3-Hydroxy-5,6,7,4′-tetramethoxyflavone; 5,6,7,8,3′,4′-Hexamethoxyflavone; 3,6,7,4′-Tetramethoxyflavone; 3,5,6,7,8,3′,4′-Hexamethoxyflavone; 5,6,7,8,4′-Pentamethoxyflavone (tangeretin)	0.09 mg/g DW; 0.27 mg/g DW; 0.28 mg/g DW; 0.32 mg/g DW; 0.14 mg/g DW; 0.63 mg/g DW; 2.19 mg/g DW	
Citrus unshiu Marcov. forma *miyagawa-wase*	Satsuma mandarin	Leaves	5,6,7,3′,4′-Pentamethoxyflavone; 6,7,8,3′,4′-Pentamethoxyflavone; 3-Hydroxy-5,6,7,4′-tetramethoxyflavone; 5,6,7,8,3′,4′-Hexamethoxyflavone; 3,6,7,4′-Tetramethoxyflavone; 3,5,6,7,8,3′,4′-Hexamethoxyflavone; 5,6,7,8,4′-Pentamethoxyflavone (tangeretin)	0.90 mg/g DW; 0.32 mg/g DW; 043 mg/g DW; 0.38 mg/g DW; 0.04 mg/g DW; 0.09 mg/g DW; 1.58 mg/g DW	[123]
		Stems	5,6,7,3′,4′-Pentamethoxyflavone; 6,7,8,3′,4′-Pentamethoxyflavone; 3-Hydroxy-5,6,7,4′-tetramethoxyflavone; 5,6,7,8,3′,4′-Hexamethoxyflavone; 3,6,7,4′-Tetramethoxyflavone; 3,5,6,7,8,3′,4′-Hexamethoxyflavone; 5,6,7,8,4′-Pentamethoxyflavone (tangeretin)	1.41 mg/g DW; 0.38 mg/g DW; 0.03 mg/g DW; 0.24 mg/g DW; 0.10 mg/g DW; 0.07 mg/g DW; 0.91 mg/g DW	
		Peels	5,6,7,3′,4′-Pentamethoxyflavone; 6,7,8,3′,4′-Pentamethoxyflavone; 3-Hydroxy-5,6,7,4′-tetramethoxyflavone; 5,6,7,8,3′,4′-Hexamethoxyflavone; 3,6,7,4′-Tetramethoxyflavone; 3,5,6,7,8,3′,4′-Hexamethoxyflavone; 5,6,7,8,4′-Pentamethoxyflavone (tangeretin)	2.25 mg/g DW; 5.89 mg/g DW; 1.25 mg/g DW; 1.03 mg/g DW; 10.13 mg/g DW; 0.66 mg/g DW; 3.76 mg/g DW	
Citrus unshiu Marcov. forma *miyagawa-wase* × *C. sinensis*	*Kiyomi tangor*	Leaves	5,6,7,3′,4′-Pentamethoxyflavone; 6,7,8,3′,4′-Pentamethoxyflavone; 3-Hydroxy-5,6,7,4′-tetramethoxyflavone; 5,6,7,8,3′,4′-Hexamethoxyflavone; 3,6,7,4′-Tetramethoxyflavone; 3,5,6,7,8,3′,4′-Hexamethoxyflavone; 5,6,7,8,4′-Pentamethoxyflavone (tangeretin)	2.08 mg/g DW; 4.93 mg/g DW; 0.71 mg/g DW; 0.89 mg/g DW; 9.24 mg/g DW; 0.03 mg/g DW; 8.41 mg/g DW	[123]
		Stems	5,6,7,3′,4′-Pentamethoxyflavone; 6,7,8,3′,4′-Pentamethoxyflavone; 3-Hydroxy-5,6,7,4′-tetramethoxyflavone; 5,6,7,8,3′,4′-Hexamethoxyflavone; 3,6,7,4′-Tetramethoxyflavone; 3,5,6,7,8,3′,4′-Hexamethoxyflavone; 5,6,7,8,4′-Pentamethoxyflavone (tangeretin)	1.39 mg/g DW; 0.71 mg/g DW; 1.48 mg/g DW; 0.17 mg/g DW; 0.50 mg/g DW; 0.09 mg/g DW; 0.35 mg/g DW	
N/A	Dancy tangerine	Leaves	5,6,7,3′,4′-Pentamethoxyflavone (sinensetin); 5,6,7,8,3′,4′-Hexamethoxyflavone (nobiletin); 5,6,7,8,4′-Pentamethoxyflavone (tangeretin); 5,7,8,3′,4′-Pentamethoxyflavanone; 5,7-Dihydroxy-6,8,3′,4′-tetramethoxyflavone; 5-Hydroxy-6,7,3′,4′-tetramethoxyflavone; 5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavone (5-*O*-desmethylnobiletin); 7-Chloro-3,5,6,8,3′,4′-hexamethoxyflavone; 7-Chloro-3,5,6,8,4′-pentamethoxyflavone	ND	[124]
Other species					
Artemisia annua L.	Qinghao	Leaves and inflorescences	Artemetin; Eupatin; Casticin; Chrysoplenetin	0.14 mg/g DW; 0.01 mg/g DW; 0.39 mg/g DW; 0.36 mg/g DW	[4]

Table 1 (*Continued*)

Latin name	Common name	PMF-containing part	Main PMFs	Yields	References
Artemisia indica	Indian wormwood	Leaves	Artemetin; Casticin; Chrysophenol-D; Chrysoplenetin; Cirsilineol; Eupatin	ND; 0.009 mg/g DW; ND; 0.016 mg/g DW; 0.021 mg/g DW; 0.045 mg/g DW	[5]
Fructus viticis	N/A	N/A	Casticin	ND	[7]
Helichrysum cassianum	N/A	Leaves and stems	3,5-Dihydroxy-6,7,8,4′-tetra-methoxyflavone	ND	[9]
Kaempferia parviflora	Black ginger		5,7-Dimethoxyflavone (DMF); 5,7,4′-Trimethoxyflavone (TMF); 3,5,7,3′,4′-Pentamethoxyflavone (PMF)	ND	[87]
Kaempferia parviflora	Black ginger	Rhizomes	5,7-Dimethoxyflavone (DMF); 5,7,4′-Trimethoxyflavone (TMF); Nobiletin	0.5 mg/g DW; 0.17 mg/g DW	[113]
Kaempferia parviflora	Black ginger	Rhizome	3,5,7,3′,4′-Pentamethoxyflavone; 3,5,7,4′-Tetramethoxyflavone; 3,5,7-Trimethoxyflavone; 5,7,3′,4′-Tetramethoxyflavone; 5,7,4′-Trimethoxyflavone; 5,7-Dimethoxyflavone; 5-Hydroxy-3,7,3′,4′-tetramethoxyflavone; 5-Hydroxy-3,7-dimethoxyflavone; 5-Hydroxy-7,4′-dimethoxyflavone; 5-Hydroxy-7-methoxyflavone; 5-Hydroxy-3,7,4′-trimethoxyflavone	19.6%; 6.2%; 3.9%; 0.91%; 12.9%; 10.1%; 2.2%; 4.1%; 2.2%; 2.0%; 5.6%	[125]
Laggera pterodonta	N/A	N/A	3,5-Dihydroxy-6,7,3′4′-tetramethoxyflavone	ND	[10]
Lantana ukambensis (Vatke) Verdc. (Verbenaceae)	N/A	Whole plant	5,6,7,3′,4′,5′-Hexamethoxyflavone; 5-Hydroxy-6,7,3′,4′,5′-hexamethoxyflavone	ND	[6]
Leucosidea sericea Eckl. & Zeyh.	Oldwood (English), ouhout (Afrikaans), and umTshitshi (Zulu)	Leaves	3,5,7,3′,4′-Pentamethoxyflavone (sinensetin); 5,6,7,8,4′-Pentamethoxyflavone (tangeretin)	ND	[11]
Mentha × *piperita citrata*	Eau de cologne mint	Leaves	5,6,4′-Trihydroxy-7,8,3′-trimethoxyflavone; 5,6-Dihydroxy-7,8,3′,4′-tetramethoxyflavone; 5,6-Dihydroxy-7,8,3′,4′-tetramethoxy-flavone	0.18 mg/g DW; 0.28 mg/g DW; 0.18 mg/g DW	[8]
Murraya paniculata	Daun kumning	Leaves	5-Hydroxy-6,7,8,3′,4′,5′-hexamethoxyflavone (gardenin A); 5,3′-Dihydroxy-6,7,8,4′,5′-pentamethoxyflavone (gardenin C); 6,7,8,4′-Tetramethoxy-5,3′,5′-trihydroxyflavone (gardenin E); 5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavone (5-*O*-desmethylnobiletin); 6,7,8,3′,4′,5′-Hexamethoxyflavone; 5-Hydroxy-6,7,3′,4′,5′-pentamethoxyflavone; 5,3′-Dihydroxy-6,7,4′,5′-tetramethoxyflavone; 5,3′,5-Trihydroxy-6,7,4′-trimethoxyflavone	2.9 mg/g DW; 0.79 mg/g DW; 0.34 mg/g DW; 0.49 mg/g DW; 0.27 mg/g DW; 0.22 mg/g DW; 0.08 mg/g DW; 0.14 mg/g DW	[126]
Nicotiana plumbaginifolia	Solanaceae	Leaves	3,3′,4′,5,5′,8-Hexamethoxy-6,7-methylenedioxyflavone; 3,3′,4′,5′,5,6,7,8-Octamethoxyflavone (exoticin); 3,3′,5,6,7,8-Hexamethoxy-4′,5-methylenedioxyflavone; 6,7,4′,5-Dimethylenedioxy-3,5,3-trimethoxyflavone	ND	[12]

Notes: ND, not detectable; PMFs, polymethoxyflavones; N/A, not applicable.

In the future, more green and advanced extraction technologies applied for other phytochemical extraction, such as tea catechins and hemp bioactive compounds[13-16], may be further utilized to extract plant PMFs.

4. Biosynthesis of PMFs

The biosynthesis of citrus flavonoids is intensively reviewed by a recent study[17], however, the biosynthesis of PMFs in citrus or other species has not been summarized. Taken citrus PMFs as an example, their biosynthesis also stems from a precursor molecule phenylalanine, like the biosynthesis of other flavones in citrus via different enzymatic reactions[17]. Afterwards, *O*-methyltransferases (OMTs) play a critical role in the methylation of flavone hydroxyl groups (−OH), but the genetic basis for the methylation process has not been fully understood in citrus.

Recent studies indicate that different OMTs can be cooperatively involved in PMF methylation with different substrate specificities and regioselectivities. For example, 5 genes encoding flavonoid *O*-methyltransferases (*CdFOMT* 1, 3, 4, 5, and 6) were isolated from *Citrus depressa*, and the *CdFOMT5* encodes an *O*-methyltransferase enzyme possessing a wide range of substrate specificity and regioselectivity for flavonoids[18]. Liu et al.[19] characterized a caffeoyl-CoA OMT-like enzyme (CrOMT1) in the *Citrus reticulata*, and the recombinant CrOMT1 could efficiently methylate flavones with neighboring OH groups, and it exhibited high catalytic

Table 2
Refined extraction technologies of polymethoxyflavones (PMFs).

Natural sources	Extraction conditions	Separation and purification conditions	Identification methods	Extraction yield	References
***Citrus* species**					
Nineteen different citrus cultivars obtained from Japan	100 g diced fresh citrus peel was homogenized in 500 mL ethanol before filtration	Purified by preparative TLC with silica gel 60 F254 glass plates and eluted with *n*-hexane/ethyl acetate (2:3) to afford 6 zones (Z1-6)	NMR and MS	ND	[107]
C. medica, *C. medica* oblong, *C. limon*, *C. maxima*, *C. sinensis*, and *C. reticulate*	1 g powdered peel was extracted by 10 mL water using a magnetic stirrer for 1 h	Capillary column (5% phenyl, 95% dimethyl polysiloxane, non-polar, 30 m × 0.25 mm, 0.25 μm, film thickness); carrier gas: N_2, 3 mL/min; carrier gas: He, 1.4 mL/min	GC-FID, GC-MS, LRI, HPTLC	(+)-Limonene (36.7%−87.54%)	[127]
Citrus reticulata	0.7 kg air-dried peel was extracted with methanol (1 L × 4) at ambient temperature	Isolated with TLC (CH_2Cl_2-/MeOH, 80:1) using silica gel (950 g, 70–230 mesh) and eluted with EtOAc-MeOH	ESI-MS, NMR	ND	[118]
Citrus unshiu	Supercritical fluid extraction: 20 g freeze-dried samples loaded into a 50 cm^3 cell, electric oven temperature 59.85 °C, total flow rate of supercritical CO_2 0.026 mol/min, temperature of 333 K and pressure of 30 MPa	ODS-column; mobile phase: methanol and phosphoric acid aqueous solution; flow rate 0.6 mL/min; column temperature 39.85 °C	HPLC-UV-vis	Nobiletin (700 μg/g)	[128]
Citrus unshiu (immature)	10 g powdered whole fruit was suspended in 50 mL MeOH and shaken at ambient temperature overnight	Isolated with silica gel and eluted with *n*-hexane/ethyl acetate	NMR	Nobiletin (0.16%); tangeletin (0.08%); 3,5,6,7,8,3′,4′-hexamethoxyflavone (0.09%)	[129]
Kiyomi tangor and *Satsuma mandarin*	20 g each plant part (peels, leaves, and stems) was extracted with MeOH (300 mL × 3) under reflux and vacuum dried	Isolated with TLC using silica gel, eluted with water/ACN	HPLC, UV/Vis	See Table 1	[123]
Long-leaf orange (*Citrus sinensis* cv. Changyecheng)	Peel was squeezed and aqueous peel oil emulsion was centrifuged at 12 000 r/min at 4 °C for 10 min	Column (4.6 mm × 150 mm, 2.7 μm); solvent: 0.05% phosphoric acid/water, methanol, and 50% tetrahydrofuran/water	GC-MS	*D*-Limonene (809.1 g/L)	[80]
Mandarin (*Citrus reticulata* Blanco cv. Egyptian), sweet orange [*C. sinensis* (L.) Osbeck cv. Olinda Valencia], white grapefruit (*C. paradisi* Macfad. cv. Duncan) and lime (*C. aurantiifolia* Swingle cv. Mexican)	Air dried peel powder was macerated in 75% alcohol for two times at room temperature and then vacuum dried	Isolated with TLC using silica gel, eluted with hexane containing 10%–100% acetone	EI/MS	Nobiletin: 202.9 μg/mL in mandarin, 72.2 μg/mL in sweetorange, 18.3 μg/mL in grapefruit, 0.09 μg/mL in lime	[86]
Ougan (*Citrus reticulata* cv. Suavissima)	100 g Ougan peel was ultrasonically extracted in 2 L of ethanol at room temperature for 30 min and this was repeated three times	Eluted with 50 BV double distilled water, 12 BV 35% aqueous methanol solution and 100% methanol successively in an Sep-pak C_{18} cartridge column	UPLC-MS	Nobiletin (purity, 99.87%), tangeretin (purity, 99.76%), 5-demethylnobiletin (purity, 98.75%)	[46]
Shiranuhi fruit (immature)	Dried peel was extracted with 80% ethanol 3 times at room temperature for 24 h	Isolated by MPLC with a C_{18} SiO_2 column and eluted with H_2O-MeOH (10%–100%)	NMR; ESI	ND	[45]
Sweet orange (*C. sinensis* L. Osbeck cv. Olinda Valencia), lime (*C. aurantiifolia* Swingle cv. Mexican), grapefruit (*C. paradisi* Macfad. cv. Duncan), mandarin (*C. reticulata* Blanco cv. Egyptian)	Powered peel was homogenized with 7 mL 100% MeOH containing 10 μg/mL umbelliferone using a Turrax mixer (11 g) for 20 s for 5 periods	Isolated with a C_{18} (500 mg) cartridge preconditioned with methanol and water and eluted with 6 mL methanol	UPLC-MS	ND	[130]
Sweet orange (*C. sinensis*)	Extracted with 2 L petroleum ether (40–60 °C) 3 times	Isolated by TLC using silica gel, and eluted with various solvent systems: S1 was *n*-hexane/*n*-butanol (85:15, *V/V*), S2 was *n*-hexane/EtOAc (4:1, *V/V*), S3 was chloroform/methanol (85:15, *V/V*), and S4 was *n*-hexane-ethyl acetate-methanol-water (5:5:5:5, *V/V*)	UV, MS, IR, ^{1}H NMR, and ^{13}C NMR	ND	[110]

Table 2 (*Continued*)

Natural sources	Extraction conditions	Separation and purification conditions	Identification methods	Extraction yield	References
	Dried rhizome powder extracted using 20 mL of 50% ethanol at room temperature overnight using a tube rotator	Isolated by preparative HPLC with different columns (YMC-DispoPack AT ODS-25 and Wakosil-II $5C_{18}$ RS-Prep) and eluents [ACN/H_2O (55:45, *V/V*) and ACN/MeOH/H_2O (20:30:35, *V/V*)]	NMR and HRMS	3,5,7,3′,4′-Pentamethoxyflavone (13.4% of KEG);5,7-dimethoxyflavone (12.5% of KEG); 5,7,4′-trimethoxyflavone (13.3% of KEG); 3,5,7-trimethoxyflavone (2.6% of KEG); 3,5,7,4′-tetramethoxyflavone (6.9% of KEG); 5-hydroxy-7-methoxyflavone (2.3% of KEG); 5-hydroxy-7,4′-dimethoxyflavone (1.5% of KEG)	[2]
Black ginger (*Kaempferia parviflora*)	Powdered rhizome soaked in hexane, ethyl acetate, acetone, ethanol, methanol, hot water (90–100 °C), or water for 24 h, after which the solutions or suspensions were filtered using quantitative filter paper	C_{18} column (4.6 mm × 250 mm, 5 µm); column temperature: 35 °C; solvent: 0.1% formic acid in ACN (solvent A) and ACN (solvent B)	HPLC	5,7-Dimethoxyflavone (0.56%–21.47% of BG); 5,7,4′-trimethoxyflavone (0.51%–26.02% of BG); 3,5,7′-trimethoxyflavone (0.10%–5.03% of BG); 3,5,7,4′-tetramethoxyflavone (0.24%–10.76% of BG)	[82]
	1 g powdered rhizome was extracted overnight with 50% aqueous ethanol (20 mL) at room temperature using a shaker. Samples were centrifuged and the supernatants were collected and filtered through a 0.2-µm membrane	Column: 150 mm × 2 mm, 5 µm; temperature 40 °C; mobile phase: 10 mmol/L ammonium acetate in water and methanol; flow rate: 0.2 mL/min	LC-MS/MS	ND	[39]
	Powdered rhizome was extracted with hot 50% ethanol (80 °C) for 2 h and evaporated	7.5 kg extract in 40% ethanol (30 L) subjected to column chromatography was eluted with 40% ethanol (70 L) and 80% ethanol (70 L)	HPLC	ND	[131]
	10 g powdered rhizome was shaken overnight at room temperature in 300 mL 50% aqueous ethanol and then filtrated and dried. The filtrate was partitioned between 100 mL *n*-hexane and 100 mL water containing 10 g sodium chloride. The process was repeated twice	A suspension of silica gel (100 g) and *n*-hexane was packed into a glass column (30 cm × 30 mm), and 190 mg extract dissolved in 5 mL ethyl acetate was loaded onto it	GC-MS, LC-MS/MS	5,7-Dimethoxyflavone (370 mg/g of extract); 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (260 mg/g of extract)	[113]
	100 g dried rhizomes extracted by methanol (200 mL × 5) under reflux for 1 h	Silica gel using hexane/acetone (20:1)	NMR	0.13 g/g DW	[132]
Other species					
Eau de cologne mint	50 g cut leaves were soaked in 100% ethanol (100 mL) 4 times, and solvent was removed under vacuum	HPLC 5 µm-ODS column with 60% methanol aqueous solution at a flow rate of 1 mL/min	NMR and MS	5,6,4-Trihydroxy-7,8,3′-trimethoxyflavone (16.9 mg/g); 4′-trihydroxy-7,8-dimethoxyflavone (23.8 mg/g); 5,6-dihydroxy-7,3′,4′-trimethoxyflavone (17.7 mg/g); 5,6-dihydroxy-7,8,3′,4′-tetramethoxy flavone (62.2 mg/g) 5,6-dihydroxy-7,8,4′-trimethoxy-flavone (36.07 mg/g)	[9]
Lantana ukambensis (Vatke) Verdc. (Verbenaceae)	50 g powdered sample was macerated with stirring for 1 day in methylene chloride (250 mL)	Isolated with TLC using silica gel and eluted with cyclohexane/ethyl acetate/ethanol/acetic acid (6:2:2:0.1)	MS, NMR	5-Hydroxyl-6,7,3′,4′,5-pentamethoxyflavone	[6]
Leucosidea sericea	3 g powdered leave were soaked in 30 mL ethanol for 72 h and placed on a shaker at room temperature	C_{18} column (150 mm × 2.1 mm, 1.8 µm); column temperature: 60 °C; solvent mixture: water (eluent A) containing 10 mmol/L formic acid (natural pH 2.3) and ACN (eluent B) containing 10 mmol/L formic acid	NMR and LC-MS	10 mg of crude ethanol extract/powdered plant material (3 g)	[11]
Qinghao (*Artemisia annua* L.)	Leaves and inflorescences were freeze dried and cut, and then extracted with 100 mL hexane for 3 days	Column RT (250 mm × 4.6 mm, 5 µm); eluents comprise: water adjusted to pH 3.2 by formic acid (A) and ACN (B); flow rate: 1.3 mL/min	HPLC/DAD and HPLC/MS	0.54 mg/g artemetin in leaves and 0.44 mg/g artemetin in the inflorescences	[4]

Notes: ACN, acetonitrile; BV, bed volume; DAD, diode array detector; ESI, electrospray ionisation; FID, flame ionisation detector; GC, gas chromatography; HPLC, high performance liquid chromatography; HPTLC, high performance thin layer chromatography; HRMS, high-resolution electrospray ionisation mass spectrometry; IR, infrared; LC, liquid chromatography; LRI, laser resonance ionisation; MPLC, medium-pressure liquid chromatography; MS, mass spectrometer; NMR, nuclear magnetic resonance; TLC, thin layer chromatography; UPLC, ultra-performance liquid chromatography; UV-vis, ultraviolet-visible.

efficiencies for 6-OH- and 8-OH-containing compounds *in vitro*. In addition, transient overexpression of *CrOMT1* gene resulted in the accumulation of three major PMFs in the citrus fruit, suggesting that *CrOMT1* is probably involved in the biosynthesis of PMFs in citrus[19]. Moreover, Liu et al.[20] again isolated another *OMT* gene, *CrOMT2*, from the fruit peel of *Citrus reticulata*. Its recombinant protein was able to efficiently catalyze the methylation of 3′-, 5′-, and 7-OH of flavonoids with vicinal hydroxyl substitutions, similar to *CrOMT1*. In addition, CrOMT2 recombinant enzyme preferred PMF-type substrates in the substrate preference assay *in vitro*[20]. Zohra et al.[21] also identified two novel OMT candidate genes, *CreOMT1* and *CreOMT4* in citrus, and the expression of *CreOMT1* gene had a positive correlation with the content of nobiletin in the flavedo of 10 citrus cultivars. Seoka et al.[22] showed that the recombinant protein of a novel *OMT* gene (*CitOMT*) had the methylation activity to transfer a methyl group to the 3′-hydroxy group of flavones. In a recent study, Ma et al.[23] also showed that *O*-methyltransferase (*CitOMT2*) was highly expressed in the citrus fruit varieties rich in nobiletin. Thus, *CitOMT* is a key gene for nobiletin biosynthesis in citrus fruits.

Based on the above results, OMTs has been seldom revealed in other plants besides certain *Citrus* species, and understanding the methylation process and related molecular basis is essential to better characterize the biosynthesis of PMFs in them. Moreover, discovering OMTs with high enzymatic activities and substrate specificities can promote the large-scale biosynthesis of specific PMFs with excellent bioactivities using advanced metabolic engineering techniques.

5. Metabolism of PMFs involving in gut microbiota

The bioavailability of natural products is a general concept of their absorption, distribution, metabolism, and excretion. A previous article reviewed the relationship between the side chains (e.g., hydroxyl and methoxy) of PMFs and their solubility as well as the permeability of PMFs[24]. Another review discussed the actions of different enzymes on PMFs and their metabolites[25]. Herein, we updated some recent progress on the metabolism of PMFs involving in gut microbiota.

Gut microbiota play an important role in the metabolism of diverse plant components. Recent studies demonstrate that gut microbiota are also involved in the metabolism of certain PMFs. An activity-guided screening strategy was used to discover PMF-metabolizing gut microbiota under anaerobic conditions, and a strict anaerobic bacterium, *Blautia* sp. MRG-PMF1, was isolated, which exhibited the demethylation activity and could metabolize different PMFs into the corresponding demethylated flavones[26]. For example, 5,7,4′-trimethoxyflavone could be completely metabolized to 5,7,4′-trihydroxyflavone (apigenin) by this strain. In addition, it was suggested that gut microbiota exhibited a stronger activity on the biotransformation of nobiletin than the host when analyzing its demethylated metabolite profiles in the urine and faeces of rats[27]. Moreover, the oral administration of PMF-rich extract from Ougan (*Citrus reticulata* cv. Suavissima) in mice led to the occurrence of 21 PMF metabolites in the intestine, mostly through the effects of demethylation, demethoxylation, hydroxylation, and glucuronidation, and gut microbiota should play an important role in metabolizing PMFs[28].

On the other hand, PMFs can also regulate the composition of gut microbiota. It was found that citrus peel extracts rich in PMFs could significantly increase the abundance of *Prevotella*, but reduce the abundance of rc4-4 bacteria in high-fat diet (HFD)-induced obese mice[29]. In a recent study, treatment of aged citrus peels (Chenpi) extract significantly decreased the abundance of *Proteobacteria* and the ratio of *Firmicutes* to *Bacteroidetes* in HFD-induced obese mice, while dose-dependently increased two beneficial bacteria, *Akkermansia* spp. and *Allobaculum* spp.[30]. Similarly, the relative abundance of two probiotics, *Lactobacillus* and *Bifidobacterium*, dramatically increased after oral administration of PMF-rich extract from Ougan (*Citrus reticulata* cv. Suavissima) in mice[28]. Therefore, PMF can overall ameliorate gut microbial dysbiosis by inhibiting pathogenic microbes and increasing beneficial or probiotic microbes in the gut, which can be important for the gut barrier and overall health[31]. Falduto et al.[32] showed that the treatment of a PMF-enriched Chenpi extract significantly increased the numbers of *Roseburia*, *Blautia*, *Subdoligranulum*, *Eubacterium*, and *Firmicutes* in the human gut. Chen et al.[33] showed that after oral administration of PMF-rich fraction from Ougan (*Citrus reticulata* cv. Suavissima), the relative abundance of *Lactobacillus* and *Bifidobacterium* was found to be increased significantly in the gut of mice. Additionally, Wu et al.[34] proved that the consumption of PMF extracts also altered the composition of gut microbiota by increasing butyrate-producing probiotics and decreasing colorectal cancer (CRC)-related bacteria.

However, there is still lack of enough evidence to fully explain the interaction of diverse PMFs with gut microbiota, and the relationships among gut microbiota, PMFs, and their bioactivities remain not very clear. Currently, most studies still focus on the interaction of PMF-enriched extracts with gut microbiota. In the future, more specific PMF compounds prepared by refined extraction should be explored about their influences on the regulation of gut microbiota, and more gut microbiota capable of metabolizing PMFs should be discovered and isolated, with intensive clarification of their interactions and related influences on gut and overall health. Besides, whether gut microbiota can influence the bioavailability of PMFs and whether the metabolites of PMFs transformed by gut microbiota have enhanced bioactivities than PMFs *per se* remain to be verified in the future.

6. Bioactivities of PMFs

PMFs have been reported to exhibit plenty of bioactivities, such as antioxidant, anti-inflammatory, anti-cancer, anti-obesity, and neuroprotective effects. In the following section, their main bioactivities and related molecular mechanisms are discussed, and the detailed information of these *in vitro* and *in vivo* studies are summarised in Tables 3, 4, and S1, respectively.

6.1 Antioxidant effect

Flavonoids are one of the main antioxidants existing in plant-based foods, such as fruits, vegetables, and teas[35-38]. PMFs as a type of uncommon flavonoids also exhibit good antioxidant effects *in vitro* and *in vivo*.

Table 3
Bioactivities of polymethoxyflavones (PMFs) based on *in vitro* studies.

PMFs	Models	Dosages	Treatment	Major effects	Molecular mechanisms	References
Antioxidant effect						
DMF and TMF	Pyocyanin-stimulated HUVECs	1 000 μg/mL	Incubated with 100 μL samples for 2 h	ROS production ↓		[39]
Tangeretin	HepG2 liver cancer cells	20 μmol/L	Incubated with tangeretin of various concentrations	Tert-butyl hydroperoxide-induced oxidative stress ↓	HO-1 ↑ and NQO1 ↑; MAPK-Nrf2-ARE signaling pathway ↑	[41]
(2*S*)-5,6,7,3′,4′-pentamethoxyflavanone	Hepa 1c1c7 murine hepatoma cells; human breast carcinoma MDA-MB-231 cells; human lung epithelial Beas-2B cells	6.25 or 12.5 μmol/L	Treated with various doses of PMF for 24 h	Arsenic- and cigarette smoke extract-induced cytotoxicity ↓	Stabilisation of Nrf2 ↑, Nrf2 ubiquitination ↓	[42]
NBT, 5-demethylnobiletin, tangeretin, and 5-demethyltangeretin	Wild-type *Saccharomyces cerevisiae* strain BY4741	25, 50 or 100 μg/mL	Incubated with PMFs at 28 °C, 160 r/min for 2 h	Oxidative damages ↓	Intracellular ROS and lipid peroxidation ↓	[44]
Anti-inflammatory effect						
NBT, tangeretin, and 5-demethylnobiletin	BV-2 cells	6.25 and 25 μg/mL	Incubated with PMFs for 1 day	Inflammation-related cytokines, NO release phosphorylation, and expression of JAK2 ↓; expression and phosphorylation of STAT3 ↓	IL-1β ↓, IL-6 ↓, TNF-α ↓, iNOS ↓, JAK2 ↓, and NF-κB ↓; IκBα ↑	
Sudachitin	Osteoblasts from C57BL/6J mice	10–50 μmol/L	Incubated with sudachitin for 1 day	Bone destruction and osteoclastogenesis ↓	Expression of c-fos, NFATc1, cathepsin K, DC-STAMP, and Atp6v0d2 ↓; ROS production ↓; MAPKs activation ↓	
Tetramethyl-*O*-scutellarin	LPS-induced RAW264.7 cells	100 μg/mL (EtOAc fraction)	Immature Shiranuhi peels (1 g) were extracted with 80% ethanol three times by stirring using a mechanical stirrer at room temperature for 24 h	Exhibited anti-inflammatory capabilities	PGE_2, TNF-α, IL-1β, and IL-6 ↓; NO production ↓	
DMF and TMF	TNF-α-stimulated HUVECs and RAW264.7 cells	50 or 1 000 μg/mL	Incubated with DMS TMF, and extracts for 1 day	Nitrite levels ↓; adhesion of THP-1 cells to HUVECs ↓	eNOS in TNF-*α*-stimulated HUVECs ↑; various cell adhesion molecules, inflammatory factors, and endothelial function-related mediators ↓	
NBT	MH7A human synovial cells	25 and 50 μmol/L	Treated with nobiletin for 48 h	IL-21 downstream inflammation-related mediators ↓; JAK1/STAT3 pathway ↓	IL-21 receptor in MH7A FLS ↓; phosphorylation of both JAK1 and STAT3 ↓	
Anti-cancer effect						
NBT	K562 leukaemia cells	10–100 μmol/L	Treated with NBT for 1 and 2 days	Cell viability ↓; cell growth ↓; megakaryocytic differentiation ↑	Regulated cyclin D2, p21 and p27; G1 phase arrest ↑; expression of CD41, CD42, CD61 and EGR1 ↑; MAPK/ERK phosphorylation ↑	[60]
NBT	Prostate cancer VCaP and PC-3 cells	0.02–0.05 μmol/L	Treated with NBT and/or bicalutamide at various concentrations for 24–72 h	Cell growth, clonogenicity and migration ↓; apoptosis ↑	NF-κB, p-STAT3/STAT3, and p-Erk/Erk ↓	[712
Casticin	DU 145 prostate cancer cells	1.25, 2.5, and 5 μmol/L	Incubation with various concentrations of casticin for 24 and 48 h	Viability, mobility, migration and invasion ↓	AKT, GSK3 αβ, Snail, MMPs, NF-κB, p65, GRB2, SOS-1, MEK, p-ERK1/2, and p-NK1/2 ↓	[69]
Tangeretin	PC-3 cells and LNCaP prostate cancer cells	75 μmol/L	Treated with tangeretin for 1, 2 and 3 days	Cell viability ↓; reprogrammed EMT	Expression of mesenchymal proteins ↓; expression of epithelial proteins ↑; PI3K/Akt/mTOR pathway ↓	[66]
5-AcTMF	T98G, U-87 MG and GBM8401 brain cancer cells	50, 100, 150, and 200 μmol/L	Treated with 5-AcTM for 48 h	Cell colony formation and viability ↓; apoptosis ↑	p-STAT3, JAK2, BCL-2 and BCL-xL ↓; phosphorylation of STAT3 ↓	[64]
Casticin	Colo 205 human colon cancer cells	0–100 μmol/L	Treated with casticin for 1 and 2 days	Morphological changes ↑, viability ↓, apoptosis ↑	ROS production ↑; activities of caspase-3, -8 and -9 ↑; CDKN1A, PAK3, MMP-2, TLR4, PRKAR2B, and CaMK4 ↓	[61]
NBT	HT29 human colorectal cancer cells	1.5–12 μmol/L	Treated with NBT and ATST co-treatment for 3 days	Cell growth ↓	Cell cycle arrest and apoptosis ↑	[72]
Regulation of metabolic syndrome						
Casticin	H446 lung cancer cells	1, 3 and 10 μmol/L	Incubation with various concentrations of casticin for 6 days	Clonogenicity ↓	uPAR and CD133 ↓; phosphorylation of AMPK and ACC ↑; decreased p-FoxO3a expression ↓	[70]

Table 3 (*Continued*)

PMFs	Models	Dosages	Treatment	Major effects	Molecular mechanisms	References
NBT	SKOV3/TAX ovarian cancer cells	20 and 40 μmol/L	Treated with NBT for 24 and 48 h	Growth and proliferation ↓; apoptosis ↑	Cleaved caspase-9/-3 and PARP ↑; AKT signaling ↑	[59]
Casticin	SCC-4 oral cancer cells	0.25, 0.5, 1.25, and 5 μmol/L	Treated with casticin for 2 days	Cell morphological alteration ↑; cell viability ↓	Activities of caspase-3, -8, and -9 ↑; migration of AIF and cytochrome c ↑; cell cycle arrest ↑	[62]
Casticin	B16F10 skin cancer cells	0.08–20 μmol/L	Treated with casticin of various concentrations for 1 day	Viability, migration, and invasion ↓	Expression of p-JNK 1/2, MMP-9, Rho A, MMP-1, FAK, 14-3-3, uPA, GRB2, Akt, MMP-2, NF-κB p65, SOS-1, p-EGFR, NDUFS4, VEGFA and DDIT3 ↓; increased expression of SCN1B and TIMP2 ↑	[7]
Casticin	B16F10 skin cancer cells	20, 30, and 40 μmol/L	Treated with casticin for 1 and 2 days	Cell viability ↓; altered morphological properties	DNA damage ↑; expression of MGMT, BRCA1, MDC1, and DNA-PK ↓; p-p53, p-ATM, p-H2A.X, and PARP ↑	[133]
Casticin	A375.S2 skin cancer cells	0–25 μmol/L	Treated with casticin for 1 day	Altered morphological properties; cell viability ↓; DNA damage and cell cycle arrest ↑	ROS production and caspase-3 activities ↑; ΔΨm level ↓; expression of AIF, Endo G, p53, p21 and CHK-1 ↑; expression of Cdc25c, CDK-1, Cyclin A and Cyclin B ↓; NF-κB binding DNA ↓	[63]
NBT	SNU-16 gastric cancer cells	12.5–50 μmol/L	Treated with NBT for 1 day	Apoptosis ↑	IRE1-α, ATF-4, CHOP, and GRP78 ↑	[58]
NBT	U2OS and HOS osteosarcoma cells	25–100 μmol/L	Treated with NBT for 1 day	Mobility, migration, and invasion ↓	MMP-2/9 ↓; NF-κB, CREB and SP-1 ↓	[67]
5-Hydroxy-6,7,3′,4′,5′-pentamethoxyflavone	Monocytic lymphoma (U937), acute T cell leukaemia (Jurkat), chronic myelogenous leukaemia (K562) cell lines	10 μg/mL	Treated with crude extracts for 1, 2, and 3 days	Exhibited cytotoxic, anti-proliferative and apoptotic effects	N/A	[6]
NBT	Human colorectal cancer cells	10, 50, 100, and 150 μg/mL	Treated with nobiletin for 48 h	Cell invasion and migration ↓; angiogenesis ↓	NF-κB/STAT3 pathway ↓; expression of VEGFA and Ang2 ↓	[68]
5HHMF	BGC-7901 gastric cancer cells	0.5, 1, 5, 10, and 20 mmol/L	Treated with 5HHMF for 1 day	Proliferation ↓; ROS and cytochrome c ↑	Procaspase-3/-9 and PARP ↓; cleaved caspase-3/-9, cleaved PARP, and Bax/Bcl-2 ratio ↑	[57]
Neuroprotection						
Kaempferia parviflora extracts	PPAR ligand-binding assay	100–500 μg/g	Final concentration of each *Kaempferia parviflora* extract was < 500 μg/mL	Methanol, ethanol, and acetone extracts showed PPAR ligand-binding capacity	N/A	[82]
NBT	HepG2 cells	5, 25, 50 μmol/L	Treated with NBT for 24 h	Hepatic lipid accumulation ↓	Expression of SREBP-1c and FAS ↓; phosphorylation of AMPK and ACC ↑	[78]
HMF	3T3-L1 cells	10–50 μmol/L	Treated with HMF for 8 days	Early stage of adipogenesis ↓	PKA-AMPK-ACC signaling ↑	[79]
Immune regulation						
DMF, 5,7,4′-trimethoxyflavone, and 3,5,7,3′,4′-pentamethoxyflavone	Human amyloid precursor protein cleaving enzyme 1	30, 50, or 100 μmol/L	Treated with PMFs	Exhibited strong inhibitory effects on BACE1 without significantly inhibition of the activities of α-secretase or other serine protease	PMFs bound with the allosteric sites instead of direct interaction with the BACE1 active site	[87]
4′-Demethylnobiletin	PC12D cells, hippocampal neuronal cultures, and Basal forebrain neuronal culture	30 μmol/L	Treated with 4′-demethylnobiletin for 2 days	Choline acetyltransferase gene transcription ↑	N/A	[87]
Skin protection						
Sudachitin	Bone narrow cells from femurs of BALB/c rats	12.5, 25, and 50 μmol/L	Treated with sudachitin for 24 h	Ability of antigen presentation ↑	Expression of CD80 and CD86 ↑	[75]
NBT	Bone marrow-derived dendritic cells	12.5, 25, 50 μmol/L	Treated with NBT for 24 h	Capacity of antigen presentation ↑	N/A	[95]
Sudachitin	HaCaT cells	30 μmol/L	Treated with sudachitin for 1 h	Cell migration and proliferation ↓; apoptosis ↑	p38MAPK ↑; ERK1/2 ↓; serum- and EGF-stimulated Raf-1-ERK1/2 activation ↓	[103]
Sudachitin and NBT	HaCaT cells	30 or 100 μmol/L	Treated with PMFs for 1 and 2 days	Sudachitin and NBT: proliferation ↓; sudachitin: apoptosis ↑; NBT: autophagy ↑	LC3-II ↑	[104]

Table 3 (*Continued*)

PMFs	Models	Dosages	Treatment	Major effects	Molecular mechanisms	References
PMF mixture	HM3KO cells	10 000 μg/mL	Treated with PMF mixture for 5 days	Tyrosinase ↓; colocalisation of tyrosinase ↓; melanogenesis ↓	Tyrosinase degradation in lysosomes ↑; acidified cell organelles and melanosomes	[101]
PMF mixture (PMFs > 80%) extracted from orange peel	HaCaT cells	10 μg/mL	Treated with PMFs mixture for 48 h	MMP-1 expression ↓	MMP-1 transcription ↓ and MMP-1 protein levels ↓ mediated by JNK activity ↓ via JNK phosphorylation ↓	[99]
NBT and chrysosplenetin	Tyrosinase inhibition assay	3 000 mmol/L	20 μL of tyrosinase (250 U/mL) and 20 μL compounds were incubated for 10 min at room temperature. After preincubation, 20 μL L-DOPA (3 mmol/L) was added and incubated for 10 min	Tyrosinase ↓	Methoxy groups on the B-ring of PMFs leaded to steric hindrance that prevented alternative binding modes	[102]
Other bioactivities						
NBT	IEC-6 cells, 293T cells, Caco-2 cells and HT-29 cells	100 μmol/L	Treated with NBT for 6 h	TEER ↑; reversed epithelial injury	N/A	[110]
Mixture of NBT, tangeretin, HMF, and TMF	IL-1-induced osteoclast	15 and 30 μg/mL	Treated with PMFs for 7 days	Bone-resorbing activity ↓	N/A	[109]
Sudachitin	INS-1D cells	0−100 μmol/L	Treated with sudachitin for 1 h	PDE activity ↓	N/A	[135]
NBT, sinensetin, HMP, and tangeretin	*Phytophthora citrophthora* fungal cultures	200−600 μg/mL (sinensetin), 50−400 μg/mL (NBT), and 100−1 000 μg/mL (heptamethoxyflavone and Tangeretin)	Treated with sinensetin, NBT, heptamethoxyflavone, and tangeretin for 100 h	NBT exhibited the strongest antimicrobial effect, followed by sinensetin, heptamethoxyflavone, and tangeretin	N/A	[106]
NBT, sinensetin, HMP, and tangeretin	*Penicillium digitatum* fungal cultures	8 000 μmol/L	Treated with PMFs for 100 h	NBT was the most effective compound	N/A	[105]
KP extracts	NIH3T3 cells	500 μg/mL	Treated with extracts for 24 h	Circadian rhythms amplitude and circadian period ↑; phase delays ↑	Expression of Per2, Cry1, and Bmal1 ↑	[2]
NBT and tangeretin	Embryonic fibroblasts from PER2:LUCIFERASE rats	0−200 μmol/L	Treated with NBT and tangeretin	Phase delay of rhythm ↑; amplitude and period of rhythm ↑	NBT: ERK phosphorylation ↑	[112]

Notes: 5HHMF, 5-hydroxy-3,6,7,8,30,40-hexamethoxyflavone; ACC, acetyl-CoA carboxylase; AIF, anti-apoptosis inducing factor; Akt, protein kinase B; AMPK, AMP-activated protein kinase; ATF, activating transcription factor; ATST, atorvastatin; BACE1, β-site amyloid precursor protein cleaving enzyme 1; BRCA1, breast cancer 1, early onset; CaMK4, calcium/calmodulin-dependent protein kinase IV; CDK, cyclin-dependent kinase; CDKN1A, cyclin-dependent kinase inhibitor 1A; CHOP, C/EBP homology protein; CREB, cAMP response element-binding protein; Cry, cryptochrome; DDIT3, DNA-damage-inducible transcript 3; DMF, 5,7-dimethoxyflavone; DNA-PK, DNA-dependent protein kinase; EMT, epithelial-mesenchymal transition; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; FAS, fatty acid synthase; FLS, fibroblast-like synoviocytes; GBM, glioblastoma multiforme; GRB2, Growth factor receptor-bound protein 2; GRP78, glucose-regulated protein 78; HMF, 3,5,6,7,8,3′,4′-heptamethoxyflavone; HO-1, heme oxygenase 1; HUVECs, human umbilical vein endothelial cells; IL, interleukin; iNOS, inducible nitric oxide synthase; IRE1-α, inositol requiring enzyme 1 alpha; JAK, janus kinase 2; JNK, c-Jun N-terminal kinase; KP, *Kaempferia parviflora*; LC3, light chain 3; MAPK, mitogen-activated protein kinase; MDC1, mediator of DNA damage checkpoint 1; MGMT, O^6-methylguanine-DNA methyltransferase; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; N/A, not applicable; NBT, nobiletin; NQO1, NADH quinone oxidoreductase 1; NDUFS4 (NADH dehydrogenase (ubiquinone) Fe-S protein 4; NF-κB, nuclear factor-kappa B; PAK3, p21 protein (Cdc42/Rac)-activated kinase 3; PARP, poly (ADPribose) polymerase; p-ATM, phospho-ataxia telangiectasia mutated kinase; Per, period; PGE_2, prostaglandin E_2; p-H2A.X, phospho-histone H2A.X; PI3K, phosphoinositide 3-kinase; PMF, polymethoxyflavones; p-p53, phospho-p53 tumor suppressor protein; PRKAR2B, protein kinase, cAMP-dependent, regulatory, type II, bet; p-STAT3, tyrosine 705-phosphorylated STAT3; ROS, reactive oxygen species; SCN1B, cell adhesion molecule 1; SP-1, specificity protein 1; SREBP-1c, sterol regulatory element-binding protein 1c; *T. brucei*, *Trypanosoma brucei*; TEER, transepithelial electrical resistance; TIMP, tissue inhibitor of metalloproteinase; TLR4, toll-like receptor 4; TMF, 5-hydroxy3,7,3′,4′-tetramethoxyflavone; TNF-α, tumour necrosis factor-alpha; uPA, urokinase-type plasminogen activator; VEGFA, vascular endothelial growth factor A. "↓": down-regulation; "↑": up-regulation. The same below.

Table 4
Bioactivities of polymethoxyflavones (PMFs) based on *in vivo* studies.

PMFs	Animal models	Dosages	Treatment	Major effects	Molecular mechanisms	Ref.
Anti-inflammatory effect						
Nobiletin	*L*-arginine-induced acute pancreatitis in C57BL/6 mice	25–50 mg/(kg·day)	Treated with NBT by intraperitoneal injection once daily for 14 days	Plasma amylase levels, pancreatic myeloperoxidase activity, pancreatic necrosis, plasma pro-inflammatory factors, ROS, and tissue damage ↓	Phosphorylation and activation of p38 MAPK and AKT ↓	[50]
Anti-cancer effect						
NBT	AOM/DSS-induced male CD-1 mice colon cancer model	0.05%	Treated withNBT for 20 weeks	Proliferation and carcinogenesis ↓	iNOS ↓; HO-1 and NQO1 ↑; Nrf2 signaling pathway ↓	[65]
NBT and its metabolites	AOM-induced male F344 rats colon cancer model	0.05% NBT + 0.02% ATST	Treated with NBT and NBT + ATST in basal diet for 40 weeks	Tumour incidence and multiplicity ↓	p21, CDK2 and CDK4 ↑; cyclin D and cyclin E ↓; p53, cleaved caspase-3/7 and cleaved PARP ↑	[71]
5HHMF	Nude BALB/c mice injected with BGC-7901 cells to induce gastric cancer	5–10 mg/(kg·day)	Treated with 5HMMF for 3 weeks	Tumour weight ↓	Expression of PI3K and phospho-Akt ↓	[58]
Casticin	A375.S2 cells implanted into right of flanks of BALB/c nu/nu mice to induce skin cancer	2–10 mg/(kg·day)	Treated with casticin in 200 μL olive oil by injection per wk for 3 weeks	Tumour growth and volume ↓	N/A	[63]
Regulation of metabolic syndrome						
PMF-rich extract	HFD-fed mice	120 mg/(kg·day)	Treated with PMF-rich extract by oral gavage for 8 weeks	Exhibited potential metabolic protective effects; gut dysbiosis ↓, regulated gut microbiome and BCAAs	mTOR/P70S6K/SREBP pathway ↓; ZO-1 expression ↑; abundance of *Bacteroides ovatus* ↑	[83]
4 PMFs	Male diabetic Nagoya-Shibata-Yasuda mice	1% BG and 0.19% BGE	Treated with BG and BGE in diets for 8 weeks	Plasma glucose ↑; fat accumulation ↓	N/A	[82]
NBT	STZ diabetic male Wistar rats	10 and 25 mg/(kg·day)	Treated with NBT by oral gavage for 4 weeks	Heart rate, arterial pressure, and body weight ↓; vascular structure and left ventricle performance ↑	Expression of MMP-2 and MMP-9 ↓; myocardial SOD and catalase activity ↓; collagen in aorta ↓	[84]
Sudachitin	C57BL/6 J mice with a high-fat diet	5 mg/(kg·day)	Treated with sudachitin by oral gavage for 12 weeks	Weight gain and fat ↓; glucose intolerance and insulin resistance ↑	N/A	[136]
5-Desmethylnobiletin	*C. elegans*	12.5–50 μmol/L	Treated with DN	Synaptic Ach and activity of nAChR ↑	Cholinergic function ↑ via acetylcholinesterase activity ↓ and unc-29 ↑; ROS production ↓	[90]
Eight PMFs	Male Sprague-Dawley rats	5–10 mL/(kg·day)	Treated with orange peel oil by oral gavage for 61 days	Systolic pressure and diastolic pressure ↓	HO-1, nNOS, and eNOS expression ↑; iNOS, ADM, and RAMP2 expression ↓	[80]
NBT	MCT treated male Wistar rats	50 mg/(kg·day)	Treated with NBT by intragastric gavage for 3 weeks	RVSP and right ventricular hypertrophy ↓; exhibited cardio protective effects	Src/STAT3 activation and PASMCs proliferation ↓; Pim1 and NFATc2 ↓	[854]
Neuroprotection						
HMF	Male ICR strain mice	50 mg/(kg·day)	Treated with HMF by i.p.	MK-801-induced locomotive hyperactivity ↓	Phosphorylation of ERK1/2 ↑	[87]
Tangeritin	Transgenic *Drosophila* model of PD	5–20 μmol/L	Treated with tangeritin in diets for 24 days	Cognitive impairments ↓	Bound with the α synuclein molecule; tyrosine hydroxylase ↓	[91]
HMF	CUMS-treated mice	50–100 mg/(kg·day)	Treated with HMF for 10–90 min	Weight loss and depressive-like behaviour ↓	BDNF expression ↑; neurogenesis and p-CaMK II level ↑	[93]
4′-Demethylnobiletin	Male ddY mice and pregnant Sprague-Dawley rats	2 600 mg/(kg·day)	Treated with PMF-rich fraction by oral gavage for 7 days	MK801-impaired memory formation ↓	N/A	[89]
Immune regulation						
Sudachitin	OVA-immunized BALB/c mice	20 mg/(kg·day)	Treated with sudachitin in 200 μL by oral gavage for 35 days	Regulated immune function	Production of IL-4, IL-10, IgE, IgG1, and IgG I ↑	[965]

Table 4 (*Continued*)

PMFs	Animal models	Dosages	Treatment	Major effects	Molecular mechanisms	Ref.
NBT	OVA-immunized BALB/c mice	20 mg/(kg·day)	Treated with 200 μL NBT solution by oral gavage for 35 days	Regulated immune function	Production of IL-4, IL-10, IgE, IgG and IgG1 ↑	[95]
HMF	C57BL/6J and 129 hybrid mice	17–50 mg/(kg·day)	Treated with HMF in corn oil by i.p., 2 times/week for 2–5 weeks	Reduced IL-4 expression	IgE↓ via IL-4 secretion ↓	[97]
HMF	OVA-immunized BALB/c mice	50 mg/(kg·day)	Treated with HMF by i.p. twice a week for 2 weeks	Inhibited the increase in total IgE levels	N/A	[98]
Other bioactivities						
Nine PMFs	Ethanol-induced gastric ulcer in male Wistar rats	250 mg/(kg·day)	Treated with petroleum ether extract by oral gavage for 7 days	The increase in gastric volume ↓; completely heal injured ulcer	GSH, SOD, SDH, LDH, IL-10, and PGE2 ↑; MDA ↓	[110]
NBT	DSS-induced intestinal epithelial injury in male C57BL/6J mice	0.01% or 0.25 nmol/(kg of diet)	Treated with NBT in diets for 7 days	Regulated intestinal mucosa function and colon Integrity	Serum FITC-dextran and iNOS ↓; claudin-7 and HNF-4α ↑	[110]
NBT	*C. elegans*	3.1–12.5 μmol/L	Treated with NBT from day 0 to death	Lifespan ↑, resistance to oxidative stress, UV radiation, and heat shock ↑	Lipofuscin accumulation ↓; DAF-16, HSF-1, and SKN-1 ↑	[114]
Mixture of NBT, tangeretin, HMF, and TMF	Ovariectomized mice	5 mg/day	Treated with PMFs by oral gavage for 4 weeks	Femoral bone loss ↓	IL-1-induced osteoclast differentiation and bone resorption ↓; bone-resorbing activity ↓	[109]
PMFs from *N. plumbaginifolia* leaves	Male Swiss albino mice	12.5–25 mg/kg	Treated with PMFs by oral gavage at 1 h before experiments	Exhibited dose-dependent antinociceptive activity and anxiolytic-like activity	Involved ATP sensitive K^+ channels and $GABA_A$ receptor	[12]

Notes: 5HHMF, 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone; ADM, adrenomedullin; Akt, protein kinase B; AMPK, AMP-activated protein kinase; AOM, azoxymethane; AP, acid phosphatase; ATST, atorvastatin; BCAA, branched-chain amino acid; BDNF, brain-derived neurotrophic factor; BG, black ginger; BGE, black ginger extract; *C. elegans*, *Caenorhabditis elegans*; CaMK4, calcium/calmodulin-dependent protein kinase IV; CDK, cyclin-dependent kinase; DAF-16, dauer formation 16; DMF, 5,7-dimethoxyflavone; DN, 5-desmethylnobiletin; DSS, sulphate sodium; ERK, extracellular signal-regulated kinase; GABA, gamma-aminobutyric acid; GSH, glutathione; HFD, high fat diet; HMF, 3,5,6,7,8,3′,4′-heptamethoxyflavone; HNF-4α, hepatocyte 20 nuclear factor 4α; HO-1, heme oxygenase-1; HSF-1, heat-shock transcription factor 1; i.p., intraperitoneal; iNOS, inducible nitric oxide synthase; KP, *Kaempferia parviflora*; LDH, lactate dehydrogenase; MCT, monocrotaline; MDA, malondialdehyde; MMP, matrix metalloproteinase 9; *N. plumbaginifolia*, *Nicotiana plumbaginifolia*; N/A, not applicable; nAChR, activity of nicotinic acetylcholine receptors; NBT, nobiletin; NQO1, NADH quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor; OVA, ovalbumin; PARP, poly (ADPribose) polymerase; p-CaMK, phosphorylated calcium-calmodulin-dependent protein kinase; PD, Parkinson's disease; Per, period; PGE_2, prostaglandin E_2; p-STAT3, tyrosine 705-phosphorylated STAT3; RAMP2, receptor activity modifying protein 2; ROS, reactive oxygen species; RVSP, right ventricular systolic pressure; SDH, succinate dehydrogenase; SKN-1, skinhead 1; SOD, superoxide dismutase; SREBP-1c, sterol regulatory element-binding protein 1c; STZ, streptozotocin; TIMP, tissue inhibitor of metalloproteinase; TMF, 5-hydroxy3,7,3′,4′-tetramethoxyflavone; TNF-α, tumour necrosis factor-alpha; UV, ultraviolet; ZO-1, zonula occludens-1.

6.1.1 Antioxidant effect in vitro

PMFs have been demonstrated to possess antioxidant effects in cell models. *K. parviflora* extracts and its two main PMFs, 5,7-dimethoxyflavone and 5-hydroxy-3,7,3′,4′-tetramethoxyflavone, exhibited good antioxidant effects through inhibiting the cellular reactive oxygen species (ROS)[39]. Since nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a major activator of a series of endogenous antioxidant enzymes such as heme oxygenase 1 (HO-1), superoxide dismutase (SOD), glutathione *S*-transferase (GST), nicotinamide adenine dinucleotide phosphate (NADPH), quinone oxidoreductase 1 (NQO1), and catalase (CAT), suppression of the ubiquitination of Nrf2 can stabilize it, contributing to its translocation to the nucleus and subsequently binding to antioxidant response element (ARE) to upregulate the expression of these related antioxidant enzymes to neutralize cellular ROS[40]. Cell-based assays suggested that PMFs, such as (2*S*)-5,6,7,3′,4′-pentamethoxyflavanone, tangeretin, and nobiletin from citrus, were novel Nrf2 activators with the potential molecular mechanism of enhancing the stabilization of Nrf2 by blocking its ubiquitination and degradation[41-43]. In addition, PMFs may have different antioxidant mechanisms compared to other common citrus flavonoids. For example, both tangeretin and neohesperidin increased the expression of Nrf2 via the inhibition of its partner protein Kelch-like ECH-associated protein 1 (KEAP1), while only tangeretin could suppress ubiquitin ligase Cullin 3 to decrease the ubiquitination of Nrf2[43].

6.1.2 Antioxidant effect in vivo

Limited studies have demonstrated the antioxidant effects of PMFs *in vivo*. PMFs from citrus peels, including nobiletin, 5-demethylnobiletin, tangeretin, and 5-demethyltangeretin, were reported to alleviate oxidative damages in *Saccharomyces cerevisiae* by reducing intracellular ROS and lipid peroxidation[44].

6.1.3 Structure-antioxidant effect relationship

For the structure-antioxidant effect relationship of PMFs, 5-demethylnobiletin and 5-demethyltangeretin were found with stronger effects to reduce the lipid peroxidation damage than their parental compounds nobiletin and tangeretin, respectively[45]. These results suggest that C-5 position demethylation of PMFs may enhance their antioxidant effects, probably due to the hydroxyl group at the C-5 position, which exhibits a higher antioxidant effect than the methoxy group.

Generally, PMFs possess antioxidant effects by reducing intracellular ROS and lipid peroxidation, and stabilizing Nrf2 via blocking its ubiquitination and degradation. However, few studies investigated the antioxidant effects of PMFs based on *in vitro* spectroscopic assays, which can be easy and useful to investigate their structure-antioxidant effect relationships. Moreover, PMFs seem to be a novel type of Nrf2 agonists, and their *in vivo* antioxidant mechanisms still need further investigation.

6.2 Anti-inflammatory effect

Inflammation is involved in the pathogenesis and progression of many chronic diseases. Several studies demonstrated that PMFs can inhibit inflammation in different *in vitro* and *in vivo* models.

6.2.1 Anti-inflammatory effect in vitro

The anti-inflammatory effects of PMFs are partially attributed to their ability to decrease the production of pro-inflammatory mediators. Decreases in certain pro-inflammatory mediators, including IL-1, IL-6, tumour necrosis factor-α (TNF-α), high-mobility group box 1 (HMGB1), prostaglandin E2 (PGE_2), and matrix metalloproteinase (MMP) 3 and 13, were observed after the administration of PMFs (e.g., tetramethyl-*O*-scutellarin, nobiletin, tangeretin, and 5-demethylnobiletin) to various cell lines[45-47]. IL-21 is an upstream inflammation-related mediator of certain inflammatory cytokines like IL-6, TNF-α, and HMGB1, the inhibition of which might be partially responsible for the anti-inflammatory effect of PMFs. For example, it was proved that nobiletinit could significantly downregulate the expression of IL-21 receptor and subsequently decrease the downstream inflammation-related mediators of IL-21[48]. In addition, inflammatory processes can upregulate inducible nitric oxide synthase (iNOS) to produce excessive nitric oxide (NO). Cell-based assays found that *K. parviflora* extracts, 5,7-dimethoxyflavone, nobiletin, tangeretin, and 5-demethylnobiletin could decrease the nitrite levels in various cell lines[39,46]. Furthermore, a study indicated that the phosphorylation of both Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) could be suppressed by tangeretin and 5-demethylnobiletin, but not by nobiletin[47]. However, a different study indicated that nobiletin exerted an inhibitory effect on the phosphorylation of JAK1 and STAT3[46]. These results suggest that the JAK-STAT signaling can be a key pathway involved in the anti-inflammatory effect of certain PMFs, but different PMFs may target different molecules in the JAK-STAT signaling. Cytokines have effects on bone cells and are linked to the development of osteoporosis[48]. Sudachitin could inhibit inflammatory bone destruction via the direct inhibition of osteoclastogenesis in the osteoclast cells, probably associated with inhibiting the gene expression of osteoclast differentiation-related signaling molecules, such as c-fos, nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), cathepsin K, and osteoclast fusion proteins, as well as blocking the activation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), and the ROS production[49].

6.2.2 Anti-inflammatory effect in vivo

PMFs also exhibited anti-inflammatory effects of some *in vivo* studies. Nobiletin was reported to alleviate *L*-arginine-induced acute pancreatitis in C57BL/6 mice by blocking the phosphorylation and activation of p38MAPK and AKT[50].

Overall, PMFs exhibit anti-inflammatory effects mainly by blocking the production of inflammation-related factors, such as NO, ROS, MMPs, PGE, and pro-inflammatory cytokines (e.g., IL-1, IL-6, IL-21, and TNF-*α*), and targeting different signaling molecules in inflammation-related pathways, such as the MAPK, NF-κB, and JAK-STAT, and AKT signaling. In the future, more animal-based *in vivo* studies are needed to further verify the anti-inflammatory effect and related mechanism of PMFs.

6.3 Anti-cancer effect

Many dietary polyphenols possess anti-cancer effects, such as epigallocatechin gallate[51], curcumin[52], resveratrol[53], rutin[54], and dihydrochalcones[55], are commonly used as dietary supplements to prevent cancer. PMFs have also been widely demonstrated to exhibit anti-cancer effects both *in vitro* and *in vivo*[56], which are illustrated in Fig. 2 and discussed below, highlighting related molecular mechanisms.

6.3.1 Inhibition of cancer cell growth and induction of cancer cell apoptosis

Recent studies demonstrate that PMFs can inhibit cancer cell growth and/or induce cancer cell apoptosis *in vitro*. The induction of G0/G1 phase arrest and reduction of G2/M phase can be caused by the inhibition of diverse cyclins by upregulating the expression of p53, p21, and p27. In a study, 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone[57], nobiletin[58-60], casticin[61-63], and 5-acetyloxy-6,7,8,4′-tetramethoxyflavone (5-AcTMF, a acetylated derivative of tangeretin)[64] have been evidenced that they can induce cell cycle arrest in various cancer cell lines, which was partially attributed to the suppression of cell proliferation caused by blocking the PI3K/Akt signaling pathway[57]. Cell-based assays also suggested that PMFs could induce cancer cell apoptosis via both intrinsic and extrinsic apoptosis pathways[6,54,57,59,61,62,64]. The intrinsic apoptosis pathway was mediated by inhibiting the phosphorylation of STAT3 at the site of tyrosine 705, leading to the blockade of JAK2/STAT3/BCL-2/BCL-xL signaling[64]. The suppression of BCL-2 and BCL-xL could increase the expression of cleaved Caspase-9/-3 and poly (ADP-ribose) polymerase (PARP) and subsequently induce apoptosis[59]. The induction of pro-apoptotic proteins, such as p53, p21, and checkpoint kinase 1 (CHK-1), and the inhibition of anti-apoptotic proteins, such as Cdc25c, were also involved in the pro-apoptotic effects[63]. Despite existing cytotoxic effects on some cancer cell lines, 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone did not exhibit cytotoxicity against peripheral blood mononuclear cells (PBMCs) from healthy human donors[6]. On the other hand, it was found that nobiletin could promote or inhibit autophagy in different cancer cells. In a study, nobiletin was reported to induce protective autophagy in SNU-16 cells, suggesting that combination of nobiletin with autophagy inhibitors, such as chloroquine, might be promising for gastric cancer treatment[58]. Also, nobiletin was found to mediate autophagic flux inhibition by activating the Akt signaling in SKOV3/TAX cells[59]. These effects were probably dependent on the different genetic background of different cancer cells as well as the specific experimental context.

The animal models, including murine gastric cancer and colon cancer models, have been used to study the ability of PMFs to inhibit tumor formation and carcinogenesis. In a murine gastric cancer model, the tumor growth in xenograft models was inhibited by 5-hydroxy-3,6,7,8,3,4′-hexamethoxyflavone and the mechanism might be associated with targeting PI3K/Akt[57]. In a murine colon cancer model, the colitis-associated colon carcinogenesis in the colonic tissue

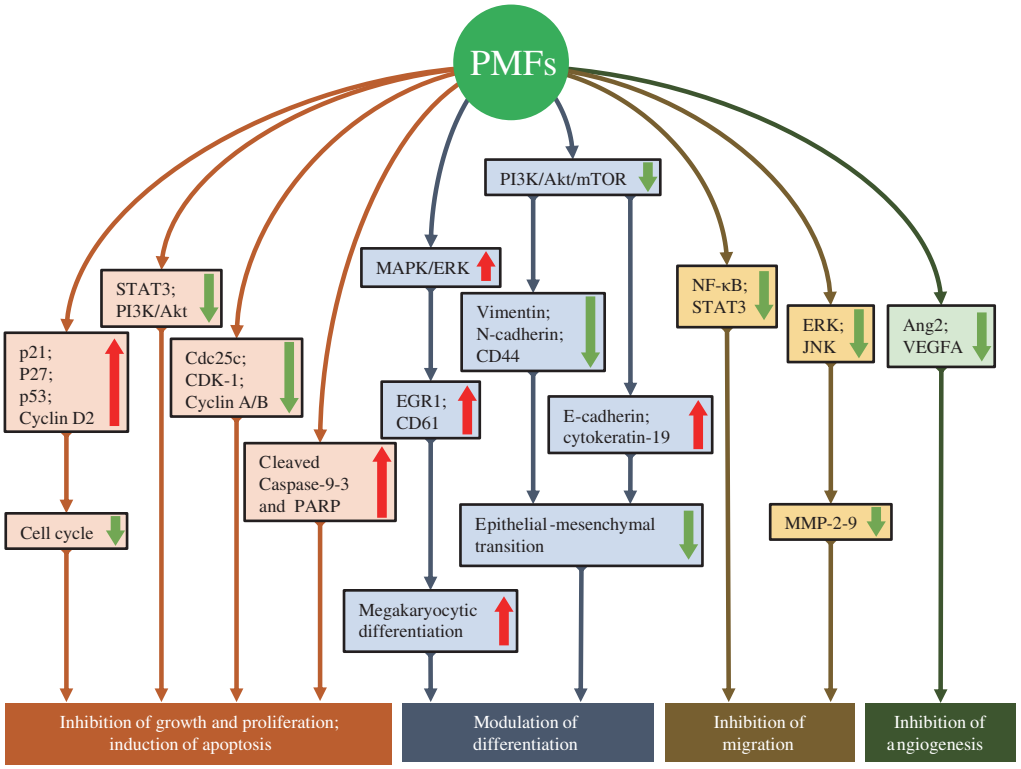

Fig. 2 Molecular mechanisms of anti-cancer effects of PMFs by inhibiting cancer cell growth, inducing cancer cell apoptosis, modulating cancer cell differentiation, suppressing cancer cell migration, and blocking cancer angiogenesis. Ang2, Angiopoietin 2; Cdc25c, cell division cycle 25c; CDK, cyclin-dependent kinase; EGR1, early growth response 1; ERK, extracellular-signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen activated protein kinase; MMP, matrix metalloproteinase; mTOR, mechanistic target of rapamycin kinase; NF-κB, nuclear factor-kappa B; PARP, poly (ADP-ribose) polymerase; PI3K, phosphatidylinositol 3-kinase; STAT3, signal transducer and activator of transcription 3; VEGFA, vascular endothelial growth factor A. Red arrows: up-regulation; green arrows: down-regulation.

of azoxymethane (AOM)/dextran sulfate sodium (DSS)-treated mice was inhibited by nobiletin and the mechanism might be associated with blocking inflammation (e.g., iNOS), inducing antioxidative enzymes (e.g., HO-1 and NQOI) and Nrf2 signaling, and arresting cancer cell cycle progression[65].

6.3.2 Modulation of cancer cell differentiation

Recent studies find that PMFs, such as tangeretin and nobiletin, can modulate the differentiation of cancer cells *in vitro*. Tangeretin blocked the epithelial-mesenchymal transition (EMT) through blocking the PI3K/Akt/mTOR signaling pathway, leading to downregulating the expression of the mesenchymal proteins, including vimentin, cluster of differentiation (CD)44, and N-cadherin, but upregulating the expression of epithelial proteins, including E-cadherin and cytokeratin-19[61]. In addition, nobiletin promoted megakaryocytic differentiation through upregulation of EGR1 and CD61 gene expression by activating MAPK/ERK signaling pathway[55].

6.3.3 Suppression of cancer cell migration, invasion, and metastasis

Certain PMFs, such as nobiletin[67,68] and casticin[7,69], have also been reported to inhibit the migration, invasion, and metastasis of cancer cells *in vitro*. These effects of PMFs could be mediated by downregulating the expression of MMPs (e.g., *MMP-2* and *MMP-9*) genes and proteins via blocking the ERK and JNK pathways and their downstream NF-κB, cAMP response element-binding protein (CREB), and specificity protein 1 (SP-1) transcription factor activities[67]. MMPs could also be inhibited via suppressing the Ras/Akt/NF-κB signaling pathway[69]. PMFs could also upregulate the gene expression of cell adhesion molecule 1 B (*SCN1B*) and metallopeptidase inhibitor 2TIMP2 (*TIMP*), downregulate the gene expression of NADH dehydrogenase (ubiquinone) Fe-S protein 4 (*NDUFS4*), vascular endothelial growth factor A (*VEGFA*), and DNA-damage-inducible transcript 3 (*DDIT3*), and block the PI3K/AKT and NF-κB/STAT3 signaling pathways[7,68].

6.3.4 Other anti-cancer effects

Several other anti-cancer effects, such as blocking the cancer angiogenesis and cancer-stem cells, have also been reported. For example, nobiletin was reported to block the angiogenesis in human colorectal cancer cells by suppressing the expression of VEGFA and angiopoietin 2 (Ang2)[68]. VEGFA and Ang2 are capable of sustaining tumor angiogenesis and limiting antitumor immunity[70]. In addition, casticin could inhibit the sphere- and colony-formation in lung cancer stem-like cells (LCSLCs) from H446 small cell lung cancer cells by activating the AMPK/FoxO3a signaling[71].

6.3.5 Synergistic anti-cancer effects with other agents

Many natural products have been reported to combine with current anti-cancer therapies to improve cancer outcomes. PMFs, especially nobiletin, have been combined with other anti-cancer agents to fight against cancer *in vitro* and *in vivo*. Cell-based assays suggested that nobiletin enhanced the anti-cancer abilities of imatinib and bicalutamide against chronic myeloid leukaemia cells[60] and prostate cancer cells[72], respectively. An animal study reported that nobiletin and atorvastatin could also synergistically inhibit colonic tumor incidence and multiplicity in an azoxymethane-induced colon cancer rat model, accompanied with dramatically modulating key cellular signaling regulators associated with inflammation, cell proliferation, cell cycle progression, apoptosis, angiogenesis, and metastasis[73]. In addition, the combined administration of atorvastatin and nobiletin increased the population of the cancer cells at G0/G1 phase by 30.4%[73]. Taken together, PMFs, especially nobiletin, have the potential to be combined with current anti-cancer therapies to reduce the side effects of anti-cancer drugs, and the synergistic anti-cancer effects of other PMFs should also be investigated in the future.

In general, PMFs exert their anti-cancer effects through multiple actions, including inhibiting cancer cell growth, inducing cancer cell apoptosis, modulating cancer cell differentiation, suppressing cancer cell migration, invasion, and metastasis, blocking cancer angiogenesis and cancer-stem cells, and strengthing anti-cancer effects of other agents. Several cancer-related signaling pathways, such as the PI3K-Akt, JAK-STAT, MAPK, NF-κB, and AMPK-FoxO3a signaling pathways, have been demonstrated to be regulated by PMFs and played critical roles in the anti-cancer effect of PMFs, which is briefly illustrated in Fig. 2.

6.4 Regulation of metabolic syndrome

Metabolic syndrome is a pathogenic condition featured by a constellation of risk factors, including obesity, dyslipidaemia, insulin resistance, hypertension, and low-grade inflammation, and can progress to more severe metabolic complications, such as type 2 diabetes and non-alcoholic fatty liver disease. Many natural products have been demonstrated to regulate metabolic syndrome and its complications[74-77]. Recent studies indicate that PMFs and PMF-rich natural products can also regulate the risk factors and complications of metabolic syndrome (Fig. 3), which are discussed below.

6.4.1 Regulation of metabolic syndrome in vitro

Several PMFs have been reported to inhibit the risk factors of metabolic syndrome *in vitro*. Cell-based assays suggested that nobiletin and 3,5,6,7,8,3′,4′-heptamethoxyflavone could inhibit lipid accumulation and adipogenesis by downregulating the protein expression of lipogenic factors, including sterol regulatory element-binding protein 1c (SREBP-1c) and fatty acid synthase (FAS)[78,79].

6.4.2 Regulation of metabolic syndrome in vivo

Moreover, pure PMFs can also block the risk factors of metabolic syndrome *in vivo*. An animal study reported that PMF-rich orange peel oil reduced the systolic pressure and diastolic pressure in hypertensive rats, probably associated with upregulating the protein expression of neuronal (nNOS) and endothelial (eNOS) NO synthase and downregulating the protein expression of iNOS[80]. A reduction in the activity of eNOS is known to be mainly responsible for the elevation of blood pressure, while eNOS/nNOS-derived NO is

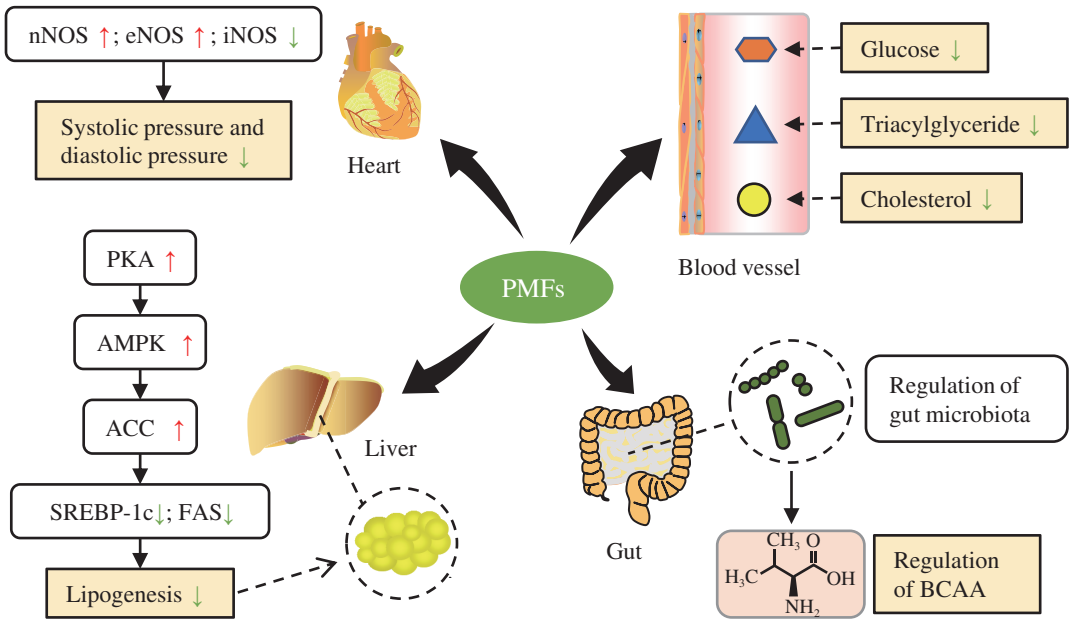

Fig. 3 Molecular mechanisms of metabolic syndrome regulatory effects of PMFs by lowering blood pressure and sugar, ameliorating dyslipidaemia, inhibiting oxidative stress and inflammation, and modulating gut homeostasis. ACC, acetyl-CoA carboxylase; AMPK, adenosine monophosphate-activated protein kinase; BCAA, branched-chain amino acid; eNOS, endothelial nitric oxide synthase; FAS, fatty acid synthase; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; PKA, protein kinase A; PMFs, polymethoxyflavones; SREBP-1c, sterol regulatory element-binding protein 1c (SREBP-1c). Red arrows: up-regulation; green arrows: down-regulation.

protective against the development of atherosclerosis[81]. Another animal study supported the inhibitory effect of PMFs on the fat accumulation in adipose tissues[82]. Gut microbiota also plays a vital role in PMF-mediated regulation of metabolic diseases. For example, a purified citrus PMF-rich extract could significantly alleviate HFD-induced metabolic syndrome in mice, accompanied with ameliorating gut dysbiosis and regulating branched-chain amino acid (BCAA) metabolism[83]. These effects were mainly associated with the regulation of gut microbiota by PMFs, especially the commensal bacterium *Bacteroides ovatus*, since its intragastric administration reduced BCAA concentrations and alleviated metabolic syndrome in HFD mice[83]. These studies suggest that PMFs may be applied as potential prebiotic agents to manage metabolic syndrome. Additionally, cardiovascular dysfunction, such as the hemodynamic parameters and vascular reactivity, could be ameliorated by nobiletin through inhibiting oxidative stress, MMP-2, and MMP-9[84]. Also, nobiletin could inhibit the proliferation of rat pulmonary artery smooth muscle cells and protect against pulmonary arterial hypertension, probably via regulating the Src/STAT3 signaling pathway[85]. High levels of cholesterol, triacylglyceride, and glucose are risk factors for metabolic disorders, which could be alleviated by the ingestion of citrus fruit peels that were rich in nobiletin and 4′,5,7,8-tetramethoxy flavone[86].

Overall, PMFs exhibit excellent effects on managing metabolic syndrome by lowering blood pressure and sugar, ameliorating dyslipidaemia, inhibiting oxidative stress and inflammation, and modulating gut homeostasis. Several key molecules, like eNOS, SREBP-1c, FAS, MMPs, and signaling pathways, such as the AMPK/ACC and Src/STAT3 signaling, can be potential molecular targets of PMFs to fight against metabolic syndrome.

6.5 *Neuroprotection*

Recent studies indicate that PMFs exhibit different neuroprotective effects (Fig. 4). They can protect from neurodegenerative diseases *in vitro* and *in vivo*.

6.5.1 *Neuroprotection in vitro*

β-Site amyloid precursor protein cleaving enzyme 1 (BACE1) is the rate-limiting enzyme for the abnormal production of *β*-amyloid peptides involving in Alzheimer's disease. PMFs, including 5,7-dimethoxyflavone, 5,7,4′-trimethoxyflavone, and 3,5,7,3′,4′-pentamethoxyflavone, exhibited strong inhibitory effects on BACE1 without significantly inhibiting the activities of *α*-secretase or other serine protease, suggesting that they were relatively specific and selective BACE1 inhibitors[87]. Acetylcholine (ACh) is the neurotransmitter synthesized by choline and acetyl coenzyme A (AcCoA) with the presence of acetyl coenzyme acetyltransferase (ChAT)[88]. The *ChAT* gene transcription could be promoted by the *Aspergillus kawachii*-fermented *Citrus reticulata* (Ponkan) fruit squeezed draffs extracts that were rich in 4′-demethylnobiletin[89]. In addition, the level of synaptic ACh and the activity of nicotinic acetylcholine receptor (nAChR) could be enhanced by 5-desmethylnobiletin[90]. Tangeretin could improve the cognitive impairments in transgenic *Drosophila* model of Parkinson's disease, and the molecular docking study suggested that it could bind with the α synuclein molecule[91].

6.5.2 *Neuroprotection in vivo*

It was reported that 3,5,6,7,8,3′,4′-heptamethoxyflavone could alleviate locomotive hyperactivity in mice by activating extracellular

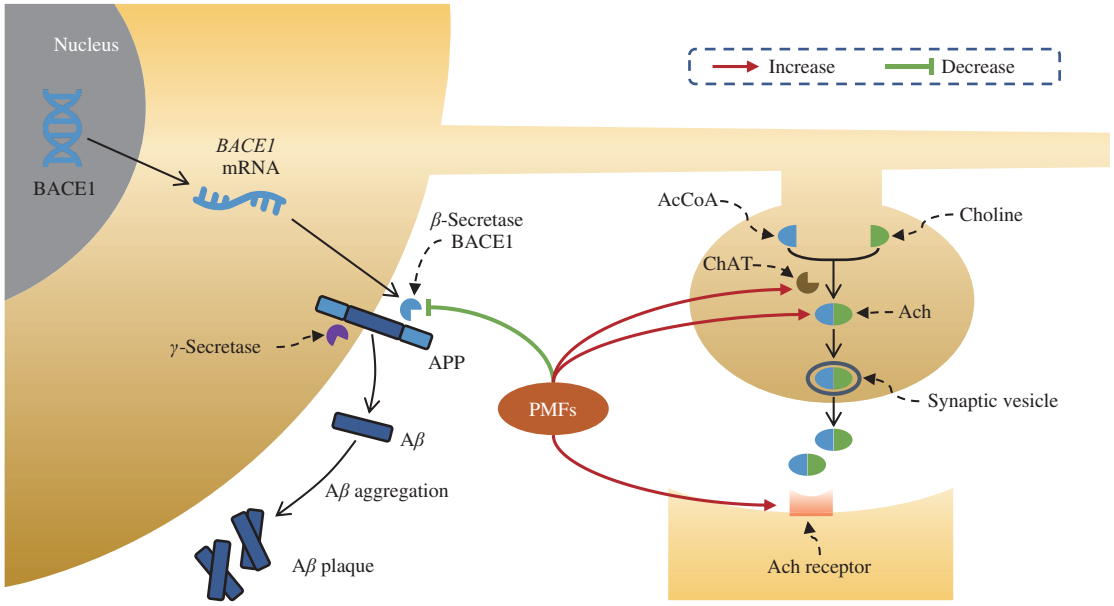

Fig. 4 Neuroprotective effects of PMFs and related molecular mechanisms by inhibiting the activities of BACE1, promoting *ChAT* gene transcription, enhancing the level of ACh and the activity of nAChR. AcCoA, acetyl coenzyme A; Ach, acetylcholine; APP, amyloid precursor protein; Aβ, amyloid β peptide; BACE1, β-site amyloid precursor protein cleaving enzyme 1; ChAT, choline acetyltransferase; nAChR, nicotinic acetylcholine receptor.

signal-regulated kinases 1/2 (ERK1/2) in the hippocampus, and it had a much higher permeability to the brain tissues than other citrus PMFs, such as nobiletin, tangeretin, and natsudaidain[92]. The depressive-like behaviour and hippocampal neurochemical changes in chronic unpredictable mild stressed mice could be ameliorated by 3,5,6,7,8,3′,4′-heptamethoxyflavone, partially owing to increasing brain-derived neurotrophic factor (BDNF) in the hippocampus via activating the ERK1/2/MAPK signaling[93].

Therefore, PMFs may be promising agents for the prevention and management of neurodegenerative diseases, such as the Alzheimer's disease, Parkinson's disease, and depression, and several molecules and signaling, like BACE1, ChAT, nAChR, BDNF, and the ERK signaling, should be their main molecular targets.

6.6 *Immune regulation*

PMFs can regulate immune functions. Antigen presentation process is pivotal for T cells to recognize antigenic epitopes[94]. Cell-based assays reported that the antigen presentation capability in bone marrow-derived dendritic cells could be enhanced by nobiletin[95] and sudachitin[96]. Animal studies also demonstrated that PMFs could modulate the production of cytokines and antibodies. The production of IL-4 could be reduced by 3,5,6,7,8,3′,4′-heptamethoxyflavone via inhibiting the activities of phosphodiesterase (PDE) and increasing the content of cyclic AMP (cAMP)[97,98]. In ovalbumin (OVA)-immunized mice, nobiletin and sudachitin were reported to increase OVA-specific IL-4, IL-10, as well as IgE, IgG, and IgG1 production[95,96]. In generally, these studies suggest that PMFs can facilitate antigen presentation, modulate inflammatory factor production, and activate antibody response to regulate immune functions.

6.7 *Skin protection*

Several studies support that PMFs can protect skin from ultraviolet (UV) B irradiation (Fig. 5). The exposure to UVB can trigger the expression of MMPs in the skin to degrade collagen, leading to the occurrence of deep wrinkles.

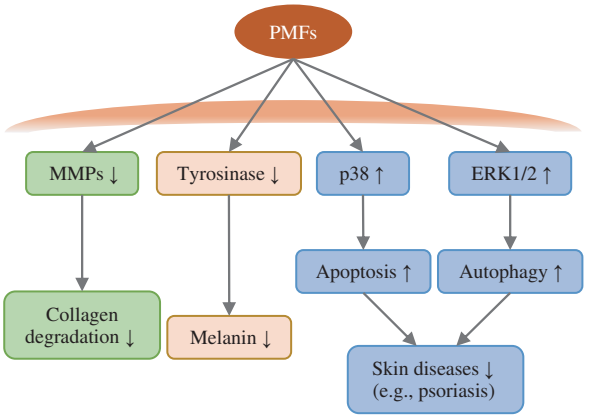

Fig. 5 Skin protective effects of PMFs and related molecular mechanisms by inhibiting the activities of MMPs and tyrosinase and activating the p38 MAPK and ERK1/2 pathway. ERK, extracellular signal-regulated kinase; MMPs, matrix metalloproteinases; PMFs, polymethoxyflavones. "↓": down-regulation; "↑": up-regulation.

First, PMFs were potential candidates of anti-photoaging agents. The UVB-induced expression of MMP-1 could be suppressed by PMFs extracted from orange peels through inhibiting JNK phosphorylation and its activity[99]. In addition, the orange peel cold-pressed oil rich in PMFs could maintain the dermal structure, enhance serum and skin tissue antioxidant defence system, and block serum and skin inflammation[100]. Intriguingly, these protective effects led to the partial decomposition of PMFs into 5-hydroxy-PMFs[100].

Second, PMFs have whitening effects on the skin (Fig. 5). Tyrosinase is a copper-containing oxidase enzyme that catalyzes the first step in the formation of melanin in the melanocytes, and PMFs can inhibit the tyrosinase, leading to the reduction of melanin production. Previous studies suggested that nobiletin, chrysosplenetin, and PMF mixtures (nobiletin, 3,3′,4′,5,6,7,8-heptamethoxyflavone, and tangeretin), could induce the degradation of tyrosinase in lysosomes[101,102]. The possible mechanism was that PMFs could acidify cell organelles, especially the melanosomes, finally resulting in the suppression of melanogenesis[101]. In addition, *in vitro* docking studies suggested that the methoxy groups on the B-ring of PMFs faced the catalytic site in the tyrosinase, and they caused steric hindrance, preventing alternative binding modes in the enzyme[102].

Third, PMFs have the potential to treat certain skin diseases, such as psoriasis (Fig. 5). For example, sudachitin, a PMF isolated from the peel of *Citrus sudachi*, was found to induce cell apoptosis via activating the p38 MAPK pathway in human keratinocyte HaCaT cells[103,104]. In addition, sudachitin could also inhibit EGF-induced cell migration and proliferation in HaCaT cells by blocking the activation of Raf-1-ERK1/2 signaling[103]. However, another common PMF, nobiletin, was reported to promote autophagy but not apoptosis in HaCaT cells[104], probably by activating ERK1/2[103], and 3′-demethoxysudachitin, on the other hand, failed to induce apoptosis or autophagy[104]. These studies suggest that different PMFs may lead to different cellular responses, probably by regulating different signaling pathways.

As a summary, PMFs can protect skins from UV irradiation, whiten skins, and treat certain skin disorders, and these effects should be attributed to antioxidant, anti-inflammatory, anti-tyrosinase, and regulation of different cellular responses. In the future, more deep insights about the skin protection mechanism of PMFs should be revealed to facilitate their applications in the cosmeceuticals.

6.8 Other bioactivities

PMFs also exhibit many other bioactivities *in vitro* and *in vivo*, such as antimicrobial effects[105,106], antitrypanosomal effects[107], antinociceptive effects[12], anxiolytic effects[12], antimutagenic effects[108], bone protective effects[109], intestinal protective effects[110,111], regulation of circadian rhythm[2,112], improving male sexual functions[113], and anti-aging effects[114], with detailed information summarized in Tables 3, 4, and S1.

The human beneficial dose equivalence of PMFs was calculated based on the body surface area (BSA) normalization method according to a previous study[115]. According to Table 4, most beneficial doses of PMFs ranged from 2 mg/(kg·day) to 50 mg/(kg·day) in mouse models. To convert the dose used in a mouse (20 g) to an equivalent dose for humans, the corresponding doses of PMFs for adults (60 kg) should be 9.6−122 mg/day. According to Table 1, the concentrations of PMFs in different plant sources ranged from 0.9−20 mg/g, therefore, the suggested level of daily PMF-rich food consumption can be 0.5−136 g for adults (60 kg) with potential health benefits. This suggests that the potential health benefits of PMFs in humans should be achieved by the consumption of plant-based foods rich in PMFs, while it still needs further verificationin by clinical trials.

7. Conclusions and perspectives

In conclusion, this review updated recent advances on the natural sources, refined extraction technologies, biosynthesis, metabolism, as well as main bioactivities and related molecular mechanisms of PMFs. Since flavonoids and *OMT* genes are widely existed in the plant kingdom, it is speculated that PMFs should exist in more plants besides the reported species, so, it is possible to discover novel PMFs and their natural sources to enrich the PMF pool. In addition, increasing evidence supports that gut microbiota play a critical role in the metabolism and bioactivities of PMFs, while the studies about the interaction between PMFs and gut microbiota are still limited, and how gut microbiota connect PMFs and their bioactivities is still not clear. Besides, whether the metabolites of PMFs transformed by gut microbiota have enhanced bioactivities than PMFs *per se* remains to be verified in more studies. Moreover, encapsulation may be an effective method to increase their bioaccessibility and bioavailability *in vivo*, and should also be explored in the future. Finally, although PMFs exhibit promising bioactivities *in vitro* and *in vivo*, especially on cancer and metabolic syndrome, the health benefits of them in humans have been much less investigated. This review still have some limitations. For example, most bioactivities summarized in this review are based on *in vitro* and animal-based studies due to the lack of human-based studies, therefore, it is recommended to verify the health benefits of PMFs in humans based on well-designed clinical trials in the future, which can promote the large-scale refined extraction of PMFs in the industries, and the PMFs with specific health benefits can be developed into nutraceuticals and pharmaceuticals to prevent and manage certain chronic diseases.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2022.9250003.

References

[1] A.M. Batenburg, T.D. Joode, R.J. Gouka, Characterization and modulation of the bitterness of polymethoxyflavones using sensory and receptor-based methods, J. Agric. Food Chem. 64(12) (2016) 2619-2626. https://doi.org/10.1021/acs.jafc.5b05833.

[2] I. Yoshida, M. Kumagai, M. Ide, et al., Polymethoxyflavones in black ginger (*Kaempferia parviflora*) regulate the expression of circadian clock genes, J. Funct. Foods 68 (2020) 103900. https://doi.org/10.1016/j.jff.2020.103900.

[3] J.Wang, C. Xu, Y.K. Wong, et al., Artemisinin, the magic drug discovered from traditional Chinese medicine, Engineering 5(1) (2019) 32-39. https://doi.org/10.1016/j.eng.2018.11.011.

[4] R. Baraldi, B. Isacchi, S. Predieri, et al., Distribution of artemisinin and bioactive flavonoids from *Artemisia annua* L. during plant growth,

Biochem. Syst. Ecol. 36(5/6) (2008) 340-348. https://doi.org/10.1016/j.bse.2007.11.002.

[5] D. Tasdemir, M. Tierney, R. Sen, et al., Antiprotozoal effect of *Artemisia indica* extracts and essential oil, Planta Med. 81(12/13) (2015) 1029-1037. https://doi.org/10.1055/s-0035-1546125.

[6] W.R. Sawadogo, C. Cerella, A. Al-Mourabit, et al., Cytotoxic, antiproliferative and pro-apoptotic effects of 5-hydroxyl-6,7,3′,4′,5′-pentamethoxyflavone isolated from *Lantana ukambensis*, Nutrients 7(12) (2015) 10388-10397. https://doi.org/10.3390/nu7125537.

[7] Y.L. Shih, H.M. Chou, H.C. Chou., et al., Casticin impairs cell migration and invasion of mouse melanoma B16F10 cells via PI3K/AKT and NF-κB signaling pathways, Environ. Toxicol. 32(9) (2017) 2097-2112. https://doi.org/10.1002/tox.22417.

[8] A. Sato, H. Tamura, High antiallergic activity of 5,6,4′-trihydroxy-7,8,3′-trimethoxyflavone and 5,6-dihydroxy-7,8,3′,4′-tetramethoxyflavone from eau de cologne mint (*Mentha* × *piperita citrata*), Fitoterapia 102 (2015) 74-83. https://doi.org/10.1016/j.fitote.2015.02.003.

[9] E. Wollenweber, J.F. Stevens, M. Ivancic, Flavonoid aglycones and a thiophene derivative from *Helichrysum cassianum*, Phytochemistry 47(7) (1998) 1441-1443. https://doi.org/10.1016/S0031-9422(97)00738-3.

[10] C.S. Cao, B.L. Liu, C.G. Zeng, et al., A polymethoxyflavone from *Laggera pterodonta* induces apoptosis in imatinib-resistant K562R cells via activation of the intrinsic apoptosis pathway, Cancer Cell Int. 14(1) (2014) 137. https://doi.org/10.1186/s12935-014-0137-1.

[11] P.F. Sehlakgwe, N. Lall, G. Prinsloo, ^{1}H-NMR metabolomics and LC-MS analysis to determine seasonal variation in a cosmeceutical plant *Leucosidea serice*, Front. Pharmacol 5(11) (2020) 219. https://doi.org/10.3389/fphar.2020.00219.

[12] M.S. Shajib, R.B. Rashid, L.C. Ming, et al., Polymethoxyflavones from *Nicotiana plumbaginifolia* (Solanaceae) exert antinociceptive and neuropharmacological effects in mice, Front. Pharmacol. 9 (2018) 85. https://doi.org/10.3389/fphar.2018.00085.

[13]Y. Liu, H.Y. Liu, S.H. Li, et al., *Cannabis sativa* bioactive compounds and their extraction, separation, purification, and identification technologies: an updated review, Trends. Analyt. Chem. 149 (2022) 116554. https://doi.org/10.1016/j.trac.2022.116554.

[14] H. Li, H. Guo, Q. Luo, et al., Current extraction, purification, and identification techniques of tea polyphenols: an updated review, Crit. Rev. Food Sci. Nutr. 27 (2021) 1-19. https://doi/10.1080/10408398.2021.1995843.

[15] Q. Yu, W. Chen, J. Zhong, et al., Purification, structural characterization, and bioactivities of a polysaccharide from *Coreopsis tinctoria*, Food Frontiers (2022) 1-13. https://doi.org/10.1002/fft2.145.

[16] Y. Liu, K.W. Kong, D.T. Wu, et al., Pomegranate peel-derived punicalagin: ultrasonic-assisted extraction, purification, and its *α*-glucosidase inhibitory mechanism, Food Chem. 374 (2022) 131635. https://doi.org/10.1016/j.foodchem.2021.131635.

[17] C.Y. Zhao, F. Wang, Y.H. Lian, et al., Biosynthesis of citrus flavonoids and their health effects, Crit. Rev. Food Sci. Nutr. 60(4) (2020) 566-583. https://doi.org/10.1080/10408398.2018.1544885.

[18] N. Itoh, C. Iwata, H. Toda, Molecular cloning and characterization of a flavonoid-*O*-methyltransferase with broad substrate specificity and regioselectivity from Citrus depressa, BMC. Plant Biol. 16(1) (2016) 180. https://doi.org/10.1186/s12870-016-0870-9.

[19] X. Liu, C. Zhao, Q. Gong, et al., Characterization of a caffeoyl-CoA *O*-methyltransferase-like enzyme involved in biosynthesis of polymethoxylated flavones in *Citrus reticulata*, J. Exp. Bot. 71(10) (2020b) 3066-3079. https://doi.org/10.1093/jxb/eraa083.

[20] X.J. Liu, Y. Wang, Y.Z. Chen, et al., Characterization of a flavonoid 3′5′7-*O*-methyltransferase from *Citrus reticulata* and evaluation of the *in vitro* cytotoxicity of its methylated products, Molecules 25(4) (2020a) 858. https://doi.org/10.3390/molecules25040858.

[21] F. Zohra, S. Takematsu, Y. Itami, et al., Accumulation of polymethoxyflavones and *O*-methyltransferase gene expression in various citrus cultivars, Sci. Hortic. 89(3) (2020) 225-236. https://doi.org/10.2503/hortj.UTD-146.

[22] M, Seoka, G. Ma, L. Zhang, et al., Expression and functional analysis of the nobiletin biosynthesis-related gene CitOMT in citrus fruit, Sci. Rep. 10(1) (2020) 15288. https://doi.org/10.1038/s41598-020-72277-z.

[23] G. Ma, L. Zhang, M. Seoka, et al., Characterization of a caffeic acid 8-*O*-methyltransferase from citrus and its function in nobiletin biosynthesis, J. Agric. Food. Chem. 70(2) (2022) 543-553. https://doi.org/10.1021/acs.jafc.1c06513.

[24] S.M. Li, M.H. Pan, C.Y. Lo, et al., Chemistry and health effects of polymethoxyflavones and hydroxylated polymethoxyflavones, J. Funct. Foods 1(1) (2009) 2-12. https://doi.org/10.1016/j.jff.2008.09.003.

[25] A. Karn, C.Y. Zhao, F.L. Yang, et al., *In vivo* biotransformation of citrus functional components and their effects on health, Crit. Rev. Food Sci. Nutr. 61(5) (2021) 756-776. https://doi.org/10.1080/10408398.2020.1746234.

[26] M. Kim, N. Kim, J. Han, Metabolism of *Kaempferia parviflora* polymethoxyflavones by human intestinal bacterium *Bautia* sp. MRG-PMF1, J. Agric. Food Chem. 62(51) (2014) 12377-12383. https://doi.org/10.1021/jf504074n.

[27] M. Zhang, Y. Xin, K. Feng, et al., Comparative analyses of bioavailability, biotransformation, and excretion of nobiletin in lean and obese rats, J. Agric. Food. Chem. 68(39) (2020) 10709-10718. https://doi.org/10.1021/acs.jafc.0c04425.

[28] J.B. Chen, Y. Wang, T.L. Zhu, et al., Beneficial regulatory effects of polymethoxyflavone-rich fraction from Ougan (*Citrus reticulata* cv. *Suavissima*) fruit on gut microbiota and identification of its intestinal metabolites, Antioxidants 9(9) (2020) 831. https://doi.org/10.3390/antiox9090831.

[29] Y.C. Tung, W.T. Chang, S.M. Li, et al., Citrus peel extracts attenuated obesity and modulated gut microbiota in mice with high-fat diet-induced obesity, Food Funct. 9(6) (2018) 3363-3373. https://doi.org/10.1039/c7fo02066j.

[30] M. Zhang, J.Y. Zhu, X. Zhang, et al., Aged citrus peel (Chenpi) extract causes dynamic alteration of colonic microbiota in high-fat diet induced obese mice, Food Funct. 11(3) (2020) 2667-2678. https://doi.org/10.1039/c9fo02907a.

[31] M.Y. Wang, H. Zhao, X. Wen, et al., Citrus flavonoids and the intestinal barrier: interactions and effects, Compr. Rev. Food Sci. Food Saf. 20(1) (2021) 225-251. https://doi.org/10.1111/1541-4337.12652.

[32] M. Falduto, F. Smedile, M. Zhang, et al., Anti-obesity effects of Chenpi: an artificial gastrointestinal system study, Microb. Biotechnol. 15(3) (2022) 874-885. https://10.1111/1751-7915.14005.

[33] J. Chen, Y. Wang, T. Zhu, et al., Beneficial regulatory effects of polymethoxyflavone-rich fraction from ougan (*Citrus reticulata* cv. *Suavissima*) fruit on gut microbiota and identification of its intestinal metabolites in mice, Antioxidants (Basel) 9(9) (2020) 831. https://10.3390/antiox9090831.

[34] J.C. Wu, M.L. Tsai, C.S. Lai, et al., Polymethoxyflavones prevent benzo[a]pyrene/dextran sodium sulfate-induced colorectal carcinogenesis through modulating xenobiotic metabolism and ameliorate autophagic defect in ICR mice, Int. J. Cancer. 142(8) (2018) 1689-1701. https://10.1002/ijc.31190.

[35] L. Fu, B.T. Xu, X.R. Xu, et al., Antioxidant capacities and total phenolic contents of 62 fruits, Food Chem. 129(2) (2011) 345-350. https://doi.org/10.1016/j.foodchem.2011.04.079.

[36] G.Y. Tang, C.N. Zhao, X.Y. Xu, et al., Phytochemical composition and antioxidant capacity of 30 Chinese teas, Antioxidants 8(6) (2019) 180. https://doi.org/10.3390/antiox8060180.

[37] D.P. Xu, Y. Li, X. Meng, et al., Natural antioxidants in foods and medicinal plants: extraction, assessment and resources, Int. J. Mol. Sci. 18(1) (2017) 96. https://doi.org/10.3390/ijms18010096.

[38] H. Khan, H. Ullah, R. Tundis, et al., Dietary flavonoids in the management of Huntington's disease: mechanism and clinical perspective, eFood 1(1) (2020) 38-52. https://doi.org/10.2991/efood.k.200203.001.

[39] S. Horigome, I. Yoshida, S. Ito, et al., Inhibitory effects of *Kaempferia parviflora* extract on monocyte adhesion and cellular reactive oxygen species production in human umbilical vein endothelial cells, Eur. J. Nutr. 56(3) (2017) 949-964. https://doi.org/10.1007/s00394-015-1141-5.

[40] X.Z. Dai, X.Q. Yan, K.A. Wintergerst, et al., Nrf2: redox and metabolic regulator of stem cell state and function, Trends Mol. Med. 26(2) (2020) 185-200. https://doi.org/10.1016/j.molmed.2019.09.007.

[41] F.Q. Liang, Y.J. Fang, W.W. Cao, et al., Attenuation of tert-butyl hydroperoxide (t-BHP)-induced oxidative damage in HepG2 cells by tangeretin: relevance of the Nrf2-ARE and MAPK signaling pathways, J. Agric. Food Chem. 66(25) (2018) 6317-6325. https://doi.org/10.1021/acs.jafc.8b01875.

[42] M.X. Zhou, G.H. Li, X.Y. Wu, et al., (2*S*)-5,6,7,3′,4′-pentamethoxyflavanone, a citrus polymethoxyflavone ameliorates arsenic- and cigarette smoke extract-induced cytotoxicity via activating Nrf2-mediated defense system, J. Funct. Foods 54 (2019) 337-347. https://doi.org/10.1016/j.jff.2019.01.019.

[43] Y. Wang, R. Jin, J. Chen, et al., Tangeretin maintains antioxidant activity by reducing CUL3 mediated NRF2 ubiquitination, Food Chem. 15(365) (2021) 130470. https://doi.org/10.1016/j.foodchem.2021.130470.

[44] M. Wang, D. Meng, P. Zhang, et al., Antioxidant protection of nobiletin, 5-demethylnobiletin, tangeretin, and 5-demethyltangeretin from citrus peel in Saccharomyces cerevisiae, J. Agric. Food Chem. 66(12) (2018) 3155-3160. https://doi.org/10.1021/acs.jafc.8b00509.

[45] J.M. Hyun, Y.J. Jo, J.E. Kim, et al., Tetramethyl-*O*-scutellarin isolated from peels of immature shiranuhi fruit exhibits anti-inflammatory effects on LPS-induced RAW264.7 cells, Trop. J. Pharm. Res. 16(9) (2017) 2197-2205. https://doi.org/10.4314/tjpr.v16i9.22.

[46] Y. Wang, W. Zang, S. Ji, et al., Three polymethoxyflavones purified from Ougan (*Citrus reticulata* cv. *Suavissima*) inhibited LPS-induced NO elevation in the neuroglia BV-2 cell line via the JAK2/STAT3 pathway, Nutrients 11(4) (2019) 1-16. https://doi.org/10.3390/nu11040791.

[47] Z.B. Liu, S.G. Guo, Q.R. Dong, Nobiletin suppresses IL-21/IL-21 receptor-mediated inflammatory response in MH7A fibroblast-like synoviocytes (FLS): an implication in rheumatoid arthritis, Eur. J. Pharmacol. 875 (2020) 172939. https://doi.org/10.1016/j.ejphar.2020.172939.

[48] J. Lorenzo, Cytokines and the pathogenesis of osteoporosis, in: Marcus and Feldman's Osteoporosis, Fifth Edition ed., Academic Press, 2021, pp. 799-831.

[49] Y. Ohyama, J. Ito, V.J. Kitano, et al., The polymethoxy flavonoid sudachitin suppresses inflammatory bone destruction by directly inhibiting osteoclastogenesis due to reduced ROS production and MAPK activation in osteoclast precursors, PLoS One 13(1) (2018) e0191192. https://doi.org/10.1371/journal.pone.0191192.

[50] B. Liu, J. Huang, B, Zhang, Nobiletin protects against murine l-arginine-induced acute pancreatitis in association with downregulating p38MAPK and AKT, Biomed. Pharmacother. 81 (2016) 104-110. https://doi.org/10.1016/j.biopha.2016.03.051.

[51] R.Y. Gan, H.B. Li, Z.Q. Sui, et al., Absorption, metabolism, anti-cancer effect and molecular targets of epigallocatechin gallate (EGCG): an updated review, Crit. Rev. Food Sci. Nutr. 58(6) (2018) 924-941. https://doi.org/10.1080/10408398.2016.1231168.

[52] X.Y. Xu, X. Meng, S. Li, et al., Bioactivity, health benefits, and related molecular mechanisms of curcumin: current progress, challenges, and perspectives, Nutrients 10(10) (2018) 1553. https://doi.org/10.3390/nu10101553.

[53] X. Meng, J. Zhou, C.N. Zhao, et al., Health benefits and molecular mechanisms of resveratrol: a narrative review, Foods 9(3) (2020) 340. https://doi.org/10.3390/foods9030340.

[54] A.K. Farha, R.Y. Gan, H.B. Li, et al., The anticancer potential of the dietary polyphenol rutin: current status, challenges, and perspectives, Crit. Rev. Food Sci. Nutr. 62(3) (2020) 832-859. https://doi.org/10.1080/10408398.2020.1829541.

[55] A. Shang, H.Y. Liu, M. Luo, et al., Sweet tea (*Lithocarpus polystachyus* rehd.) as a new natural source of bioactive dihydrochalcones with multiple health benefits, Crit. Rev. Food Sci. Nutr. 62(4) (2020) 917-934 https://doi.org/10.1080/10408398.2020.1830363.

[56] J.X. Goh, L.T. Tan, J.K. Goh, et al., Nobiletin and derivatives: functional compounds from citrus fruit peel for colon cancer chemoprevention, Cancers (Basel) 11(6) (2019) 867. https://doi.org/10.3390/cancers11060867.

[57] X. Wang, M. Xia, 5-Hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone, a polymethoxyflavone, exerts antitumor effect on PI3K/Akt signaling pathway in human gastric cancer cell BGC-7901, J. Recept. Sig .Transd. 36(5) (2016) 471-477. https://doi.org/10.3109/10799893.2015.1122046.

[58] J.Y. Moon, S.K. Cho, Nobiletin induces protective autophagy accompanied by ER-stress mediated apoptosis in human gastric cancer SNU-16 cells, Molecules 21(7) (2016) 914. https://doi.org/10.3390/molecules21070914.

[59] Y.P. Jiang, H. Guo, X.B. Wang, Nobiletin (NOB) suppresses autophagic degradation via over-expressing AKT pathway and enhances apoptosis in multidrug-resistant SKOV3/TAX ovarian cancer cells, Biomed. Pharmacother. 103 (2018) 29-37. https://doi.org/10.1016/j.biopha.2018.03.126.

[60] J.H. Yen, C.Y. Lin, C.H. Chuang, et al., Nobiletin promotes megakaryocytic differentiation through the MAPK/ERK-dependent EGR1 expression and exerts anti-leukemic effects in human chronic myeloid leukemia (CML) K562 cells, Cells 9(4) (2020) 877. https://doi.org/10.3390/cells9040877.

[61] H.S. Shang, J.Y. Liu, H.F. Lu, et al. Casticin induced apoptotic cell death and altered associated gene expression in human colon cancer colo 205 cells, Environ. Toxicol. 32(8) (2017) 2041-2052. https://doi.org/10.1002/tox.22381.

[62] G.L .Chou, S.F. Peng, C.L. Liao, et al., Casticin impairs cell growth and induces cell apoptosis via cell cycle arrest in human oral cancer SCC-4 cells, Environ. Toxicol. 33(2) (2018) 127-141. https://doi.org/10.1002/tox.22497.

[63] Y.W. Shiue, C.C. Lu, Y.P. Hsiao, et al., Casticin induced apoptosis in A375.S2 human melanoma cells through the inhibition of NF-κB and mitochondria-dependent pathways *in vitro* and inhibited human melanoma xenografts in a mouse model *in vivo*, Am. J. Chinese Med. 44(3) (2016) 637-661. https://doi.org/10.1142/S0192415X1650035X.

[64] Y.P. Cheng, S. Li, W.L. Chuang, et al., Blockade of STAT3 signaling contributes to anticancer effect of 5-acetyloxy-6,7,8,4′-tetra-methoxyflavone, a tangeretin derivative, on human glioblastoma multiforme cells, Int. J. Mol. Sci. 20(13) (2019) 3366. https://doi.org/10.3390/ijms20133366.

[65] X. Wu, M.Y. Song, Z.L. Gao, et al., Nobiletin and its colonic metabolites suppress colitis-associated colon carcinogenesis by down-regulating iNOS, inducing antioxidative enzymes and arresting cell cycle progression, J. Nutr. Biochem. 42 (2017) 17-25. https://doi.org/10.1016/j.jnutbio.2016.12.020.

[66] W.B. Zhu, N. Xiao, X.J. Liu, Dietary flavonoid tangeretin induces reprogramming of epithelial to mesenchymal transition in prostate cancer cells by targeting the PI3K/Akt/mTOR signaling pathway, Oncol. Lett. 15(1) (2018) 433-440. https://doi.org/10.3892/ol.2017.7307.

[67] H.L. Cheng, M.J. Hsieh, J.S. Yang, et al., Nobiletin inhibits human osteosarcoma cells metastasis by blocking ERK and JNK-mediated MMPs expression, Oncotarget. 7(23) (2016) 35208-35223. https://doi.org/10.18632/oncotarget.9106.

[68] J. Li, Z. Li, F. Zheng, Nobiletin inhibits proliferation, invasion, migration and angiogenesis in colorectal cancer cells, J. Biomater. Tissue. Eng. 9(6) (2019) 662-667. https://doi.org/10.1166/jbt.2019.2022.

[69] C.C. Lin, K.B. Chen, C.H. Tsai, et al., Casticin inhibits human prostate cancer DU 145 cell migration and invasion via Ras/Akt/NF- κ B signaling pathways, J. Food. Biochem. 43(7) (2019) e12902. https://doi.org/10.1111/jfbc.12902.

[70] M. Schmittnaegel, N. Rigamonti, E. Kadioglu, , et al., Dual angiopoietin-2 and VEGFA inhibition elicits antitumor immunity that is enhanced by PD-1 checkpoint blockade, Sci. Transl. Med. 9(385) (2017) eaak9670. https://doi.org/10.1126/scitranslmed.aak9670.

[71] Q. Gong, X.Z. Cao, J.G. Cao, et al., Casticin suppresses the carcinogenesis of small cell lung cancer H446 cells through activation of AMPK/FoxO3a signaling, Oncol. Rep. 40(3) (2018) 1401-1410. https://doi.org/10.3892/or.2018.6547.

[72] Y.R. Ma, X. Ren, N.D.N. Patel, et al., Nobiletin, a citrus polymethoxyflavone, enhances the effects of bicalutamide on prostate cancer cells via down regulation of NF-κB, STAT3, and ERK activation, RSC. Advances 10 (17) (2020) 10254-10262. https://doi.org/10.1039/C9RA10020B.

[73] X. Wu, M. Song, P. Qiu, et al., Synergistic chemopreventive effects of nobiletin and atorvastatin on colon carcinogenesis, Carcinogenesis 38(4) (2017) 455-464. https://doi.org/10.1093/carcin/bgx018.

[74] G.R. Gandhi, A.B.S Vasconcelos, D.T. Wu, et al., Citrus flavonoids as promising phytochemicals targeting diabetes and related complications: a systematic review of *in vitro* and *in vivo* studies, Nutrients 12 (2020) 2907. https://doi.org/10.3390/nu12102907.

[75] H.Y. Li, R.Y. Gan, A. Shang, et al., Plant-based foods and their bioactive compounds on fatty liver disease: effects, mechanisms, and clinical application, Oxid. Med. Cell Longev. 2021 (2021) 6621644. https://doi.org/10.1155/2021/6621644.

[76] A. Noce, M.D. Lauro, F.D. Daniele, et al., Natural bioactive compounds useful in clinical management of metabolic syndrome, Nutrients 13(2) (2021) 630. https://doi.org/10.3390/nu13020630.

[77] A. Shang, R.Y. Gan, X.Y. Xu, et al., Effects and mechanisms of edible and medicinal plants on obesity: an updated review, Crit. Rev. Food Sci. Nutr. 61(12) (2020) 2061-2077. https://doi.org/10.1080/10408398.2020.1769548.

[78] T. Yuk, Y. Kim, J. Yang, et al., Nobiletin inhibits hepatic lipogenesis via activation of AMP-activated protein kinase, Evid. Based. Complement Alternat. Med. 2018 (2018) 7420265. https://doi.org/10.1155/2018/7420265.

[79] A. Sawamoto, M. Nakanishi, S. Okuyama, et al., Heptamethoxyflavone inhibits adipogenesis via enhancing PKA signaling, Eur. J. Pharmacol. 865 (2019) 172758. https://doi.org/10.1016/j.ejphar.2019.172758.

[80] G.J. Li, J. Wang, Y.J. Cheng, et al., Prophylactic effects of polymethoxyflavone-rich orange peel oil on N^{ω}-nitro-*L*-arginine-induced hypertensive rats, Appl. Sci. 8(5) (2018) 752. https://doi.org/10.3390/app8050752.

[81] J. Lee, E.H. Bae, S.K. Ma, et al., Altered nitric oxide system in cardiovascular and renal diseases, Chonnam. Med. J, 52(2) (2016) 81-90. https:// doi.org/10.4068/cmj.2016.52.2.81.

[82] M. Ochiai, T. Takeuchi, T. Nozaki, et al., *Kaempferia parviflora* ethanol extract, a peroxisome proliferator-activated receptor γ ligand-binding agonist, improves glucose tolerance and suppresses fat accumulation in diabetic NSY mice, J. Food Sci. 84(2) (2019) 339-348. https://doi.org/10.1111/1750-3841.14437.

[83] S.L. Zeng, S.Z. Li, P.T. Xiao, et al., Citrus polymethoxyflavones attenuate metabolic syndrome by regulating gut microbiome and amino acid metabolism, Sci. Adv. 6(1) (2020) eaax6208. https://doi.org/10.1126/sciadv.aax6208.

[84] N.A. Parkar, L.K. Bhatt, V. Addepalli, Efficacy of nobiletin, a citrus flavonoid, in the treatment of the cardiovascular dysfunction of diabetes in rats, Food Funct. 7(7) (2016) 3121-3129. https://doi.org/10.1039/c6fo00294c.

[85] X. Cheng, Q. Li, J. Liu, et al., Nobiletin protects against monocrotaline-induced pulmonary arterial hypertension in rats by regulating Src/STAT3 signaling pathway, Int. J. Clin. Exp. Med. 10(7) (2017) 10342-10350.

[86] N.M. Fayek, A.H. El-Shazly, A.R. Abdel-Monem, et al., Comparative study of the hypocholesterolemic, antidiabetic effects of four agro-waste *Citrus* peels cultivars and their HPLC standardization, Rev. Bras. Farmacogn. 27(4) (2017) 488-494. https://doi.org/10.1016/j.bjp.2017.01.010.

[87] K.M. Youn, J. Lee, C.T. Ho, et al., Discovery of polymethoxyflavones from black ginger (*Kaempferia parviflora*) as potential *β*-secretase (BACE1) inhibitors, J. Funct. Foods. 20 (2016) 567-574. https://doi.org/10.1016/j.jff.2015.10.036.

[88] S.D. Meriney, E.E. Fanselow, Acetylcholine, in: Synaptic Transmission, Academic Press, 2019, pp. 345-367.

[89] I. Kawahata, T. Suzuki, E.G. Rico, et al., Fermented *Citrus reticulata* (ponkan) fruit squeezed draff that contains a large amount of 4′-demethylnobiletin prevents MK801-induced memory impairment, J. Nat. Med. 71(4) (2017) 617-631. https://doi.org/10.1007/s11418-017-1091-8.

[90] S. Trivedi, P. Maurya, S.R. Sammi, et al., 5-Desmethylnobiletin augments synaptic ACh levels and nicotinic ACh receptor activity: a potential candidate for alleviation of cholinergic dysfunction, Neurosci. Lett. 657 (2017) 84-90. https://doi.org/10.1016/j.neulet.2017.08.010.

[91] A. Fatima, Y.H. Siddique, Role of tangeritin against cognitive impairments in transgenic drosophila model of Parkinson's disease, Neurosci. Lett. 705 (2019) 112-117. https://doi.org/10.1016/j.neulet.2019.04.047.

[92] S. Okuyama, K. Miyazaki, R. Yamada, et al., Permeation of polymethoxyflavones into the mouse brain and their effect on MK-801-induced locomotive hyperactivity, Int. J. Mol. Sci. 18(3) (2017) 489. https://doi.org/10.3390/ijms18030489.

[93] A. Sawamoto, S. Okuyama, Y. Amakura, et al., 3,5,6,7,8,3′,4′-Heptam ethoxyflavone ameliorates depressive-like behavior and hippocampal neurochemical changes in chronic unpredictable mild stressed mice by regulating the brain-derived neurotrophic factor: requirement for ERK activation, Int. J. Mol. Sci. 18(10) (2017) 2133. https://doi.org/10.3390/ijms18102133.

[94] F. Wang, A. Ullah, X. Fan, et al., Delivery of nanoparticle antigens to antigen-presenting cells: from extracellular specific targeting to intracellular responsive presentation. J Control Release. 333 (2021) 107-128. https://doi.org/10.1016/j.jconrel.2021.03.027.

[95] A. Nakamoto, M. Mitani, K. Urayama, et al., Nobiletin enhances induction of antigen-specific immune responses in BALB/c mice immunized with ovalbumin, J. Nutr. Sci. Vitaminol 65(3) (2019) 278-282. https://doi.org/10.3177/jnsv.65.278.

[96] M. Mitani, Y. Minatogawa, A. Nakamoto, et al., Sudachitin, polymethoxyflavone from *Citrus sudachi*, enhances antigen-specific cellular and humoral immune responses in BALB/c mice, J. Clin. Biochem .Nutr. 64(2) (2019) 158-163. https://doi.org/10.3164/jcbn.

[97] M. Nakajima, M. Ogawa, Y. Amakura, et al., 3,5,6,7,8,3′,4′-Heptamethoxyf lavone reduces interleukin-4 production in the spleen cells of mice, Biomed. Res. 37(2) (2016) 95-99. https://doi.org/10.2220/biomedres.37.95.

[98] Y. Hamada, M. Nakajima, K. Tsuzuki, et al., Heptamethoxyflavone reduces phosphodiesterase activity and T-cell growth *in vitro*, Int. Arch. Allergy Immunol. 174(3/4) (2017) 113-120. https://doi.org/10.1159/000481094.

[99] N. Yoshizaki, T. Fujii, R. Hashizume, et al., A polymethoxyflavone mixture, extracted from orange peels, suppresses the UVB-induced expression of MMP-1, Exp. Dermatol. 25(3) (2016) 52-56. https://doi.org/10.1111/exd.13087.

[100] G. Li, F. Tan, Q. Zhang, et al., Protective effects of polymethoxyflavone-rich cold-pressed orange peel oil against ultraviolet B-induced photoaging on mouse skin, J. Funct. Foods 67 (2020) 103834. https://doi.org/10.1016/j.jff.2020.103834.

[101] N. Yoshizaki, R. Hashizume, H. Masaki, A polymethoxyflavone mixture extracted from orange peels, mainly containing nobiletin, 3,3′,4′,5,6,7,8-heptamethoxyflavone and tangeretin, suppresses melanogenesis through the acidification of cell organelles, including melanosomes, J. Dermatol Sci. 88(1) (2017) 78-84. https://doi.org/10.1016/j.jdermsci.2017.06.008.

[102] R.R.J. Arroo, S. Sari, B. Barut, et al., Flavones as tyrosinase inhibitors: kinetic studies *in vitro* and in silico, Phytochem. Anal. 31(3) (2020) 314-321. https://doi.org/10.1002/pca.2897.

[103] S. Abe, K. Yuasa, Sudachitin, a polymethoxyflavone from Citrus sudachi, induces apoptosis via the regulation of MAPK pathways in human keratinocyte HaCaT cells, Biochem. Biophys. Res. Commun. 519(2) (2019) 344-350. https://doi.org/10.1016/j.bbrc.2019.09.010.

[104] S. Abe, S. Hirose, M. Nishitani, et al., Citrus peel polymethoxyflavones, sudachitin and nobiletin, induce distinct cellular responses in human keratinocyte HaCaT cells, Biosci. Biotechnol Biochem. 82(12) (2018) 2064-2071. https://doi.org/10.1080/09168451.2018.1514246.

[105] A. Ortuño, A. Báidez, P. Gómez, et al., *Citrus paradisi* and *Citrus sinensis* flavonoids: their influence in the defence mechanism against *Penicillium digitatum*, Food Chem. 98(2) (2006) 351-358. https://doi.org/10.1016/j.foodchem.2005.06.017.

[106] A. Ortuño, M.C. Arcas, J.M. Botía, et al., Increasing resistance against *Phytophthora citrophthora* in tangelo Nova fruits by modulating polymethoxyflavones levels, J. Agric. Food Chem. 50(10) (2002) 2836-2839. https://doi.org/10.1021/jf011382a.

[107] M. Nakanishi, M. Hino, M. Yoshimura, et al., Identification of sinensetin and nobiletin as major antitrypanosomal factors in a citrus cultivar, Exp. Parasitol. 200 (2019) 24-29. https://doi.org/10.1016/j.exppara.2019.03.008.

[108] M. Miyazawa, Y. Okuno, M. Fukuyama, et al., Antimutagenic activity of polymethoxyflavonoids from *Citrus aurantium*, J. Agric. Food Chem. 47(12) (1999) 5239-5244. https://doi.org/10.1021/jf990176o.

[109] S. Matsumoto, T. Tominari, C. Matsumoto, et al., Effects of polymethoxyflavonoids on bone loss induced by estrogen deficiency and by LPS-dependent inflammation in mice, Pharmaceuticals. 11(1) (2018) 7. https://doi.org/10.3390/ph11010007.

[110] A. Aboul Naser, E. Younis, A. El-Feky, et al., Management of *Citrus sinensis* peels for protection and treatment against gastric ulcer induced by ethanol in rats, Biomarkers 25(4) (2020) 349-359. https://doi.org/10.1080/1354750X.2020.1759693.

[111] X. Wen, H. Zhao, L. Wang, et al., Nobiletin attenuates DSS-induced intestinal barrier damage through the HNF4α-claudin-7 signaling pathway, J. Agric. Food Chem. 68(16) (2020) 4641-4649. https://doi.org/10.1021/acs.jafc.0c01217.

[112] A. Shinozaki, K. Misawa, Y. Ikeda, et al., Potent effects of flavonoid nobiletin on amplitude, period, and phase of the circadian clock rhythm in PER2::LUCIFERASE mouse embryonic fibroblasts, PLoS One 12(12) (2017) e0170904. https://doi.org/10.1371/journal.pone.0170904.

[113] S. Horigome, M. Maeda, H.J. Ho, et al., Effect of *Kaempferia parviflora* extract and its polymethoxyflavonoid components on testosterone production in mouse testis-derived tumour cells, J. Funct. Foods 26 (2016) 529-538. https://doi.org/10.1016/j.jff.2016.08.008.

[114] X Yang, H Wang, T Li, et al., Nobiletin delays aging and enhances stress resistance of *Caenorhabditis elegans*, Int. J. Mol. Sci. 21(1) (2020) 341. https://doi.org/10.3390/ijms21010341.

[115] S. Reagan-Shaw, M. Nihal, N. Ahmad, et al., Dose translation from animal to human studies revisited, FASEB J. 22(3) (2008) 659-661. https://doi.org/0.1096/fj.07-9574LSF.

[116] M.C. Arcas, J.M. Botía, A.M. Ortuño, et al., UV irradiation alters the levels of flavonoids involved in the defence mechanism of *Citrus aurantium* fruits against *Penicillium digitatum*, Eur. J. Plant Pathol. 106 (2000) 617-622. https://doi.org/10.1023/a:1008704102446.

[117] T. Yasuda, Y. Yoshimura, H. Yabuki, et al., Urinary metabolites of nobiletin orally administered to rats, Chem. Pharm. Bull. 51(12) (2003) 1426-1428. https://doi.org/10.1248/cpb.51.1426.

[118] Y.W. Ting, Y.K. Jiang, Y.Q. Lan, et al., Viscoelastic emulsion improved the bioaccessibility and oral bioavailability of crystalline compound: a mechanistic study using *in vitro* and *in vivo* models, Mol. Pharm. 12(7) (2015) 2229-2236. https://doi.org/10.1021/mp5007322.

[119] A.M. Ortuño, M.C. Arcas, O. Benavente-García, et al., Evolution of polymethoxy flavones during development of tangelo Nova fruits, Food Chem. 66(2) (1999) 217-220. https://doi.org/10.1016/S0308-8146(99)00047-3.

[120] B. Weber, B. Hartmann, D. Stöckigt, et al., Liquid chromatography/mass spectrometry and liquid chromatography/nuclear magnetic resonance as complementary analytical techniques for unambiguous identification of polymethoxylated flavones in residues from molecular distillation of orange peel oils (*Citrus sinensis*), J. Agric. Food Chem. 54(2) (2006) 274-278. https://doi.org/10.1021/jf051606f.

[121] H.C. Ko, M.G. Jang, C.H. Kang, et al., Preparation of a polymethoxyflavone-rich fraction (PRF) of *Citrus sunki* Hort. ex Tanaka and its antiproliferative effects, Food Chem. 123(2) (2010) 484-488. https://doi.org/10.1016/j.foodchem.2010.04.028.

[122] S. Han, H.M. Kim, J.M. Lee, et al., Isolation and identification of polymethoxyflavones from the hybrid Citrus, hallabong, J. Agric. Food Chem. 58(17) (2010) 9488-9491. https://doi.org/10.1021/jf102730b.

[123] S. Han, H.M. Kim, S. Lee, Simultaneous determination of polymethoxyflavones in *Citrus* species, *Kiyomi tangor* and *Satsuma mandarin*, by high performance liquid chromatography, Food Chem. 134(2) (2012) 1220-1224. https://doi.org/10.1016/j.foodchem.2012.02.187.

[124] J. Chen, A.M. Montanari, Isolation and identification of new polymethoxyflavonoids from *Dancy tangerine* leaves, J. Agric. Food Chem. 46(4) (1998) 1235-1238. https://doi.org/10.1021/jf970606f.

[125] N. Wongsrikaew, H. Kim, K. Vichitphan, et al., Antiproliferative activity and polymethoxyflavone composition analysis of *Kaempferia parviflora* extracts, J. Korean Soc. Appl. Biol. Chem. 55 (2012) 813-817. https://doi.org/10.1007/s13765-012-2175-5.

[126] T. Kinoshita, K. Firman, Highly oxygenated flavonoids from *Murraya paniculata*, Phytochemistry 42(4) (1996) 1207-1210. https://doi.org/10.1016/0031-9422(96)00058-1.

[127] S.A Nair, R.K. Sr, AS Nair, et al., Citrus peels prevent cancer, Phytomedicine 50 (2018) 231-237. https://doi.org/10.1016/j.phymed.2017.08.011.

[128] C. Oba, M. Ota, K. Nomura, et al., Extraction of nobiletin from *Citrus Unshiu* peels by supercritical fluid and its CRE-mediated transcriptional activity, Phytomedicine 27 (2017) 33-38. https://doi.org/10.1016/j.phymed.2017.01.014.

[129] H. Ihara, H. Yamamoto, T. Ida, et al., Inhibition of nitric oxide production and inducible nitric oxide synthase expression by a polymethoxyflavone from young fruits of *Citrus unshiu* in rat primary astrocytes, Biosci. Biotechnol Biochem. 76(10) (2012) 1843-1848. https://doi.org/10.1271/bbb.120215.

[130] N.M. Fayek, M.A. Farag, A.R. Abdel Monem, et al., Comparative metabolite profiling of four *Citrus* peel spectrometry and multivariate data analyses, J. Chromatogr. Sci. 57(4) (2019) 349-360. https://doi.org/10.1093/chromsci/bmz006.

[131] M. Hidaka, K. Horikawa, T. Akase, et al., Efficacy of *Kaempferia parviflora* in a mouse model of obesity-induced dermatopathy, J. Nat. Med. 71(1) (2017) 59-67. https://doi.org/10.1007/s11418-016-1027-8.

[132] T. Horikawa, T. Shimada, Y. Okabe, et al., Polymethoxyflavonoids from *Kaempferia parviflora* induce adipogenesis on 3T3-L1 preadipocytes by regulating transcription factors at an early stage of differentiation, Biol. Pharm. Bull. 35(5) (2012) 686-692. https://doi.org/10.1248/bpb.35.686.

[133] Y.L. Shih, J. Chou, M.Y. Yeh, et al., Casticin induces DNA damage and inhibits DNA repair-associated protein expression in B16F10 mouse melanoma cancer cells, Oncol Rep. 36(4) (2016) 2094-2100. https://doi.org/10.3892/or.2016.5027.

[134] F. Ning, X. Wang, H. Zheng, et al., Improving the bioaccessibility and *in vitro* absorption of 5-demethylnobiletin from chenpi by se-enriched peanut protein nanoparticles-stabilized pickering emulsion, J. Funct. Foods 55 (2019) 76-85. https://doi.org/10.1016/j.jff.2019.02.019.

[135] H. Sagara, M. Kanakogi, Y. Tara, et al., Concise synthesis of polymethoxyflavone sudachitin and its derivatives, and biological evaluations, Tetrahedron Lett. 59(19) (2018) 1816-1818. https://doi.org/10.1016/j.tetlet.2018.03.064.

[136] Y. Nii, Y. Shikishima, T. Sakai, Improvement of lipid metabolism in mice fed a high-fat diet treated with sudachitin and development of sudachi peel extract powder, Nippon Shokuhin Kagaku Kogaku Kaishi 66 (7) (2019) 233-237.