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Millettia isoflavonoids: a comprehensive review of structural diversity, extraction, isolation, and pharmacological properties

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Abstract There are approximately 260 known species in the genus *Millettia*, many of which are used in traditional medicine to treat human and other animal ailments in various parts of the world. Being in the Leguminosae (Fabaceae) family, *Millettia* species are rich sources of isoflavonoids. In the past three decades alone, several isoflavonoids originating from *Millettia* have been isolated, and their pharmacological activities have been evaluated against major diseases, such as cancer, inflammation, and diabetes. Despite such extensive research, no recent and comprehensive review of the phytochemistry and pharmacology of *Millettia* isoflavonoids is available. Furthermore, the structural diversity of isoflavonoids in *Millettia* species has rarely been reported. In this review, we comprehensively summarized the structural diversity of *Millettia* isoflavonoids, the methods used for their extraction and isolation protocols, and their pharmacological properties. According to the literature, 154 structurally diverse isoflavonoids were isolated and reported from the various tissues of nine well-known *Millettia* species. Prenylated isoflavonoids and rotenoids were the most dominant subclasses of isoflavonoids reported. Other subclasses of reported isoflavonoids include isoflavans, aglycone isoflavones, glycosylated isoflavones, geranylated isoflavonoids, phenylcoumarins, pterocarpans and coumaronochromenes. Although some isolated molecules showed promising pharmacological properties, such as anticancer, anti-inflammatory, estrogenic, and antibacterial activities, others remained untested. In general, this review highlights the potential of *Millettia* isoflavonoids and could improve their utilization in drug discovery and medicinal use processes.

Keywords Isoflavonoids · Isolation · *Millettia* species · Pharmacological effect · Structural diversity

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Abbreviations

AChE	Acetylcholinesterase
BChE	Butyrylcholinesterase
CC	Column chromatography
CCC	Countercurrent chromatography
CD	Circular dichroism
COSY	Correlation spectroscopy
COVID	Coronavirus disease
ECD	Electronic circular dichroism
HMBC	Heteronuclear multiple bond correlation
HPLC	High-performance liquid chromatography
HR-MS	High resolution mass spectrometry
HSCCC	High-speed countercurrent chromatography
HSQC	Heteronuclear single quantum coherence
IR	Infrared spectroscopy
MIC	Minimum inhibitory concentration
MS	Mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
P-HPLC	Preparative high-performance liquid chromatography
P-TLC	Preparative thin layer chromatography
RP-HPLC	Reverse-phase high-performance liquid chromatography
TLC	Thin layer chromatography
UV–Vis	Ultraviolet–visible spectroscopy
VLC	Vacuum-liquid chromatography

Introduction

Isoflavonoids, also known as 3-phenylchromanes, are a class of flavonoids found in the Leguminosae (Fabaceae) family (Veitch 2013). Although isoflavonoids are considered biomarkers of the legume species, their presence has been confirmed in more than 20 non-leguminous plant families, including Rutaceae, Iridaceae, Asteraceae, Cyperaceae, Convolvulaceae, and Asclepiadaceae, among others (Mackova et al. 2006; Raynaud et al. 2005). Structurally, flavonoids contain a 15-carbon backbone arranged as C_6-C_3-C_6, where the two phenyl rings (known as the *A*-ring and *B*-ring) are linked by a heterocyclic pyran ring called the *C*-ring. Isoflavonoids also share this basic chromophore in their structure. However, unlike the other classes of flavonoids, the phenyl *B*-ring is attached to position 3 of the heterocyclic *C*-ring rather than position 2 (Fig. 1), giving rise to the name 3-phenylchromane flavonoids (Botta et al. 2009; Tsimogiannis and Oreopoulou 2019).

As in many flavonoids, the oxidation state and hydroxylation pattern of the *C*-ring make isoflavonoids structurally diverse (Sisa et al. 2010; Tsimogiannis and Oreopoulou 2019). As illustrated in Fig. 1, at least ten subclasses of isoflavonoids are known, including isoflavans, isoflavanones, isoflav-3-ens, isoflavan-4-ols, isoflavones, rotenoids, coumarins, coumestans, coumaronochromenes, and pterocarpans. Such structural diversity of naturally occurring isoflavonoids makes them exert several health-promoting and disease-prevention properties (Bhargavan et al. 2009; Farajzadeh-Dehkordi et al. 2021; Yamaki et al. 2002). For example, in the aftermath of the recent outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-*CoV*-2), also known as coronavirus disease (COVID-19), several flavonoids have been studied for their ability to combat the spread of the virus. As a result, isoflavonoids such as glisoflavanone, 5,7,3',4'-tetrahydroxy-2'-(3,3-dimethylallyl) isoflavone, and kraussianones 2 have been shown to bind to angiotensin-converting enzyme 2 (ACE-2), making them ideal drug candidates for the treatment of COVID-19 (Alesawy et al. 2021; Prasansuklab et al. 2021; T-ul et al. 2020). Furthermore, other isoflavonoids have been discovered to have anticancer, anti-HIV, and antidiabetic properties, highlighting the potential of natural isoflavonoids in combating the most difficult human ailments (Cayetano-Salazar et al., 2021; Hussain and Green 2017; Stevenson et al. 2018). The biosynthesis of flavonoids in legumes follows the phenylpropanoid pathway, where various enzymes participate in the production of diverse classes of flavonoids (Sohn et al. 2021). As shown in Scheme 1, the interdependent actions of two enzymes called chalcone isomerase (CHI) and isoflavone synthase (IFS) led to the synthesis of the basic chromophore of isoflavonoids, and the subsequent actions of other enzymes led to the synthesis of their subclasses. Other articles provide additional information, such as the actions of specific enzymes and intermediates involved in each step (Jung et al. 2000; Yu and McGonigle 2005).

Millettia is a genus of over 260 species in the legume family. *Millettia* species, as members of the legume family, are known to be rich sources of several

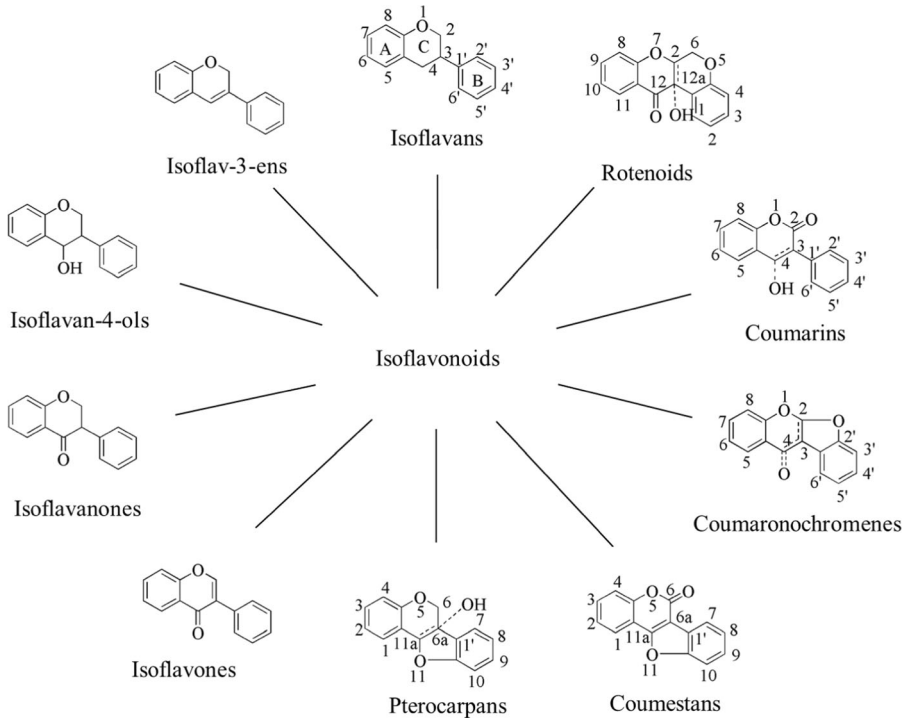
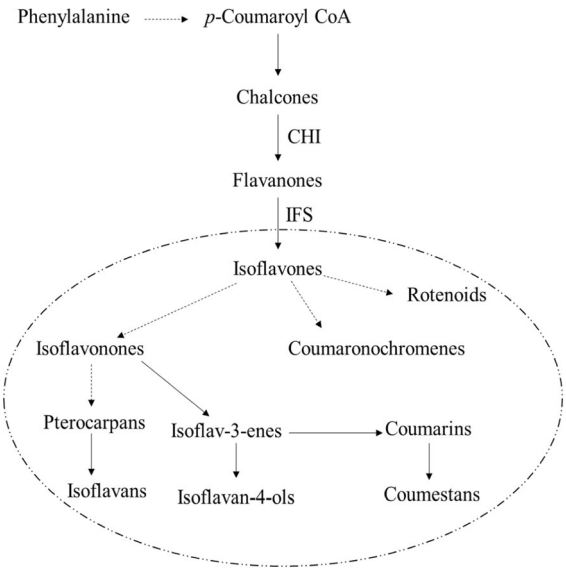


Fig. 1 Structural chromophores and classification of isoflavonoids. Isoflavones, isoflavanones, isoflavan-4-ols and isoflav-3-ens follow a similar carbon numbering as isoflavans



Scheme 1 General biosynthetic pathways of isoflavonoids in legumes CHI: Chalcone isomerase; IFS: isoflavone synthase

classes of isoflavonoids. Many of the *Millettia* species are widely distributed in tropical and subtropical regions and have been used in traditional medicine to treat a variety of human and other animal ailments. For instance, the different tissues of *Millettia dura* are used to treat menstrual irregularities in different parts of Africa (Ngameni et al. 2013). Likewise, *M. pachycarpa* is used to control agricultural pests and for the treatment of cancer in several Asian countries (Ningombam et al. 2017; Roy and Bharti 2020). Previously, several review papers documented the medicinal uses and geographical distributions of *Millettia* species (Banzouzi et al. 2008; Jena et al. 2020). In recent years, there has been much interest in isolating metabolites from *Millettia* species and evaluating their pharmacological properties. This is partly because of technological advancements in the analysis of medicinal plants (Fitzgerald et al. 2020). In contrast, only a few review papers covering the progress of *Millettia* isoflavonoid research are available. Furthermore, the literature lacks depth in demonstrating the structural diversity and characterization of *Millettia* isoflavonoids. For instance, a recent review on the genus *Millettia* by Jena et al. (2020) covered less than 30 individual isoflavonoids and barely highlighted their structural diversity and

pharmacological properties. Another review covered only two *Millettia* species, *M. dura* and *M. ferruginea,* and the isoflavonoids were barely characterized (Buyinza et al. 2020). Because of their abundance in *Millettia* species, isoflavonoids require separate consideration to provide a clear picture of their structural diversity, pharmacological properties, and advancements in their extraction and isolation processes. Therefore, a more comprehensive review that takes into account a large number of *Millettia* species could provide a better understanding of the structural diversity, distribution, and pharmacological properties of *Millettia* isoflavonoids. Furthermore, such a review could identify the research gaps and spark further research into the potential and applications of *Millettia* species and their isoflavonoids in drug discovery and medical applications. Accordingly, we focused on nine popular *Millettia* species, including *M. brandisiana*, *M. griffithii*, *M. extensa*, *M. dielsiana*, *M. dura*, *M. griffoniana*, *M. nitida*, *M. pachycarpa*, and *M. usaramensis*, and found a total of 154 isoflavonoids isolated from their various tissues over the last three decades (1990–2021). The structural diversity, distributions, extraction and isolation protocols, and pharmacological properties of these isoflavonoids are all covered in this review.

Data extraction and methodology

Scientific search engines such as PubMed, Google Scholar, Web of Science, SciFinder, and Scopus were used to find and collect literature. In addition, some journal databases were searched, including Science Direct, Springer Link, and Wiley Online. Several search words and terms, including 'isoflavonoids', '*Millettia'* (accompanied by species names including *brandisiana, griffithii, extensa, dielsiana, dura*, *griffoniana, nitida, pachycarpa, and usaramensis*), 'phytochemistry', 'pharmacology', 'bioactivity' and their combinations, were used to extract the target literature. Only articles written in English and published between January 1990 and December 2021 were kept, while preprints and unpublished papers were excluded.

Structural diversity of *Millettia* isoflavonoids

In the past three decades alone, at least 154 isoflavonoids have been isolated from the different tissues of these *Millettia* species. These include several classes and subclasses of isoflavonoids, including isoflavans, isoflavones (aglycones, methoxylated, prenylated, glycosylated, and geranylated), rotenoids, pterocarpans and coumaronochromenes. Table 1 summarizes the classes, names, and sources of these 154 isoflavonoids, and Figs. 2, 3, 4, 5, 6 and 7 show their structures. The scientific names of the isoflavonoids according to the International Union of Pure and Applied Chemistry (IUPAC) guidelines can be found in Supplementary Table S1.

Isoflavans

Isoflavans are characterized by the absence of C = C and/or C = O bonds at the *C*-ring. Three isoflavans, (*3R*)-isovesitol (**1**), (*R*)-vestitol (**2**), and (*S*)-vestitol (**3**), have been isolated thus far, signifying the rarity of these classes of isoflavonoids in the *Millettia* species (Dat et al. 2019; Liao et al. 2013). Interestingly, (*R*)-vestitol and (*S*)-vestitol are enantiomers of each other and were isolated from the stems of two different species (*M. nitida* and *M. dielsiana,* respectively) (Fig. 2, Table 1). Brazilian red propolis, a resinous material produced by honeybees, has recently been identified as an excellent source of vestitol and isovestitol. Several studies established the botanical origin of this material to be *Dalbergia ecatosphyllum*, a species in the legume family (Bueno-Silva et al. 2013; Franchin et al. 2016). As a result, some *Millettia* species may serve as alternative sources of these molecules. Furthermore, due to their diverse pharmacological activities, both (*R*)-vestitol and (*S*)-vestitol are synthetically targeted (Ciesielski & Metz 2020; Luniwal and Erhardt 2011; Yalamanchili et al. 2018). Despite these signs of progress, studies on the relative pharmacological activities of these molecules are scarce, which could provide a research opportunity.

Isoflavones

Isoflavones, as opposed to isoflavans, have both C = C and C = O bonds at the *C*-ring and are the most diverse class of isoflavonoids isolated from these *Millettia* species. A total of 112 isoflavones were reported, with

Table 1 List and class of isoflavonoids isolated from the different tissues of *Millettia* species

Isoflavonoid class	Compound no	Compound name	Source		Collection country	References
			Millettia Species	Plant part		
Isoflavan	1	(3*R*)-Isovesitol	*M. nitida*	Vine stem	China	Liao et al. (2013)
	2	(3*R*)-Vestitol	*M. nitida*	Vine stem	China	Liao et al. (2013)
	3	(3*S*)-Vestitol	*M. dielsiana*	Stem	Vietnam	Dat et al. (2019)
Isoflavone (Aglycones)	4	Daidzein	*M. nitida; M. dielsiana*	Vine stem; stem	China, Vietnam	Liao et al. (2013); Gong et al. (2009)
	5	Genistein	*M. nitida,*	Unspecified	China	Ye et al. (2012a, b)
	6	Prunetin	*M. nitida*	Vine stem	China	Liao et al. (2013)
	7	2'-hydroxygenistein	*M. nitida*	Vine stem	China	Liao et al. (2013)
	8	Cladrastin	*M. dielsiana*	Stem	Vietnam	Gong et al. (2009)
	9	Formononetin	*M. nitida*	Vine stem	China	Liao et al. (2013)
	10	7,3'-Dihydroxyl-5'-methoxyisoflavone	*M. nitida; M. griffithii*	Vine stem; stem	China	Liao et al. (2013) Tang et al. (2016)
	11	7-Hydroxy-8,3',4'-trimethoxyisoflavone	*M. usaramensis*	Root bark	Kenya	Deyou et al. (2015)
	12	Robustigenin	*M. brandisiana*	Leaves	Thailand	Pancharoen et al. (2008)
	13	Olibergin A	*M. brandisiana*	Leaves	Thailand	Kikuchi et al. (2007)
	14	Afromosin	*M. dielsiana*	Stem	Vietnam	Gong et al. (2009)
	15	Maxamaisoflavone-D	*M. dura*	Seed pod	Kenya	Yenesewe et al. (1996)
	16	Maximaisoflavone-H	*M. dura*	Seed pod	Kenya	Yenesewe et al. (1996)
	17	Cuneatin methylether	*M. griffoniana*	Root bark	Cameroon	Yankep et al. (1997)
	18	Odorantin	*M. griffoniana*	Root bark	Cameroon	Yankep et al. (2003); Yankep et al. (1997)
	19	8-Methylretusin	*M. nitida; M. brandisiana*	Whole plant; roots	China, Thailand	Ye et al. (2012a, b); Pailee et al. (2019)
	20	Calycosin	*M. nitida*	Whole plant	China	Ye et al. (2012a, b)
	21	Caviunin	*M. dielsiana*	Stem	Vietnam	Gong et al. (2009)
	22	Gliricidin	*M. nitida*	Whole plant	China	Ye et al. (2012a, b)
	23	7,2′-Dimethoxy-4′,5′-methylenedioxyisoflavone	*M. dura*	Root barks	Kenya	Marco et al. (2017)
	24	7-Hydroxy-2',4',5'-trimethoxyisoflavone	*M. pachycarpa*	Seeds	China	Tu et al. (2020)
	25	Maximaisoflavone G	*M. griffoniana, M usaramensis*	Root bark, Stem bark	Cameroon, Kenya	Yankeb et al. (2001); Yenesew eta. (1998)
	26	7-Hydroxy-6-methoxy-3',4'-Methylenedioxyisoflavone	*M. griffoniana*	Root bark	Cameroon	Yankeb et al. (2001)

Table 1 continued

Isoflavonoid class	Compound no	Compound name	Source		Collection country	References
			Millettia Species	Plant part		
	27	3'-Methylorobol	*M. extensa, M. nitida*	stems	Thailand, China	Raksat et al. (2018); Ye et al. (2012a, b)
Isoflavones (Glycosylated)	28	Mildiside A	*M. dielsiana*	stem	Thailand	Kikuchi et al. (2007)
	29	Ononin	*M. dielsiana*	stem	Thailand	Kikuchi et al. (2007)
	30	Dalpatin	*M. dielsiana*	vine stems	China	Gong et al. (2014)
	31	Odoratin-7-*O*-glucopyranoside	*M. dielsiana*	vine stems	China	Gong et al. (2014)
	32	Genistin	*M. dielsiana, M. nitida*	vine stems	China	Gong et al. (2014); Ye et al. (2012a, b)
	33	Glycitin	*M. dielsiana*	vine stems	China	Gong et al. (2014)
	34	Daidzin	*M. dielsiana*	vine stems	China	Gong et al. (2014)
	35	Wistin	*M. dielsiana*	vine stems	China	Gong et al. (2014)
	36	Hirsutissimiside A	*M. nitida*	Stem	China	Cheng et al. (2005)
	37	Hirsutissimiside C	*M. nitida*	Stem	China	Cheng et al. (2005)
	38	Hirsutissimiside B	*M. nitida*	Stem	China	Cheng et al. (2005)
	39	Sphaerobioside	*M. nitida*	Unspecified	China	Ye et al. (2012a, b)
	40	Formononetin-7-*O*-*β*-D-apiofuranosyl-(1, 6)–*O*-D-glucopyranoside	*M. nitida*	Unspecified	China	Ye et al. (2012a, b)
	41	Millesianin G	*M. dielsiana*	vine stems	China	Gong et al. (2014)
	42	Millesianin F	*M. dielsiana*	vine stems	China	Gong et al. (2014)
	43	Lanceolarin	*M. nitida*	Unspecified	China	Ye et al. (2012a, b)
Isoflavones (Prenylated)	44	5-Hydroxy-7-methoxy-4'-*O*-(3-methylbut-2-enyl)isoflavone	*M. extensa*	Stems, roots	Thailand	Raksat et al. (2018, 2019)
	45	5,7,5'-Trimethoxy-4'-*O*-prenylisoflavone	*M. griffithii*	stems	China	Tang et al. (2016)
	46	Brandisianin A	*M. brandisiana*	Leaves	Thailand	Pancharoen et al. (2008)
	47	7,4'-Di-*O*-prenylgenistein	*M. extensa; M. brandisiana*	Stems, roots; Leaves	Thailand Thailand	Raksat et al. (2018, 2019); Pancharoen et al. (2008)
	48	Viridiflorin	*M. brandisiana*	Leaves	Thailand	Pancharoen et al. (2008); Kikuchi et al. (2007)
	49	Predurallone	*M. dura*	Seed pod	Kenya	Yenesewe et al. (1996)
	50	Furowanin B	*M. pachycarpa*	Leaves	Japan	Ito et al. (2006)
	51	Millesianin H	*M. dielsiana*	stems	China	Ye et al. (2014)
	52	Millesianin A	*M. dielsiana*	Vine stems	Vietnam	Gong et al. (2009)
	53	Millesianin I	*M. dielsiana*	stems	China	Ye et al. (2014)

Table 1 continued

Isoflavonoid class	Compound no	Compound name	Source		Collection country	References
			Millettia Species	Plant part		
	54	Brandisianin C	*M. brandisiana*	Leaves	Thailand	Kikuchi et al. (2007)
	55	Brandisianin E	*M. brandisiana*	Leaves	Thailand	Kikuchi et al. (2007)
	56	6,8-Diprenyorobol	*M. extensa;*	roots	Thailand	Raksat et al. (2019)
	57	Millewanins G	*M. pachycarpa*	Leaves	Japan	Ito et al. (2006)
	58	Millewanins H	*M. pachycarpa*	Leaves	Japan	Ito et al. (2006)
	59	Griffonianone C	*M. griffoniana*	Root, stem; Root bark/seed pod	Camerroon	Wanda et al. (2006, 2007)
	60	Millexatin A	*M. extensa*	stems	Thailand	Raksat et al. (2018)
	61	Millipurone	*M. extensa*	Leaves	Thailand	Raksat et al. (2019)
	62	Millexatin B	*M. extensa*	stems	Thailand	Raksat et al. (2018)
	63	Millexatin C	*M. extensa*	stems	Thailand	Raksat et al. (2018)
	64	Millexatin D	*M. extensa*	Roots	Thailand	Raksat et al. (2019)
	65	Millexatin E	*M. extensa*	stems	Thailand	Raksat et al. (2018)
	66	Millexatin F	*M. extensa*	stems	Thailand	Raksat et al. (2018)
	67	Millexatin G	*M. extensa*	Leaves	Thailand	Raksat et al. (2019)
	68	Millexatin H	*M. extensa*	Leaves	Thailand	Raksat et al. (2019)
	69	Millexatin I	*M. extensa*	Roots	Thailand	Raksat et al. (2019)
	70	Millexatin J	*M. extensa*	Leaves	Thailand	Raksat et al. (2019)
	71	Millesianin B	*M. dielsiana*	Vine stems	Vietnam	Gong et al. (2009)
	72	Millesianin C	*M. dielsiana*	Vine stems	Vietnam	Gong et al. (2009)
	73	Millesianin D	*M. dielsiana*	Vine stems	Vietnam	Gong et al. (2009)
	74	Millesianin E	*M. dielsiana*	Vine stems	Vietnam	Gong et al. (2009)
	75	Ferrugone	*M. dura*	Seed pod	Kenya	Yenesew et al. (1997)
	76	Brandisianin D	*M. brandisiana*	Leaves	Thailand	Kikuchi et al. (2007)
	77	Auriculatin	*M. extensa*	Stems; leaves	Thailand	Raksat et al. (2018, 2019)
	78	Scandenone	*M. extensa; M. pachycarpa*	Roots, stems; seeds	Thailand, China	Raksat et al. (2018, 2019); Ye et al. (2012a, b)
	79	Auriculasin	*M. extensa*	Stems; leaves	Thailand	Raksat et al. (2018, 2019)
	80	Isoauriculasin	*M. extensa*	Roots, stems	Thailand	Raksat et al. (2018, 2019)
	81	Isoauroculatin	*M. extensa*	Stems, roots	Thailand	Raksat et al. (2018, 2019)
	82	2'-deoxyisoauriculatin	*M. extensa*	Stems, roots	Thailand	Raksat et al. (2018, 2019)

Table 1 continued

Isoflavonoid class	Compound no	Compound name	Source		Collection country	References
			Millettia Species	Plant part		
	83	Durallone	*M. dielsiana; M. dura*	Stems; Seed pods	China, Kenya	Ye et al. (2014); Yenesewe et al. (1996)
	84	6-Methoxycalpogonium isolfavone A	*M. dielsiana; M. dura*	Stems; seed pods	China, Kenya	Ye et al. (2014); Yenesew et al. (1997)
	85	Calopogoniumisolfavone A	*M. dura; M. dielsiana*	Seed pod; stems	Kenya, China	Yenesewe et al. (1996); Ye et al. (2014)
	86	6-Demethyldurallone	*M. dura*	Seed pod	Kenya	Yenesewe et al. (1996)
	87	Hydroxy-6-methoxy-3–4-methylenedioxy-8–3-3-Dimethylallylisoflavone	*M. dielsiana*	stems	China	Ye et al. (2014)
	88	Elongatin	*M. extensa*	stems	Thailand	Raksat et al. (2018)
	89	4'-Methylalpinumisofiauone	*M. extensa*	stems	Thailand	Raksat et al. (2018)
	90	5-*O*-methyl-4'-*O*-(3-methyl-2-butenyl)alpinumisoflavone	*M. extensa*	Leaves	Thailand	Raksat et al. (2019)
	91	Barbigerone	*M. pachycarpa, M. pachycarpa; M. usaramensis; M. dielsiana*	Seeds; Stem bark; Stems	China, Kenya, Vietnam	Ye et al. (2010); Tu et al. (2019); Yenesew et al. (2003b); Ye et al. (2014); Gong et al. (2009)
	92	Isoerythrinin-A-4′-(3-methylbut-2-enyl) ether	*M. dura*	Seed pod; Root barks	Kenya	Yenesewe et al. (1996); Marco et., al. (2017)
	93	2'-*O*-Methylisoauriculatin	*M. extensa*	stems	Thailand,	Raksat et al. (2018)
	94	Durmillone	*M. griffoniana; M. dielsiana; M. dura*	Root bark; Stems; Seed pod	Cameroon, China, Kenya	Yankep et al. (2003); Yankep et al. (1997); Ye et al. (2014); Yenesewe et al. (1996)
	95	Calopogoniumisoflavone B	*M. griffoniana*	Root barks	Cameroon, Kenya	Yankep et al. (1997); Marco et al. (2017)
	96	Jamaicin	*M. usaramensis; M. dura; M. griffoniana*	Root bark; Seed pods; root bark	Kenya, Cameroon	Deyou et al. (2015); Yenesew et al. (1997); Yankep et al. (1997)
	97	Ichthynone	*M. dielsiana*	Stems	China	Ye et al. (2014)
	98	6'',6''-Dimethyl-5-hydroxy-3'-methoxy-4'-hydroxypyrano[2,'',3'':7,6]isoflavone	*M. pachycarpa*	Seeds	China	Ye et al. (2012a, b)
	99	4',5'-Dimethoxy-6,6-dimethylpyranoisoflavone	*M. pachycarpa*	Seeds	China	Tu et al. (2019)

Table 1 continued

Isoflavonoid class	Compound no	Compound name	Source		Collection country	References
			Millettia Species	Plant part		
	100	4'-Demethyltoxicarol isoflavone	*M. brandisiana*	leaves	Thailand	Kikuchi et al. (2009)
	101	Toxicarolisoflavone	*M. brandisiana*	Leaves	Thailand	Pancharoen et al. (2008)
	102	6'',6''-Dimethyl-5-hydroxy-3',4'-dimethoxypyrano[2'',3'':7,6]isoflavone	*M. pachycarpa*	Seeds	China	Ye et al. (2012a, b)
	103	Milletenone A	*M. pachycarpa*	Seeds	China	Tu et al. (2019)
	104	*cis*-3'',4''-Dihydro-3'',4''-Dihydroxylonchocarpusone	*M. Pachycarpa*	Seeds	China	Tu et al. (2020)
	105	Griffonianone B	*M. griffoniana*	Root bark	Cameroon	Yankeb et al. (2001)
	106	Brandisianin B	*M. brandisiana*	Leaves	Thailand	Kikuchi et al. (2007)
	107	Milletenol A	*M. Pachycarpa*	Seeds	China	Tu et al. (2020)
	108	Norisojamaicin	*M. usaramensis*	Stem bark	Kenya	Yenesew et a. (1998)
	109	Maximaisoflavone B	*M. dura*	Root barks	Kenya	Marco et al. (2017)
Isoflavones (Geranylated)	110	7-*O*-Geranylformononetin	*M. griffoniana*	Root bark; seed pods	Cameroon	Yankep et al. (1997); Wanda et al. (2006)
	111	3',4'-Dihydroxy-7-*O*-[(*E*)-3,7-dimethyl-2,6-octadienyl]isoflavone	*M. griffoniana*	Root bark	Cameroon	Yankep et al. (1997)
	112	7-*O*-Geranylpseudobaptigenin	*M. griffoniana*	Root bark	Cameroon	Yankep et al. (1997)
	113	Griffonianone D	*M. griffoniana*	Root bark	Cameroon	Yankep et al. (2003)
	114	4'-*O*-Geranylisoliquiritigenin	*M. griffoniana*	Root bark/seed pod	Cameroon	Wanda et al. (2006)
	115	4'-Methoxy-7-*O*-[(E)-3-methyl-7-hydroxymethyl-2,6-octadienyl]isoflavone	*M. griffoniana*	Root bark/seed pod	Cameroon	Wanda et al. (2006)
Rotenoids	116	12a-Hydroxyrotenone	*M. pachycarpa; M. brandisiana*	Seeds; Roots	China, Thailand	Tu et al. (2019); Pailee et al. (2019)
	117	Villosinol	*M. brandisiana*	Roots	Thailand	Pailee et al. (2019)
	118	Tephrosin	*M. brandisiana; M. dura; M. pachycarpa; M. usaramensis*	Roots; seed pods, seeds; seeds; Root bark	Thailand, Kenya, China	Pailee et al. (2019); Yenesew et al. (1997, 2003b); Tu et al. (2019); Ye et al. (2008; 2012a, b); Deyou et al. (2015)
	119	α-Toxicarol	*M. brandisiana*	Leaves	Thailand	Pancharoen et al. (2008)
	120	12a-Hydroxy-α-toxicarol	*M. brandisiana*	Leaves	Thailand	Pancharoen et al. (2008)
	121	6a,12a-Dehydro-α–toxicarol	*M. brandisiana; M. extensa*	Leaves; stems	Thailand	Pancharoen et al. (2008); Raksat et al., 2018

Table 1 continued

Isoflavonoid class	Compound no	Compound name	Source		Collection country	References
			Millettia Species	Plant part		
	122	6-Hydroxy-6a,12a-dehydro-α -toxicarol	*M. brandisiana*	Leaves	Thailand	Pancharoen et al. (2008)
	123	6a,12a-Dehydrosermundone	*M. brandisiana*	Leaves	Thailand	Pancharoen et al. (2008)
	124	Stemonal	*M. brandisiana*	Leaves	Thailand	Pancharoen et al. (2008)
	125	Sermundone	*M. brandisiana*	Leaves	Thailand	Pancharoen et al. (2008)
	126	6-Deoxyclitoriacetal	*M. brandisiana*	Leaves	Thailand	Pancharoen et al. (2008)
	127	Deguelin	*M. dura, M. pachycarpa*	Seeds	Kenya, China	Yenesew et al. (2003a); Tu et al. (2019); Ye et al. (2008; 2012a, b)
	128	Millettone	*M. dura*	Seed pod	Kenya	Yenesew et al. (1997)
	129	Millettosin	*M. dura; M. usaramensis*	Seed pod; Root bark	Kenya, China	Yenesew et al. (1996); Deyou et al. (2015)
	130	Rotenone	*M. dura*	Seeds	Kenya	Yenesew et al. (2003a)
	131	Sumatrol	*M. extensa*	stems	Thailand	Raksat et al. (2018)
	132	Usararotenoid A	*M. usaramensis*	Root bark; Stem bark		Deyou et al. (2015); Yenesew et al. (2003b)
	133	Usararotenoid C	*M. usaramensis*	Root bark; Stem bark	Kenya	Deyou et al. (2015); Yenesew et al. (2003b)
	134	12-Dihydrousararotenoid A	*M. usaramensis*	Root bark	Kenya	Deyou et al. (2015)
	135	12-Dihydrousararotenoid B	*M. usaramensis*	Root bark	Kenya	Deyou et al. (2015)
	136	13-Homo13-oxa-6a,12a-dehydrodeguelin	*M. pachycarpa*	Seeds	China	Ye et al. (2010, 2012a, b)
	137	6a,12a-Dehydrodeguelin	*M. pachycarpa*	Seeds	China	Ye et al. (2008, 2012a, b)
	138	6a,12a-Dehydromillettone	*M. usaramensis*	Stem bark	Kenya	Yenesew et al. (2003b)
	139	12α-Hydroxy-12-dihydro-(+)-usararotenoid-A	*M. usaramensis*	Stem bark	Kenya	Yenesew et al. (1998)
	140	12-Dihydrousararotenoid C	*M. usaramensis*	Root bark	Kenya	Deyou et al. (2015)
	141	Epimilletosine	*M. usaramensis*	Root bark; Stem bark	Kenya	Deyou et al. (2015); Yenesew et al. (2003b)
	142	*trans*-4',5'-Dihydro-4',5'-dihydroxytephrosin	*M. pachycarpa*	Seeds	China	Tu et al. (2020)
	143	(+)-Usararotenoid B	*M. usaramensis*	Stem bark	Kenya	Yenesew et al. (1998)
	144	Griffonianone A	*M. griffoniana*	Root bark	Cameroon	Yankeb et al. (2001)
	145	*cis*-4',5'-Dihydro-4',5'-dihydroxytephrosin	*M. pachycarpa*	Seeds	China	Tu et al. (2020)

Table 1 continued

Isoflavonoid class	Compound no	Compound name	Source		Collection country	References
			Millettia Species	Plant part		
Coumarin	146	4-Hydroxy-5,6,7-trimethoxy-3-(3',4'-Methylenedioxy)phenylcoumarin	*M. griffoniana*	Root bark	Cameroon	Yankeb (1998)
Pterocarpans	147	Brandisianin F	*M. brandisiana*	Leaves	Thailand	Kikuchi et al. (2007)
	148	(-)-Medicarpin	*M. brandisiana*	Roots	Thailand	Pailee et al. (2019)
	149	(-)-Maackiain	*M. brandisiana; M. extensa; M. nitida*	Roots; stems	Thailand, China	Pailee et al. (2019; 2019); Ye et al. (2012a, b)
	150	Eryristagallin	*M. extensa*	Leaves	Thailand	Raksat et al. (2019)
	151	3-*O*-Prenylmaackiain	*M. dura*	Root barks	Kenya	Marco et al. (2017)
Chromanochromenes	152	Millexatin K	*M. extensa*	Roots	Thailand	Raksat et al. (2019)
	153	Millexatin L	*M. extensa*	Roots	Thailand	Raksat et al. (2019)
	154	Millexatin M	*M. extensa*	Roots	Thailand	Raksat et al. (2019)

24 being aglycones (**4**–**27**), 16 being glycosylated (**28**–**43**), 66 being prenylated (**44**–**109**), and the remaining 6 being geranylated (**110**–**115**) isoflavones.

Aglycone isoflavones

As previously stated, 24 aglycones (**4**–**27**) were reported from these *Millettia* species. Except for *M. extensa* and *M. pachycarpa*, each of the other species had at least two aglycone isoflavones isolated from their different tissues, indicating their abundance. Among the various tissues of these *Millettia* species, the greatest number of aglycones were isolated from the stem parts (Table 1). With the exceptions of daidzein (**4**), genistein (**5**), and 2'-hydroxygenistein (**7**), the remaining aglycones were either mono- (9 compounds), di- (6 compounds) or poly- (6 compounds) methoxylated. Figure 2 also demonstrates the structural diversity of aglycone isoflavones, and one can easily observe that methylation can occur at several positions, including 5, 6, 7, and 8 (*A*-ring) and 2', 3', 4', 5' and 6' (*B*-ring) of *Millettia* isoflavones. In addition, some of these molecules, such as **15**–**18**, **23**, **25**, and **26,** contain an additional methylenedioxy substituent, which adds to their structural diversity. In general, methylation of flavonoids is thought to increase their bioavailability and chemopreventive effects (Walle 2009). As a result of these discoveries, the laboratory synthesis of naturally occurring methoxylated isoflavones, including those isolated from *Millettia* species, has become a research priority intending to study structure–activity relationships (SARs). For example, formononetin (**9**), the simplest of all methoxylated isoflavones, is synthetically known and has been used as a substrate to synthesize several analog derivatives (Mutai et al. 2015). Recently, Tay et al. (2019) published a review that revealed formononetin's diverse pharmacological activities, including anticancer properties. Similarly, the synthesis and pharmacological potentials of other aglycone isoflavones, such as genistein (**5**), prunetin (**6**), and robustigenin (**12**), have been reported (Li et al. 2006; Nakayama et al. 1980). Despite these successes, methylation of positions 5 and 6' and hence the laboratory synthesis of their derivatives remains a challenge. This is due in part to the energy barrier of such molecules as a result of their stability, as well as the possibility of chelation of these positions to the carbonyl oxygen at the *C*-ring (Dixon 19,993). In this

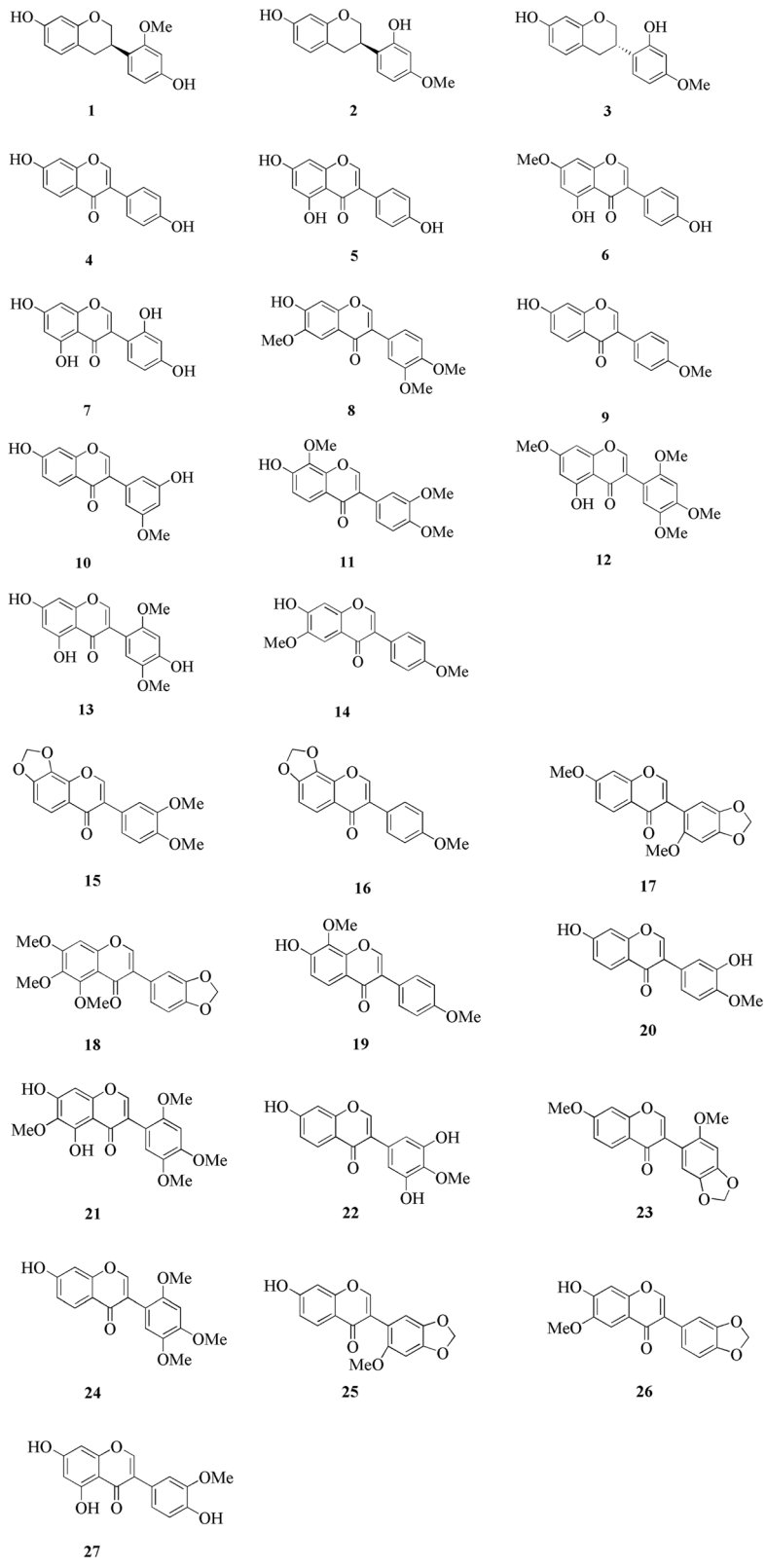


Fig. 2 Chemical structures of isoflavans (**1**–**3**) and aglycone isoflavones (**4**–**27**) isolated from *Millettia* species

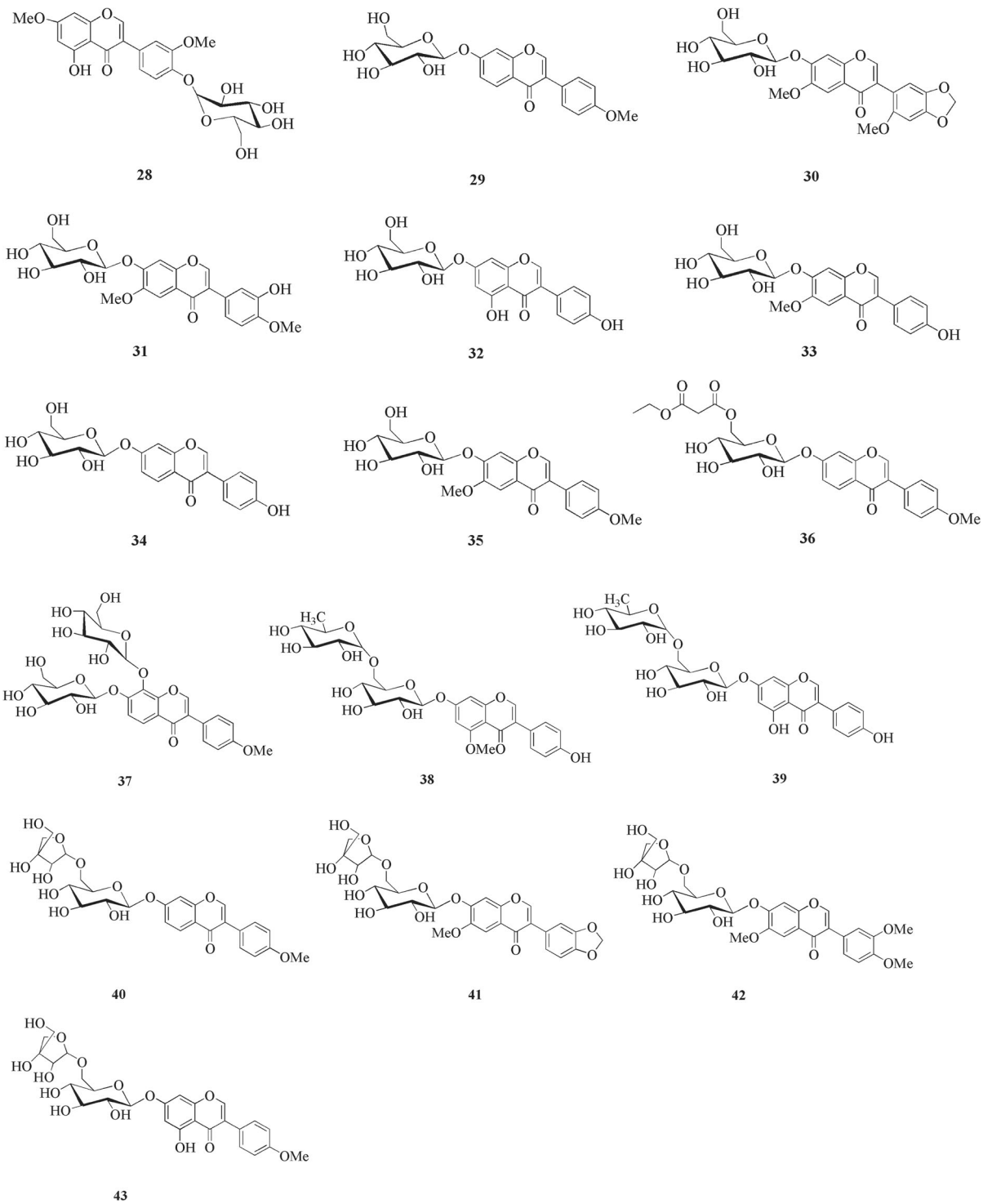


Fig. 3 Glycosylated isoflavonoids isolated from *Millettia* species

regard, the isolation of cuneatin methylether (**17**), a 5-methoxy isoflavone, and odoratin (**19**), a 6'-methoxy isoflavone, from the root bark of *M. griffoniana* could open up a new range of possibilities for studying the enzymatic synthesis of such molecules for SAR studies (Chebil et al. 2006; Yankep et al. 1997; 2003). Furthermore, a 5-*O*-methyltransferase enzyme responsible for the biosynthesis of such classes of isoflavonoids in other legumes, such as lupin, was purified (Khouri et al. 1988). This could open up new avenues for research into the specific candidate genes responsible for the biosynthesis of such isoflavonoids in *Millettia* species.

Glycosylated isoflavones

Glycosylated isoflavones contain one or more sugar units in their structure. A total of 16 glycosylated isoflavones (**28**–**43**) were reported from these *Millettia* species (Fig. 3). Structurally, position 7 was found to be the glycosylation site in all of the molecules except for mildiside A (**28**) and hirsutissimiside C (**37**), which were glycosylated at positions 4' and 8, respectively. Apiosyalted molecules (**40–43**), one of the synthetically rare glycosylated isoflavones, were also reported (Gong et al. 2014; Ye et al. 2012a, b). Among all the *Millettia* species, the stems of *M. dielsiana* and *M. nitida* were found to be rich sources of glycosylated isoflavones (Table 1). Genistin (**32**), a chemoprotective isoflavone abundant in soybeans together with glycitin (**33**) and daidzin (**34**), was also isolated from the stem vines of these two species (Gong et al. 2014; Ye et al. 2012a, b). Glycosylation is one of the most important factors considered in modifying the solubility, bioavailability, and therapeutic potential of bioactive molecules of synthetic and natural origin (Hanh et al. 2020; Szeja et al. 2017). As a result, the synthesis of glycosylated isoflavones has long been a research focus (Szeja et al. 2017). In this regard, attempts have been made to synthesize many of the glycosylated isoflavones isolated from *Millettia* species, including dalpatin (**30**), a 5'-methoxylated molecule, although some are inefficient for commercialization (Lewis et al. 1998). To the best of our knowledge, efficient laboratory synthesis protocols for hirsutissimisides A-C (**36–38**) and millesianins F-G (**41**, **42**) have yet to be reported.

Prenylated isoflavones

A prenyl structure denotes the *3*,*3*-dimethyl allyl unit, and isoflavones containing one or more of this substituent are known as prenylated isoflavones (Santos and Silva 2020; Simons et al. 2012). These classes of isoflavones are the most diverse classes of isoflavones found in the *Millettia* species and were isolated from the leaves, stems, roots, and seed parts (Fig. 4, Table 1). Among these plant tissues, the maximum number of prenylated isoflavones was isolated from the stems, followed by leaves. They were also structurally diverse. Among the 66 prenylated isoflavones (**44**–**109**) reported from the different tissues of these species, 41 molecules were *mono*-prenylated, and 23 were *di*-prenylated (Fig. 4). In addition, two tri-prenylated isoflavones, millexatin A (**60**) and millipurone (**61**), were isolated from the leaves of *M. extensa*. Such tri-prenylated isoflavonoids have also rarely been reported in other legumes (Veitch 2013). The prenyl group can also be linear or cyclic (such as pyran or furan), and it can be *O*-prenylated or *C*-prenylated. In this regard, positions 4′, 6, 7, and 8 were found to be potential prenylation sites. Furthermore, many of the prenylated molecules are methoxylated, proving the structural diversity and complexity of *Millettia* isoflavones. Such structural diversity could provide an excellent opportunity for SAR research (Kalli et al. 2021). Prenylation is thought to increase the lipophilic nature of organic molecules, which improves their affinity toward protein interactions in biological membranes (Botta et al. 2009; Simons et al. 2012; Veitch 2013). Because of these factors, several naturally occurring prenylated isoflavones have been synthesized (Mukne et al. 2011). It is worth noting that some of the prenylated isoflavonoids from *Millettia* species, including the newly discovered milletenone A (**103**), are synthetically unknown and have received little attention in SAR research (Tu et al. 2019). As a result, such molecules could provide a great research opportunity and a clue to the enzymatic synthesis of prenylated isoflavones in laboratories (Mora-Pele et al. 2013).

Geranylated isoflavones

Geranylated isoflavones contain a C_{10}-isoprenoid substituent called geranyl ([*E*]-3, 7-dimethyl-2,6-octadienyl unit). The pyrophosphate derivative of this

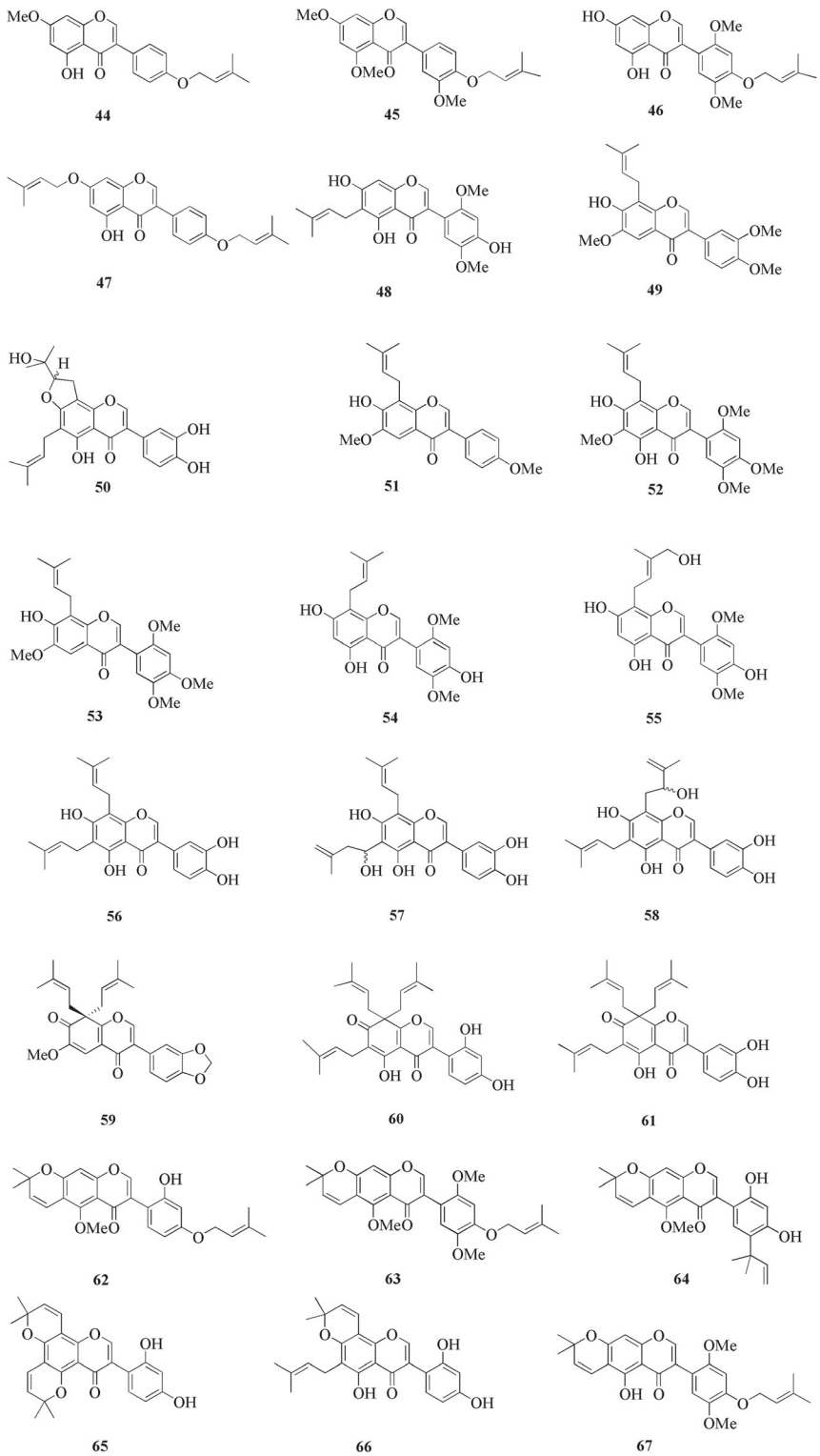


Fig. 4 Prenylated isoflavonoids isolated from *Millettia* species

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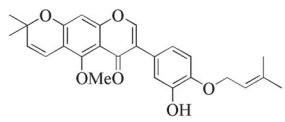

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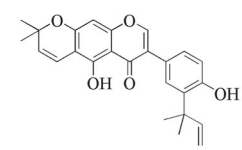

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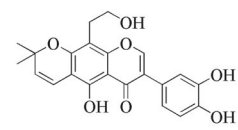

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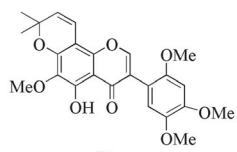

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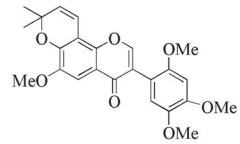

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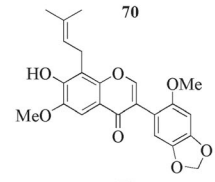

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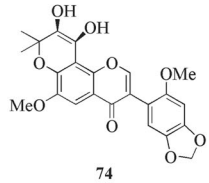

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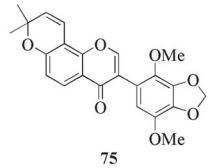

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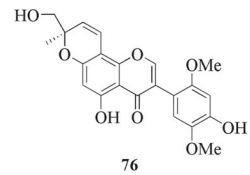

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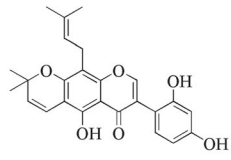

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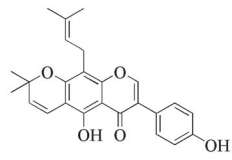

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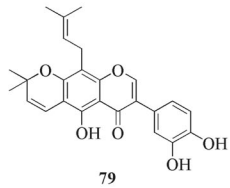

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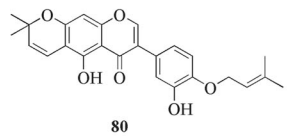

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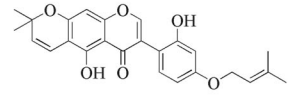

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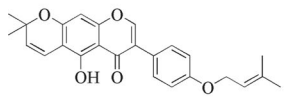

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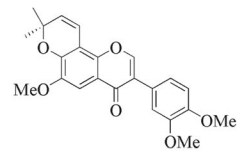

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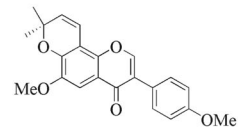

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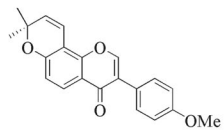

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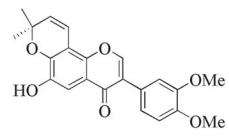

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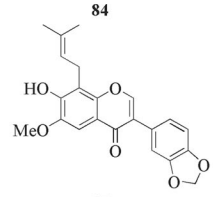

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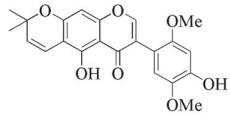

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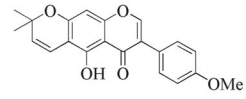

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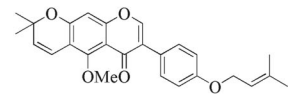

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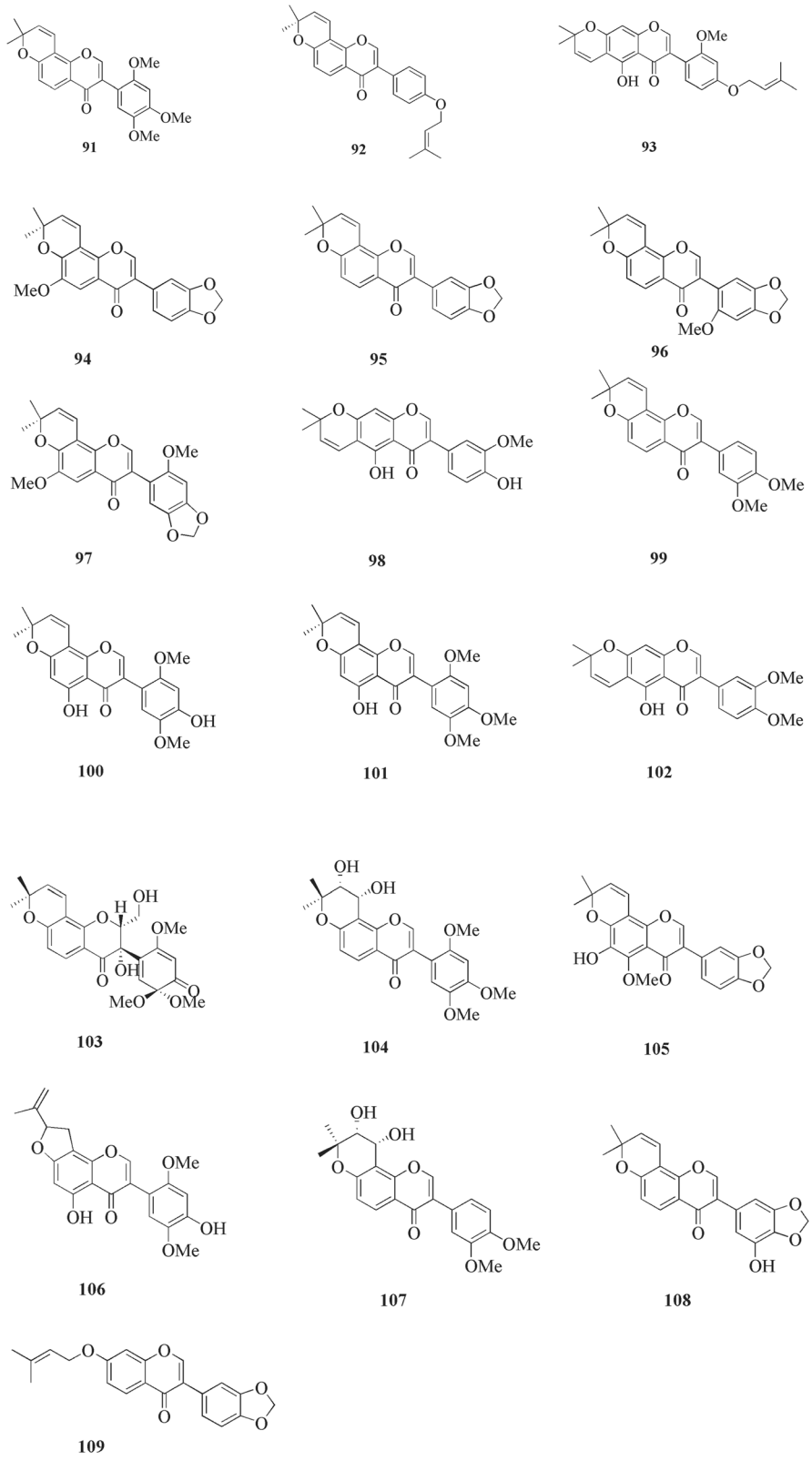

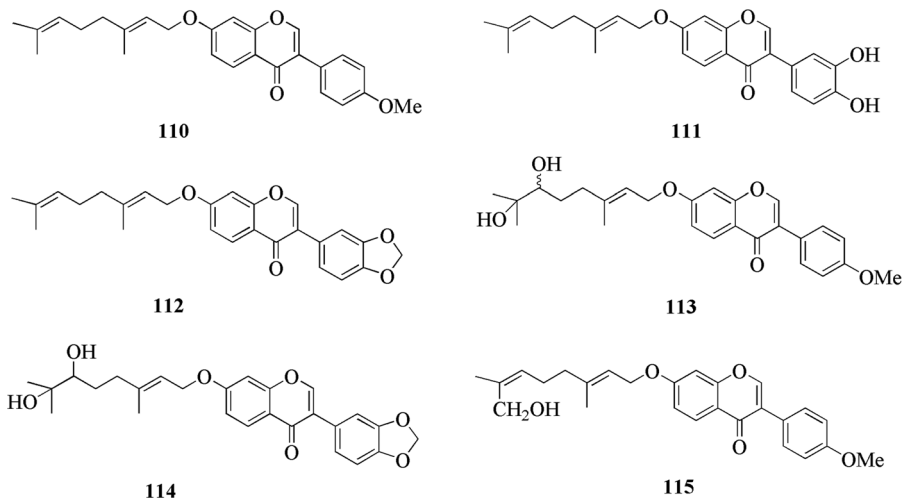

Fig. 5 Geranyl isoflavonoids isolated from *Millettia* species

substituent is an intermediate in the phenylpropanoid pathway, and its bioavailability heavily influences the distribution of geranylated isoflavones in plants. In comparison to the aforementioned classes of isoflavones, geranylated isoflavones were rarely observed in these *Millettia* species, with only six isolated. Structurally, all were exclusively 7-*O*-geranylated (Fig. 5) and derived from the root bark or seed pods of *M. griffoniana* (Wanda et al. 2006; Yankep et al. 1997, 2003). This is unlike *Caragan pruinosa*, another legume species known for *C*-geranlylated isoflavonoids (Al-Maharik 2019). There are also other legume species, such as those in the genus *Campylotropis,* where *C*-geranylated isoflavones are widely distributed (Al-Maharik 2019; Felpin et al. 2007; Sun et al. 2015). Several geranylated isoflavones have been synthesized. For example, the synthesis of 7-*O*-geranylformonentin (**110**) and its dihydroxylation product, Griffonianone D (**113**), has been reported, demonstrating the importance of *Millettia* isoflavones in drug discovery (Felpin et al. 2007; Selepe and Heerden 2013). However, the pharmacological properties of many of these isoflavonoids have rarely been investigated, and this could be a promising future research area.

Rotenoids

Rotenoids are another class of isoflavonoids widely distributed in *Millettia* species. A total of 30 structurally diverse rotenoids (**116–145**) were isolated in these *Millettia* species alone and are widely distributed across the different tissues of these species, including leaf, stem, root and seed parts (Table 1). *Millettia* species' rotenoids are structurally diverse, containing methoxy, prenyl, or methylenedioxy substituents, and two or more of these groups in their structures (Fig. 6). Tephrosin (**118**) is the most abundant rotenoid in the *Millettia* species and was isolated from *M. brandisiana* roots, *M. dura* seeds and seed pods, *M. pachycarpa* seeds and seed pods, and *M. usaramensis* root bark (Deyou et al. 2015; Pailee et al. 2019; Tu et al. 2019; Ye et al. 2012a, b; Yenesew et al. 1997, 2003a, 2003b). This molecule is also synthetically known, having been used as a lead molecule in the synthesis of several analogs and having been reported to have a variety of pharmacological properties, making it ideal for structural modification and SAR analysis (Xu et al. 2018). Another popular rotenoid, rotenone (**130**), was isolated from the seeds of *M. dura*. Synthetically, it is used as a precursor in the preparation of villosinol (**117**) (Russell et al. 2018). Likewise, sumatrol (**131**) and deguelin (**127**) are popular rotenoids that have been used in the synthesis of pharmacologically active analogs (Russell et al. 2018; Xu et al. 2018). Interestingly, millettosine (**129**) and epimilletosine (**141**) are synthetically known diasteriomers isolated from root barks of *M. usaramensis* (Deyou et al. 2015; Perveen et al. 2019; Yenesew et al. 2003b). The former was also isolated from *M. dura* seeds, while the latter was isolated from *M. usaramensis* stem bark (Deyou et al.

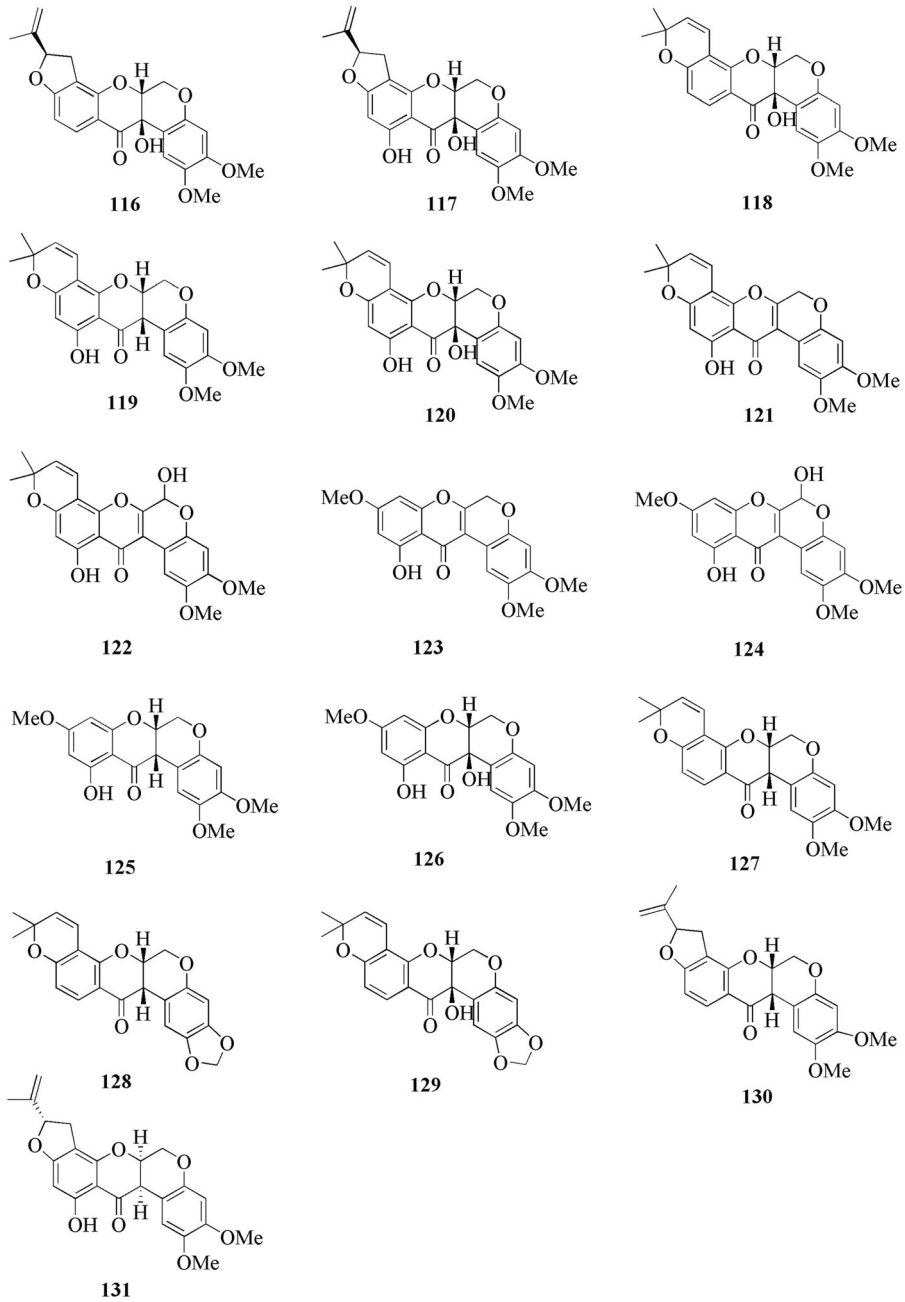

Fig. 6 Rotenoids isolated from *Millettia* species

2015; Yenesew et al. 1996). A structurally unique rotenoid, 13-homo13–oxa-6a,12a-dehydrodeguelin (**136**), was also isolated from the seeds of *M. pachycarpa* (Ye et al. 2012a, b, 2010). In general, *Millettia* rotenoids are structurally diverse, which could lead to exciting multidisciplinary research opportunities in the future.

Phenylcoumarins

Coumarins, also known as 1, 2-benzopyrones, are another important class of isoflavonoids and are widely distributed in fruits such as cherries and berries (Kumar et al. 2021; Wu et al. 2009). The only phenylcoumarin isolated from the *Millettia* species

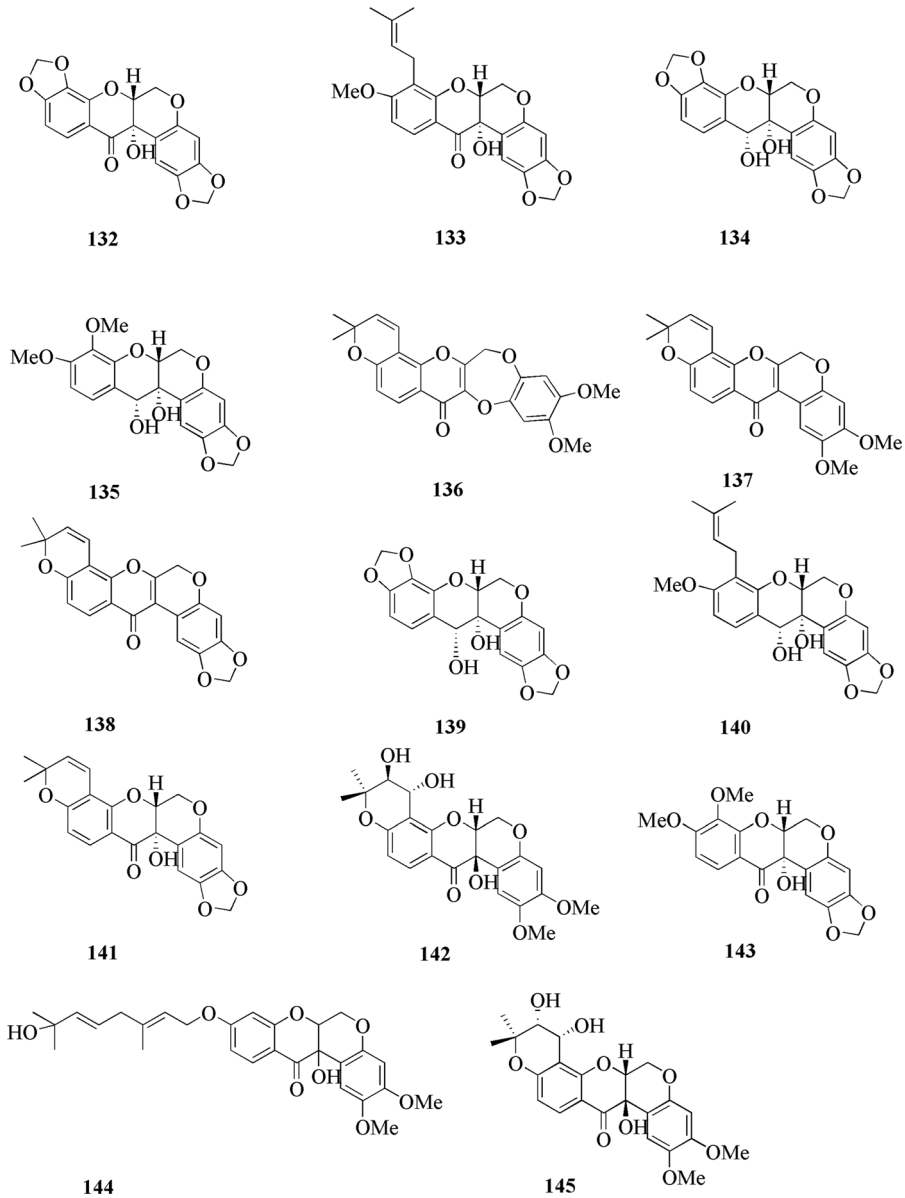

Fig. 6 continued

considered in this review was 4-hydroxy-5, 6, 7-trimethoxy-3-(3',4'-methylenedioxy)phenyl-coumarin (**146**) (Fig. 7). The molecule was isolated from the root bark of *M. griffoniana* (Yankeb et al. 1998), and the discovery could point to the rarity of such subclasses of isoflavonoids in *Millettia* species.

Pterocarpans

This review discovered only five pterocarpans (**147**–**151**) reported from the nine species considered to signify their rarity in the genus *Millettia* (Fig. 7). This is in contrast to other genera in the legume family, such as *Erythrina,* which are known for having a high concentration of such isoflavonoids in their tissues (Fahmy et al. 2018). Maackiain (**149**) was isolated from *M. brandisiana* and *M. extensa* roots, as well as

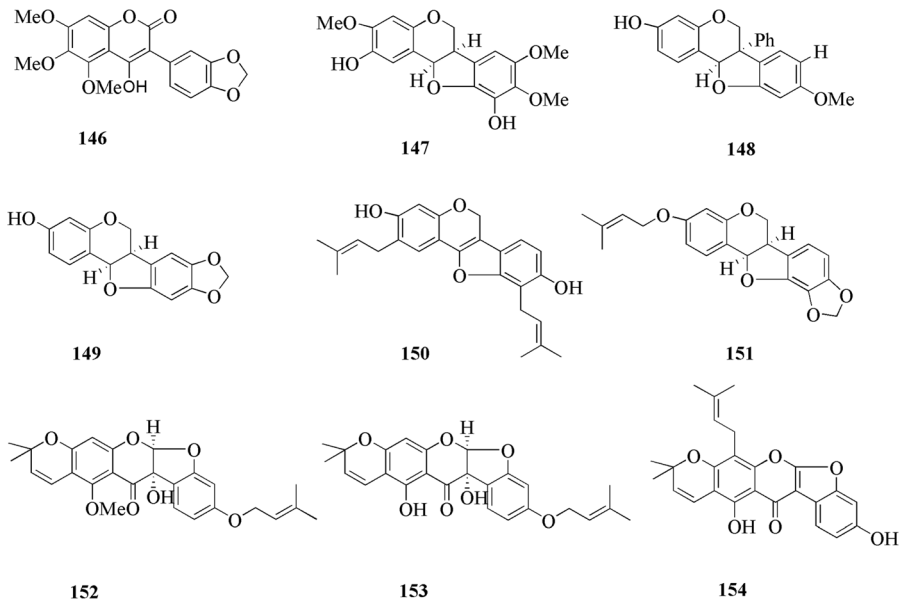

Fig. 7 Coumarin (**146**), pterocarpans (**147–151**) and coumaronochromenes (**152–154**) isolated from *Millettia* species

M. nitida stems (Pailee et al. 2019; Raksat et al. 2018; Ye et al. 2012a, b). On the other hand, (-)-medicarpin (**148**) and brandisianin F (**147**) were isolated from the leaves and roots of *M. brandisiana*, whereas erycristagallin (**150**) and 3-*O*-prenylmaackiain (**151**) were isolated from *M. extensa* leaves and *M. duara* root bark, respectively (Kikuchi et al. 2007; Marco et al. 2017; Pailee ete al. 2019; Raksat et al. 2019). Natural pterocarpans such as (-)-maackiain (**149**) and (-)-medicarpin (**148**) have been synthetically targeted (Feng et al. 2015; Goel et al. 2013; Ozaki et al. 1989; Yang et al. 2017). The laboratory synthesis of other *Millettia* pterocrapans, such as eryrcristagalin (**150**), a di-prenylated pterocrapan, could be of great interest.

Coumaronochromenes

Coumaronochromenes are also uncommon in *Millettia* species (Fig. 7). This review also found only three molecules: millexatin K (**152**), millexatin L (**153**), and millexatin M (**154**). All of these molecules were isolated from *M. extensa* roots (Raksat et al. 2019). A targeted investigation of other tissues of the plant or species could provide detailed information about the diversity of coumaronochromenes in *Millettia* species.

Extraction and isolation of isoflavonoids from *Millettia* species

The choice of an ideal solvent system, as well as appropriate extraction and isolation techniques, are important factors in determining the efficiency of plant metabolite extraction and isolation (Ivanovic et al. 2020). Because isoflavonoids are polar molecules, they are typically extracted with pure or mixtures of polar organic solvents. Many studies conducted on *Millettia* species also used polar solvents such as ethanol, methanol, chloroform, dichloromethane, acetone, and ethyl acetate (Table 2). Furthermore, liquid–liquid extraction procedures were also used to obtain a variety of extracts with varying polarities. Among all the solvents, the most widely used solvent system was 95% ethanol, followed by dichloromethane and chloroform. In a rare case, Yankeb et al. (1997) isolated geranylated (**110**, **112**) and prenylated (**95**) isoflavones from hexane-crude extracts of *M. griffoniana* root bark. Except for a few studies, direct extraction of the plant parts was conducted without a predefatting step (Yankep 2003). This indicates that isoflavonoid-rich extracts can be obtained from *Millettia* species without the need for an additional defatting step.

During extraction, maceration at room temperature was the most widely used technique (Cheng et al. 2005; Dat et al. 2019; Kikuchi et al. 2007; Pancharoen

Table 2 Common solvents and extraction methods used to obtain isoflavonoid-rich extracts from *Millettia* species

Solvent used/ Extraction condition	Isolation method	Class of isoflavonoid	References
Methanol/RT	RP-HPLC	Prenylated isoflavones (**46**, **54**, **106**)	Kikuchi et al. (2007)
Ethanol/RT	RP-HPLC	Prenylated isoflavones (**91**, **78**, **102**, **98**); Rotenoid (**136**)	Ye et al. (2012a)
	CCC	Rotenoid (**127**, **118**, **137**); Prenylated isoflavone (**99**)	Ye et al. (2012a)
	CC, VLC, P-TLC	Aglycone isoflavones (**12**); Prenylated isoflavones (**46**, **47**, **101**, **48**); Rotenoid (**119–126**)	Pancharoen et al. (2008)
95% Ethanol/RT	Silica gel packed CC	Isoflavan (**3**)	Dat et al. (2019)
		Aglycone isoflavones (**9**)	Dat et al. (2019)
		Glycosylated isoflavones (**28**, **29**, **37**, **40**)	Cheng et al. (2005); Dat et al. (2019)
		Prenylated isoflavones (**53**, **91**, **94**, **84**, **85**, **87**, **72**)	Ye et al. (2014)*
	Sephadex LH-20 packed CC	Glycosylated isoflavones (**36, 37**)	Cheng et al. (2005)
		Aglycone isoflavone (**10**)	Tang et al. (2016)
	CCC/HSCCC	Prenylated isoflavones (**45**,**45**, **83**, **97**, **99**, **73**) Rotenoid (**127**, **118**,**137**)	Ye et al. (2014)*; Ye et al (2012a); Tang et al. (2016)*; Ye et al. (2008; 2012a)
	SemiP-HPLC	Prenylated isoflavone (**91**)**;** Rotenoid (**136**)	Ye et al. (2010)
			Ye et al. (2010)
95% Ethanol/Reflux	P-HPLC	Prenylated isoflavones (**52**)	Gong et al. (2009)
	ODS-CC	Aglycone isoflavone (**21**)	
	Silica gel-CC	Prenylated isoflavones (**72–74**); Aglycone isoflavones (**14**, **8**, **4**)	
	HSCCC	Rotenoids (**118**, **127**, **137**); Prenylated isoflavones (**99**)	Ye te al. (2008)
	Sephadex LH-20 CC and P-HPLC	Aglycone isoflavone (**4–6**)	Liao et al. (2013)
		Isoflavan (**3**)	
	P-HPLC	Aglycone isoflavone (**9**)	Liao et al. (2013)
	P-HPLC	Glycosylated isoflavones (**30–35**, **41**, **42**)	Gong et al. (2014)
	Sephadex LH-20 CC	Glycosylated isoflavone (**35**)	
95% Ethanol/ Ultrasonic bath, RT	Toyopearlgel HW-40F CC	Prenylated isoflavone (**99**, **103**)	Tu et al. (2019)*
		Rotenoids (**118**, **127**, **116**)	
	P-TLC	Prenylated isoflavone (**107**, **104**)	Tu et al. (2020)
		Rotenoids (**142**, **145**)	Tu et al. (2020)
	Toyopearl gel HW-40F column chromatography	Aglycone isoflavone (**24**)	Tu et al. (2020)
	P-HPLC	Prenylated isoflavone (**55, 107**, **104**); Rotenoid (**116–118**); Pterocarpans (**147–149**)	Tu et al. (2020)
CHCl3/cold percolation	Silica-CC	Prenylated isoflavone (**49**, **75**, **83–86, 92**, **94–96**); Aglycone isoflavone (**9, 15**,**16**); Rotenoids (**127–129**, **118**, **137, 128**, **118**)	Yenesew et al. (1997); Yenesew et al. (2003b); Yenesew et al. (1996)

Table 2 continued

Solvent used/ Extraction condition	Isolation method	Class of isoflavonoid	References
Chloroform/RT (defatted with hexane)	VLC-silica gel	Prenylated isoflavone (**94**); Aglycone isoflavone (**18**)	Yankeb et al. (2003)
	Sephadex LH-20 CC	Geranylated isoflavone (**113**)	Yankeb et al. (2003)
	VLC-silica gel and P-TLC	Aglycone isoflavone (**18**, **23**); Prenylated isoflavone (**94, 96**)	Yankeb et al. (1997)
	Silica gel-CC	Prenylated isoflavone (**59**); Geranylated isoflavone (**114**, **112**, **111**, **115**)	Wanda et al. (2006)
	Silica-CC, Prep-TLC	Geranylated isoflavone (**115**, **111**); Coumarins (**146**)	Yankeb et al. (1998)
	Silica-CC, Prep-TLC	Rotenoids (**144**); Prenylated isoflavones (**59**, **105**); Aglycone isoflavone (**26**)	Yankeb et al. (2001)
	P-TLC	Prenylated isoflavones (**91**)	Tu et al. (2019)*
Dichloromethane	Silica-CC	Rotenoids (**132**, **133**, **141**, **138**); Prenylated isoflavones (**91**); Geranylated isoflavones (**114**)	Yenesew et al. (2003b)
	Sephadex-LH-20 CC	Prenylated isoflavone (**108**, **96**, **91**); Aglycone isoflavone (**25**)	Yenesew et al. (1998)
Dichloromethane/RT	P-HPLC	Aglycone isoflavone (**19**)	Pailee et al. (2019)
CH_2Cl_2/MeOH (1:1),	CC-silica gel	Rotenoids (**118**, **135**, **133**, **129**); Aglycone isoflavones (**16**)	Deyou et al. (2015)
	Sephadex LH-20	Rotenoids (**132**, **134, 140**); Aglycone isoflavones (**16**)	
	RP-HPLC	Rotenoid (**141**, **133**, **118**)	
	P-TLC	Aglycone isoflavone (**11**)	
Dichloromethane/ methanol (1:1 v/v)/cold percolation	CC-silica gel	Prenylated isoflavone (**83–85, 96**, **94**, **97**); Aglycone isoflavone (**9**)	Buyinza et al. (2021)
CH_2Cl_2/CH_3OH (1:1),	CC on Sephadex LH-20; preparative HPLC	Pterocarpans (**151**)	Marco et al. (2017)
	Silica gel-CC	Prenylated isoflavone (**95**, **109**, **92**)	
	RP-HPLC	Prenylated isoflavone (**94**)	
	CC on silica gel	Aglycone isoflavones (**23**, **11**)	
Acetone/RT Acetone/RT	Silica gel-CC	Prenylated isoflavone (**44**, **47**, **56**, **60–66**, **77**, **78**, **79**, **88**, **93**, **80**, **81**, **89**); Aglycone isoflavones (**27**); Rotenoid (**121**); Pterocarpans (**149**)	Raksat et al. (2018)
	Silica-CC, P-TLC	Prenylated isoflavone (**50**, **57**, **58**)	Ito et al. (2006)
EtOAc by cold percolation	Silica-CC	Rotenoids (**132**, **143**)	Yenesew et al. (1998)
Ethyl acetate	Silica gel-CC	Prenylated isoflavones (**44**, **47**, **60–62**, **64**, **66–70**, **78**, **80–82, 90**); Chromanochromenes (**152–154**); Pterocarpans (**150**)	Raksat et al. (2019)
n-hexane/RT	VLC-silica gel, CC and P-TLC	Geranylated isoflavones (**110**, **112**); Prenylated isoflavones (**95**)	Yankeb et al. (1997)

*Bioassay-guided isolations/fractionations

et al. 2008; Tang et al. 2016; Ye et al. 2010, 2012a). Other techniques, such as reflux, sonication, and cold percolation, were also used to obtain isoflavonoid-rich crude extracts (Table 2). For the isolation of individual molecules, the thin-layer chromatography (TLC)-guided column chromatography (CC) technique was mostly used, and components were eluted using mixtures of both polar and nonpolar organic solvents. Silica-gel packed CC was the most popular isolation technique, which may be due to its low cost and ease of application. Moreover, there were occasions where resins such as Sephadex LH-20 and Toyopearlgel HW-40F were used in place of silica gel (Cheng et al. 2005; Gong et al. 2014; Liao et al. 2013; Tu et al. 2020). Individual isoflavonoids have also been isolated using other techniques, such as countercurrent chromatography (CCC), high-speed countercurrent chromatography (HSCCC), and vacuum liquid chromatography (VLC) (Pancharoen et al. 2008; Tang et al. 2016; Ye et al. 2014, 2008, 2012a). Preparative high-performance liquid chromatography (P-HPLC) was another popular isolation technique used to isolate individual isoflavonoids. P-HPLC is more efficient than column chromatography methods and can isolate even small amounts of metabolites from bulk matrices (Deyou et al. 2015; Gong et al. 2009, 2014; Marco et al. 2017; Tu et al. 2020). Many of the studies combined two or more of the aforementioned isolation techniques, either in a bioassay-guided or untargeted isolation of *Millettia* isoflavonoids (Liao et al. 2013; Marco et al. 2017; Pancharoen et al. 2008; Ye et al. 2014; Tang et al. 2016). In general, our findings indicated that no single extraction technique or solvent system was applied to extract or isolate a specific class of isoflavonoids in *Millettia* species. Future studies on *Millettia species* could benefit from the use of more efficient and recently developed extraction and isolation techniques, such as supercritical fluid extraction, microwave-assisted extraction, pressurized liquid extraction, and enzyme-assisted extraction (Fitzgerald et al. 2020; Ivanovic et al. 2020).

Structural characterization techniques

The structural characterizations of isoflavonoids isolated from the various tissues of *Millettia* species include determining molecular weight, chromophore structure, and substituents. Several chromatographic and spectrometric techniques, including high-performance liquid chromatography (HPLC), one-dimensional (^{1}H-NMR and ^{13}C-NMR) and two-dimensional (COSY, HMBC, and HSQC) nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS), infrared spectroscopy (IR), and ultraviolet–visible spectroscopy (UV–Vis), have been used to achieve these goals (Dat et al. 2019; Tang et al. 2016; Tu et al. 2019; Yankeb et al. 2001). Nonspectrometric techniques such as *X*-ray crystallography, circular dichroism (CD) and computational methods have also been used to help determine chiral center configurations (Liao et al. 2013; Marco et al. 2017; Pailee et al. 2019). Because of their sensitivity and application in complex mixtures, hyphenated techniques such as LC–MS/MS, LC-NMR, and LC–MS-NMR have recently gained popularity for the structural analysis of plant metabolites (Gathungu et al., 2020). As a result, the use of such techniques in future studies could aid in the identification of more *Millettia* isoflavonoids, as well as their isolation and pharmacological investigation.

Pharmacological activities of *Millettia* isoflavonoids

The chemical structure of isoflavonoids influences their pharmacokinetic properties, which in turn determines their bioavailability and biological activities (Vitale et al. 2012). Numerous studies have been conducted to investigate the pharmacological properties of *Millettia* isoflavonoids. Owing to their structural diversity, several disease-protecting properties, including antibacterial, anti-inflammatory, anticancer, and estrogenic activities, among others, have been reported. The sections that follow summarize the pharmacological properties of *Millettia* isoflavonoids. Table 3 summarizes the active compounds and their biological activities, as well as their concentration giving 50% inhibition (IC_{50}) and/or minimum inhibitory concentration (MIC).

Anti-inflammatory activities

Several in vitro and in vivo studies have shown that the various classes of *Millettia* isoflavonoids have anti-inflammatory properties. In vitro studies with macrophage cells (RAW264.7) showed that (*3S*)-vestitol (**3**), Brandisianin A (**46**), scandenone (**78**), diprenyorobol (**56**), *cis*-3'',4''-Dihydro-3'',4''-

Table 3 Biological activities of isoflavonoids isolated from *Millettia* species

Biological activity	Compound name	Assay/Model	Effect (MIC, IC_{50})	Reference
Anti-inflammatory activity	Scandenone	RAW264.7 Macrophages	Inhibition of NO-production (IC_{50}: 8.5 μM)	Raksat et al. (2019)
	6,8-Diprenyorobol	RAW264.7 Macrophages	Inhibition of NO-production (IC_{50}: 14.3 μM)	Raksat et al. (2019)
	Brandisianin A	RAW264.7 Macrophages	Inhibition of NO-production (IC_{50}: 35.7 μM)	Tang et al. (2016)
	Griffoninaone D	PLA2 and TPA-induced mouse ear Edema (Female Wistar rat)	Inhibition of swelling (0.25 mg/ear, 5 mg/kg)	Yankep et al. (2003)
	(3*S*)-vestitol	RAW264.7 Macrophages	Inhibition of NO-production (IC_{50}: 16.0 μM)	Dat et al. (2019)
	6-Deoxyclitoriacetal	Male Sprague–Dawley rats	inhibited the ear edema formation (1 mg/ear)	Pancharoen et al. (2008)
	α-Toxicarol	Male Sprague–Dawley rats	inhibited the ear edema formation (1 mg/ear)	Pancharoen et al. (2008)
	cis-3'',4''-Dihydro-3'',4''-Dihydroxylonchocarpusone	RAW264.7 macrophages	Dose dependent inhibition of NO-production	Tu et al. (2020)
	Milletenol A	RAW264.7 macrophages	Dose dependent inhibition of NO-production	Tu et al. (2020)
Anticancer activity	Griffonianone C	MCF-7 cells	Upregulation of mRNA expression of Ki-67 (10^{-8} M) and CD1 (10^{-7} M)	Wanda et al. (2006)
	Usararotenoid A	MDB-MB-231 cancer Cells	Cytotoxic (IC_{50}: 87.3 μM)	Deyou et al. (2015)
	Millettosin	MDB-MB-231 cancer Cells	Cytotoxic to (IC_{50}: 61.7 μM)	Deyou et al. (2015)
	12a-Epimillettosin	MDB-MB-231 cancer Cells	Cytotoxic (IC_{50}: 100.7 μM)	Deyou et al. (2015)
	Usararotenoid C	MDB-MB-231 cancer Cells	Cytotoxic (IC_{50}: 25.7 μM)	Deyou et al. (2015)
	4'-O-geranylisoliquiritigenin	MDB-MB-231 cancer Cells	Cytotoxic (IC_{50}: 125.5 μM)	Deyou et al. (2015)
	Brandisianin B	HeLa cancer Cells	Cytotoxic (IC_{50}: 9.7 μM)	Kikuchi et al. (2007)
	Brandisianin C	HeLa cancer Cells	Cytotoxic (IC_{50}: 19.1 μM)	Kikuchi et al. (2007)
	Brandisianin E	HeLa cancer Cells	Cytotoxic (IC_{50}: 21.7 μM)	Kikuchi et al. (2007)
	Barbigerone	HepG2, C26, LL2 B16 cancer cell lines	Induction of aptosis (HepG2 (IC_{50}: 0.61 μM), C26 (IC_{50}: 7.81 μM), LL2 (IC_{50}: 0.36 μM), and B16 (IC_{50}: 5.47 μM)	Ye et al. (2012a, b)

Table 3 continued

Biological activity	Compound name	Assay/Model	Effect (MIC, IC_{50})	Reference
	Deguelin	HepG2, C26, LL2, B16 cancer cell lines	Induction of aptosis (HepG2 (IC_{50}: 1.55 μM), C26 (IC_{50}: 8.93 μM), LL2 (IC_{50}: 0.51 μM), and B16 (IC_{50}: 0.20 μM)	Ye et al. (2012a, b)
	3-Homo13-oxa-6a,12a-dehydrodeguelin	HepG2, C26, LL2, B16 cancer cell lines	Induction of aptosis (HepG2 (IC_{50}: 17.48 μM), C26 (IC_{50}: 10.10 μM), LL2 (IC_{50}: 3.33 μM), and B16 (IC_{50}: 10.15 μM)	Ye et al. (2012a, b)
	Tephrosin	HepG2, C26, LL2, B16 cancer cell lines	Induction of aptosis (HepG2 (IC_{50}: 1.41 μM), C26 (IC_{50}: 6.49 μM), LL2 (IC_{50}: 0.56 μM), and B16 (IC_{50}0: 15.95 μM)	Ye et al. (2012a, b)
	6a,12a-dehydrodeguelin	HepG2, C26, LL2, B16 cancer cell lines	Induction of aptosis (HepG2 (IC_{50}: 2.93 μM), C26 (IC_{50}: 7.55 μM), LL2 (IC_{50}: 1.35 μM), and B16 (IC_{50}: 8.85 μM)	Ye et al. (2012a, b)
	Maximaisoflavone B	MDA-MB-231 breast cancer cells	Cytotoxic (IC_{50}: 153.5 μM)	Marco et al. (2017)
	7,2′-Dimethoxy-4′,5′-dimethylenedioxy isoflavone	MDA-MB-231 breast cancer cells	Cytotoxic (IC_{50}: 174.1 μM)	Marco et al. (2017)
	Durmillone	A549 cancer cell line	Cytotoxic (IC_{50}: 6.6 ± 1.2 mM)	Buyinza et al. (2021)
	Jamaicin	A549 cancer cell line	Cytotoxic (IC_{50}: 11.4 ± 5.0 mM)	Buyinza et al. (2021)
Antibacterial activity	Millexatin A	Bacterial strains	Growth inhibition: *S. aureus, S. epidermidis* and *B. subtilis* (2 μg/mL); *S. typhyimurium and Ps. aeruginosa* (128 μg/mL)	Raksat et al. (2018)
	Millexatin F	Bacterial strains	Growth inhibition: *S. aureus, S. epidermidis,* and *B. subtilis* (2 μg/mL); *S. typhyimurium and Ps. aeruginosa (*128 2 μg/mL)	Raksat et al. (2018)
	Auriculatin	Bacterial strains	Growth inhibition: *S. aureus, S. epidermidis,* and *B. subtilis* (2 μg/mL); *S. typhyimurium and Ps. aeruginosa* (128 μg/mL)	Raksat et al. (2018)
	3'-Methylorobol	Bacterial strains	Growth inhibition: *S. aureus* (32 μg/mL*), S. epidermidis* and *B. subtilis* (64 μg/mL*); S. typhyimurium and Ps. Aeruginosa* (128 μg/mL)	Raksat et al. (2018)
	Scandenone	Bacterial strains	Growth inhibition: *S. aureus and B. subtilis* (*2* μg/mL); *S. epidermidis* (*4* μg/mL*); S. typhyimurium and Ps. aeruginosa* (128 μg/mL)	Raksat et al. (2018)
	Auriculasin	Bacterial strains	Growth inhibition: *S. aureus and S. epidermidis* (4 μg/mL*); B. subtilis* (8 μg/mL); *S. typhyimurium and Ps. Aeruginosa* (128 μg/mL)	Raksat et al. (2018)
	2'-Deoxyauriculasin	Bacterial strains	Growth inhibition: *S. typhyimurium and Ps. aeruginosa* (128 μg/mL)	Raksat et al. (2018)

Table 3 continued

Biological activity	Compound name	Assay/Model	Effect (MIC, IC_{50})	Reference
	Isoauroculatin	Bacterial strains	Growth inhibition: *S. typhyimurium and Ps. aeruginosa* (128 μg/mL)	Raksat et al. (2018)
	Sumarol	Bacterial strains	Growth inhibition: *S. typhyimurium and Ps. aeruginosa* (128 μg/mL)	Raksat et al. (2018)
	Maackiain	Bacterial strains	Growth inhibition: *S. typhyimurium and Ps. aeruginosa* (128 μg/mL)	Raksat et al. (2018)
	Millexatin I	Bacterial strains	Growth inhibition: *M. luteus* (64), *S. mutans* (128 μg/mL), *S. epidermidis* (128 μg/mL), *S. cereus* (64 μg/mL), *S. aureus* (64 μg/mL); *S. typhimurium* (128 μg/mL), *S. flexneri* (128 μg/mL)	Raksat et al. (2019)
	Millexatin J	Bacterial strains	Growth inhibition: *M. luteus* (32 μg/mL), *S. mutans* (128 μg/mL), *S. epidermidis* (128 μg/mL), *S. typhimurium* (128 μg/mL), E. coli (128 μg/mL)	Raksat et al. (2019)
	Millexatin K	Bacterial strains	Growth inhibition: *M. luteus* (64 μg/mL), *S. mutans* (128 μg/mL), *S. epidermidis* (128 μg/mL), *S. cereus* (32 μg/mL), *S. aureus* (32 μg/mL); *S. typhimurium* (128 μg/mL)	Raksat et al. (2019)
	Millexatin L	Bacterial strains	Growth inhibition: *M. luteus* (64 μg/mL), *S. mutans* (128 μg/mL), *S. epidermidis* (128 μg/mL), *S. cereus* (32 μg/mL), *S. aureus* (32 μg/mL); *S. typhimurium* (128 μg/mL)	Raksat et al. (2019)
	Millexatin M	Bacterial strains	Growth inhibition: *S. epidermidis* (128 μg/mL), *S. cereus* (128 μg/mL), *S. aureus* (128 μg/mL); *S. typhimurium* (128 μg/mL), *S. flexneri* (128 μg/mL)	Raksat et al. (2019)
	Millexatin B	Bacterial strains	Growth inhibition: *S. epidermidis* (128 μg/mL), *S. aureus* (128 μg/mL); *S. typhimurium* (128 μg/mL),	Raksat et al. (2019)
	Millexatin D	Bacterial strains	Growth inhibition: *M. luteus* (32 μg/mL), *S. mutans* (128 μg/mL), *S. epidermidis* (128 μg/mL), *S. cereus* (8 μg/mL), S. Aureus (8 μg/mL); *S. typhimurium* (128 μg/mL), *S. aeuruginosa* (128 μg/mL), *E. coli* (128 μg/mL)	Raksat et al. (2019)
	Millipurone	Bacterial strains	Growth inhibition: *M. luteus* (2 μg/mL), *S. mutans* (16 μg/mL), *S. epidermidis* (4 μg/mL), *S. cereus* (32 μg/mL), *S. aureus* (4 μg/mL); *S. typhimurium* (128 μg/mL), *S. aeuruginosa* (128 μg/mL), *E. coli* (128 μg/mL), *S. flexneri* (128 μg/mL)	Raksat et al. (2019)
	5,7,3',4'-tetrahydroxy-6,8-diprenylisoflavone (6,8-Diprenyorobol)	Bacterial strains	Growth inhibition: *M. luteus* (16 μg/mL), *S. mutans* (16 μg/mL), *S. epidermidis* (128 μg/mL), *S. cereus* (16 μg/mL), *S. aureus* (32 μg/mL); *S. typhimurium* (128 μg/mL), *S. aeuruginosa* (128 μg/mL), *E. coli* (128 μg/mL), *S. flexneri* (128 μg/mL)	Raksat et al. (2019)

Table 3 continued

Biological activity	Compound name	Assay/Model	Effect (MIC, IC_{50})	Reference
	5-Hydroxy-7-methoxy-4'-O-(3-methylbut-2-enyl)isoflavone	Bacterial strains	Growth inhibition: *S. aureus* (32 μg/mL); *S. epidermidis* (128 μg/mL), *S. typhimurium* (128 μg/mL)	Raksat et al. (2019)
	7,4'-di-O-prenylgenistein	Bacterial strains	*S. aureus* (32 μg/mL); *S. epidermidis* (128 μg/mL), *S. typhimurium* (128 μg/mL); *S. flexneri* (128 μg/mL)	Raksat et al. (2019)
Estrogenic activity	Millewanin G	17 *β*-estradiol induced yeast	Inhibition of *β*-galactosidase activity (IC_{50}: 29 μM)	Ito et al. (2006)
	Millewanin H	17 *β*-estradiol induced yeast	Inhibition of *β*-galactosidase activity IC_{50}: 18 μM)	Ito et al. (2006)
	Furowanin B	17 *β*-estradiol induced yeast	Inhibition of *β*-galactosidase activity (IC_{50}: 13 μM)	Ito et al. (2006)
	4'-methoxy-7-O-[(E)-3-methyl-7-hydroxymethyl-2,6-octadienyl]isoflavone	Yeast strain; MVLN cells; Ishikawa cells	Induction of estradiol activity (*S. sereviseae*) (10^{-8} M); Induction of luciferase activity (10–6 M); AlkP induction (10^{-6} M)	Wanda et al. (2006)
	3',4'-dihydroxy-7-O -[(*E*)-3,7-dimethyl-2,6-octadienyl]isoflavone M	Yeast strain; MVLN cells; Ishikawa cells	Induction of estradiol activity (10–8 M); Induction of luciferase activity (10^{-5} M); AlkP induction (10^{-5} M)	Wanda et al. (2006)
	4'-O-geranylisoliquiritigenin	Yeast strain; MVLN cells; Ishikawa cells	Induction of estradiol activity (10^{-8} M); Induction of luciferase activity (10^{-6} M); AlkP induction (10^{-6} M)	Wanda et al. (2006)
	7-O-geranylformononetin	Yeast strain; MVLN cells; Ishikawa cells	Induction of estradiol activity (10^{-8} M); Induction of luciferase activity (10^{-6} M); AlkP induction (10^{-7} M)	Wanda et al. (2006)
	Griffonianone C			Wanda et al. (2007)
Anti-Alzheimer 's	Deguelin	In vitro assay	Inhibition of AChE activity (IC_{50}: 126.70 μM)	Tu et al. (2019)
	Tephrosin	In vitro assay	Inhibition of AChE activity (IC_{50}: 127.18 μM)	Tu et al. (2019)
	Barbigerone	In vitro assay	Inhibition of AChE (IC_{50}: 121.60 μM) and BChE (IC_{50}: 21.77 μM) activities	Tu et al. (2019)
	4',5-dimethoxy-6,6-dimethylpyrano isoflavone	In vitro assay	Inhibition of AChE (IC_{50}: 131.17 μM) and BChE (IC_{50}: 2.34 μM) activities	Tu et al. (2019)
Larvaecidal activity	Deguelin	Aedes aegypti larvae	Potent (IC_{50}: 1.6 μg/mL)	Yenesew et al. (2003a)
	Tephrosin	Aedes aegypti larvae	Potent (IC_{50}: 1.4 μg/mL)	Yenesew et al. (2003a)
Anticoagulative activities	Genistein	Male New Zealand white rabbits	Dose-dependent antithrobin activity	Liao et al. (2013)
	Daidzein	Male New Zealand white rabbits	Dose-dependent antithrobin activity	Liao et al. (2013)
	5,7,2',4'-tetrahydroxyisolfavone	Male New Zealand white rabbits	Dose-dependent antithrobin activity	Liao et al. (2013)

Table 3 continued

Biological activity	Compound name	Assay/Model	Effect (MIC, IC_{50})	Reference
Antioxidant activity	Robustigenin, Viridiflorin, 12a-hydroxy-a -toxicarol	TLC-spot DPPH-Assay	Radical scavenging activity	Pancharoen et al. (2008)
Antiplasmodial activity	calopogonium isoflavone B;	3D7 and the Dd2 strains of *Plasmodium falciparum*	70–90% inhibition (40 μM)	Marco et al. (2017)
	Isoerythrin A-4′-(3-methylbut-2-enyl) ether		70–90% inhibition (40 μM)	Marco et al. (2017)

Dihydroxylonchocapusone (**104**) and 6,8-milletenol A (**107**) inhibit the production of NO, one of the known inflammatory mediators (Dat et al. 2019; Pancharoen et al. 2008; Raksat et al. 2019; Tang et al. 2016; Tu et al. 2020). The IC_{50} values of these molecules ranged from 8.5 to 35.7 μM (Table 2), with scandenone being the most potent molecule (Raskat et al. 2019). Other in vivo studies have shown that griffonianone D (**113**) inhibits swelling in ATP and PLA2-induced edema assays (Yankep et al. 2003). 6-Deoxyclitoriacetal (**126**) and α-toxicarol (**119**) have also been shown to have similar activities (Pancharoen et al. 2008). Despite these promising results, many of the isolated molecules from these nine *Millettia* species have rarely been studied for their anti-inflammatory activities, suggesting that there is a huge research potential for future studies.

Anticancer activities

The anticancer activities of several *Millettia* isoflavonoids have been assessed using different kinds of cancer cells. For example, 7,2-dimethyl-4',5'-dimethylenedioxyisoflavone (**23**), maximaisoflavone B (**109**), millettosin (**129**), 4'-*O*-geranylisoliquiritigenin (**114**), usararotenoid A (**132**), usararotenoid C (**133**), griffonianone C, and 12a-epimillettosin (**141**) were found to be cytotoxic to breast cancer cells, of which the former was the most potent (IC_{50}: 25.7 μM) to MDB-MB-231 cancer cells (Deyou et al. 2015; Marco et al. 2017; Wanda et al. 2011). In other studies, brandisianin B (**106**), brandisianin C (**54**) and brandisianin E (**55**) were found to be cytotoxic to colon cancer cells, while barbigerone (**91**), deguelin (**127**), 13-homo13-oxa-6a,12a-dehydrodeguelin (**136**), tephrosin (**118**), and 6a,12a-dehydrodeguelin (**137**) were found to induce aptosis in liver, lung and colorectal cancer cells (Kikuchi et al. 2007; Ye et al. 2012a, b). A recent study by Buyinza et al. (2021) also showed the cytotoxicity of durmillone (**94**) and jamaicin (**96**) to adenocarcenomic human alveolar cancer cells at low concentrations (IC_{50}: 6.6 and 11.4 μM, respectively). These findings indicate the potential of *Millettia* isoflavonoids in cancer treatment and, as such, warrant close attention in the future. Furthermore, many of the anticancer studies were conducted in vitro, and additional in vivo studies are strongly advised to support the observed in vitro results.

Estrogenic activities

In comparison to their anti-inflammatory and anticancer activities, the estrogenic activities of *Millettia* isoflavonoids have received little attention. Ito et al. (2006) demonstrated in vitro that furowanin B (**50**), millewanin G (**57**) and millewanin H (**58**) inhibit *β*-galactosidase activity in *β*-estradiol-induced yeast cells, with the former being the most active inhibitor (IC_{50}: 13 μM). In another study, Wand et al. (2006) showed the simultaneous induction of estradiol activity and luciferase activity by 4'-methoxy-7-*O*-[(*E*)-3-methyl-7-hydroxymethyl-2,6-octadienyl]isoflavone (**115**), 3',4'-dihydroxy-7-*O*-[(*E*)-3,7-dimethyl-2,6-octadienyl]isoflavone M (**111**), 4'-O-geranylisoliquiritigenin (**114**), and 7-*O*-geranylformononetin (**110**) in different cells. These findings highlight the importance of *Millettia* isoflavonoids in combating the negative health effects of endocrine disruption. The lack of detailed studies in this area suggests a great

opportunity for future research on unstudied isolated isoflavonoids.

Antibacterial activities

Compared to the aforementioned biological activities, the antibacterial activities of the isolated *Millettia* isoflavonoids have rarely been investigated. However, two studies by Raksat et al. (2018, 2019) extensively investigated the antibacterial activities of isoflavonoids isolated from *M. extensa* and found that they inhibited the growth of both gram-positive and gram-negative bacterial strains (Table 2). For example, scandenone (**78**) inhibited the growth of *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. subtilis*) at MICs of 2 µg/mL and *S. epidermidis* at MICs of 4 µg/mL (Raksat et al. 2018). Similarly, several millexatin isoflavonoids inhibited the growth of several bacterial strains at different MIC levels (Table 3). These findings suggest that isoflavonoid-rich extracts of *Millettia* species could be used as food preservatives. Moreover, further research into the antibacterial properties of *Millettia* isoflavonoids is highly recommended.

Other pharmacological properties

In addition to the pharmacological activities mentioned above, some *Millettia* isoflavonoids were tested for other biological activities. For example, independent in vitro studies by Tu et al. (2019) and Yenesew et al. (2003a) showed the anti-Alzheimer's and larvicidal potentials of both degulin (**127**) and tephrosin (**118**), respectively (Table 2). In another study, genistein (**5**) and daidzein (**4**) demonstrated dose-dependent antithrobin activity, indicating their potential as anticoagulant molecules (Liao et al. 2013). The antioxidant activities of robustigenin (**12**), viridflorin (**48**), and 12a-hydroxy-α-toxicarol (**120**) (Pacharoen et al. 2008) and the antiplasmodial activities of calopoginiumisoflavone B (**95**) and isoerythrinin-A-4′-(3-methylbut-2-enyl) ether (**92**) (Marco et al. 2017) were among the other biological activities studied. Many rotenoids have pesticide activity and are used to control a variety of insects (Lin et al. 2016). Rotenoids isolated from *Millettia* species, such as tephrosin (**118**) and rotenone (**130**), were also discovered to possess these critical properties. In general, these findings highlight the fact that most of the isolated isoflavonoids have not been studied for various pharmacological activities and thus could be a future research focus.

Conclusion and prospects

Millettia species have received much attention in recent years because of their wide distribution, diverse pharmacological properties and rich metabolite contents. Isoflavonoids are one of the most important secondary metabolites found in *Millettia* species, and they have several disease-deterrence and health-promoting properties. This review revealed that structurally diverse classes of isoflavonoids are abundant in all plant tissues of *Millettia* species. Flowers and seed coats are rarely studied in comparison to other plant parts, so future research into these tissues is highly encouraged. Furthermore, in vivo assays and clinical trials would be critical for validating the promising pharmacological activities of *Millettia* isoflavonoids. Overall, this review summarizes the structural diversity, trends in extraction and isolation protocols, structural analysis techniques, and pharmacological properties of *Millettia* isoflavonoids, potentially paving the way for fruitful and impactful research lines in *Millettia* species and their isoflavonoids.

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Declarations

Conflict of interest The authors declare they have no conflicts of interest.

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