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A comparative review on phytochemical constituents and biological effects of *Melilotus indicus* (L.) All. and *Melilotus messanensis* (L.) All., (Fabaceae): evidence for chemosystematic analysis

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Abstract

Some *Melilotus* species have been well acknowledged in worldwide traditional medicine, where its folk uses not only heal simple ailments but also used in vital disorders. The current review considers the comparative phytochemical and biological prospective survey of *Melilotus messanensis* (L.) All. and a medicinal *Melilotus* species; *Melilotus indicus* (L.) All., aiming to highlight the medicinal significance for the former species. A chemical survey of both species revealed around 135 metabolites (phenolic acids, coumarins, flavonoids, terpenoids, saponins, fatty acid esters, and others). Also, their bioactivity studies have been directed to potential antioxidant, antidiabetic, and/or antimutagenic activities, while the folk medicinal properties, as well as the anti-microbial and anti-inflammatory assays were limited to *M. indicus*. Thus, this review draws attention to further biological research for *M. messanensis* to discover its medicinal uses. From the chemosystematics point of view, the fruit morphology and the structural chemical variations confirmed the significance of the two species. As well as their chemical resemblances with a well-known commercial medicinal species; *Melilotus officinalis* (L.) Pall., which predict the significance medicinal outcomes of *M. messanensis*.

Keywords: Chemosystematics, Fabaceae, *Melilotus indicus, Melilotus messanensis*, Pharmacological, Phytochemical

1. Introduction

Melilotus Mill. (Sweet clovers) is a genus in the family Fabaceae (=Leguminosae), subfamily Faboideae (=Papilionoideae). Various species are known as grassland plants and weeds of cultivated ground. The popularity of traditional values of sweet clovers goes back many centuries. The Ancient Egyptians reported that the prepared tea from *Melilotus* was used to treat intestinal worms and earache. The Greek physician (Galen) recommended using its infusion in wrapping for inflammations and swollen joints. Also, it is used as a reputation for preserving eyesight, in Anglo-Saxon England [1]. Moreover, *Melilotus* species are acknowledged in Ethiopian, Tibetan and Chinese traditional medicine [2–4]. *Melilotus officinalis* (L.) Pall. is a commercially medicinal *Melilotus* species, which is the main ingredient of some market drugs that are used in the treatment of many diseases [4]. The genus *Melilotus* comprises about 22 accepted species that are widely distributed in the temperate regions of the world with slight extensions into subtropics and America [5]. Among them are *Melilotus indicus* (L.) All. and *Melilotus messanensis* (L.) All. that have been selected as a subject of interest for the current review. Their comparative phytochemical and biological prospective are reviewed with the hope of predicting the medicinal and chemosystematic significance of *M. messanensis.*

2. Experimental

Literature search was accompanied from databases such as Chemical Abstracts Services (Scifinder), Google, Google Scholar, Pubmed, Science Direct and

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Scopus, in addition other sources including books, thesis and official websites. Accepted species number and names were authenticated using "The Plant List" (www.theplantlist.org). Keywords such as "*Melilotus indicus"; "Melilotus messanensis*"; "Ethnomedicinal uses"; "Folk medicine" "Phytochemistry"; "Phenolics"; "Flavonoids"; "Coumarins"; "Terpenoids"; "Fatty acids"; "Pharmacological activities"; "Antioxidant"; "Antidiabetes"; "Antimicrobial"; "Anti-inflammatory"; "Antitumor"; and "Wound-healing" were carried out. All chemical structures were drawn using ChemDraw Pro 7.0 software.

3. Folk medicine

M. indicus is used by traditional healers to treat many diseases such as asthma, hemorrhoid, bowel complaints and infantile diarrhea and lacerated wounds [6,7]. Crushed seeds are used as anthelmintic, antipyretic, for curing heart diseases, bronchitis and leprosy [7]. Also, it is externally applied as poultice or plaster on swellings [8]. *M. indicus* has some economical uses in several countries, it considered as natural substitute of synthetic agriculture fungicides [9]. Moreover, its herbal preparation extract has been used as a folk medicine in inflammation-related therapy by Africans, Asians, Arabs, and Indians [7, 10]. The fresh leafy vegetable of *M. indicus* has been used as a rich source of vitamin C (approximately 88.15 mg/100g) [11]. Whereas there are no reports of folk medicine for *M. messanensis.*

4. Phytochemical constituents

Various phytochemical compounds have been isolated and reported of both species from the whole plant, aerial parts, leaves, roots and/or seeds. A comprehensive survey of both species revealed around 135 natural metabolites. Compounds reported comprise phenolic acids (8 compounds), coumarins (10 compounds), flavonoids (34 compounds), terpenoids (32 compounds), saponins (two compounds), fatty acid esters (13 compounds), simple benzene derivatives (19 compounds) and others. Phenolic compounds are the main phytochemical group in *M. indicus* and *M. messanensis*.

Phenolic acids

The distribution of phenolic acids mainly of hydroxyl cinnamic acid derivatives is restrictive to both species and listed in (Table 1)

Table 1. The reported phenolic acids for *M. indicus* and *M. messanensis*

Compound	Organ	Species	Ref.
p-hydroxybenzoic acid (**1**)	Wp	*M. messanensis*	[12]
Vanillic acid (**2**)	Wp	*M. messanensis* *M. indicus*	[12] [13]
O-coumaric acid (**3**)	Ap	*M. indicus*	[14]
m-coumaric acid (**4**)	Wp	*M. indicus*	[13]
P-coumaric acid (**5**)	Wp	*M. messanensis*	[12]
P-coumaric acid-*O*-glucoside (**6**)	Wp	*M. indicus* *M. messanensis*	[15] [16]
Ferulic acid (**7**)	Wp	*M. indicus*	[13]
Chlorogenic acid (**8**)	Wp	*M. indicus* *M. messanensis*	[13]

Ap; Aerial parts, Wp; Whole plant

Coumarins and *chromene derivatives*

Melilotus species are well-known coumarin comprising herbs [17], the coumarin content acquired them their sweet smell trait [1] which can be confirmed through the phytochemical screening [18]. Ten coumarin derivatives were reported from the two species. The whole plant of *M. messanensis* is the only source of coumestan-type coumarin (Table 2).

Flavonoids

Flavonoids are the main phenolic constituents in the genus *Melilotus*. They comprise a wide variety of classes including flavonols, flavone, isoflavones, flavanones and dihydroflavonols, where flavonols appeared to be the most dominant class in the genus. The common flavonols found in *M. indicus* and *M. messanensis* are derivatives of kaempferol, quercetin and isorhamnetin. The *O*-glycoside linkage is mostly established at position 3. Only one flavone glycoside was isolated from the seed of *M. indicus* [8]. The *C*-glycosides are characterized for *M. messanensis* [16]. Isoflavones and isoflavanones were also informed. The classes of the reported flavonoids from the two species are collected in Table (3) and organized based on their chemical structure according to Harborne and Mabry [20].

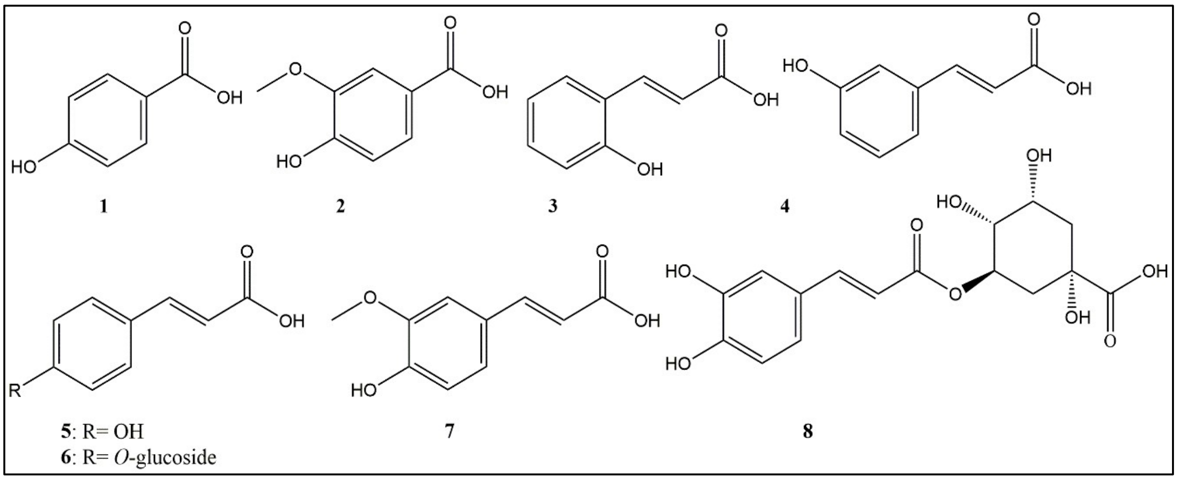

Figure 1. The reported phenolic acids from *M. indicus* and *M. messanensis*

Table 2. The reported coumarins and chromene derivatives from *M. indicus* and *M. messanensis*

Compound	Organ	Species	Ref.
Coumarin (**9**)	Ap	*M. indicus*	[14]
3-Hydroxycoumarin (**10**)	Wp	*M. messanensis*	[12]
3-(4′-methoxy-2′-hydroxy-phenyl)-7-hydroxycoumarin (**11**)			
Melimessanol B; 3-(4′-hydroxy-2′-methoxyphenyl)-4,7-dihydroxycoumarin (**12**)			
Coumestrol (7, 4′-Dihydroxy-coumestan) (**13**)			
4′-*O*-methylcoumestrol (**14**)			
7-hydroxy-4′,5′-dimethoxy-coumestan (**15**)			
Melimessanol A; (6, 4′-Dihydroxy-7-methoxy-coumestan) (**16**)			
5,5,8a-Trimethyl-3,5,6,7,8,8a hexahydro-2H-chromene (**17**)			
2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H chromen-4a-yl hydroperoxide (**18**)	Nr	*M. indicus*	[19]

Ap; Aerial parts, Wp; Whole plant, Nr; Not reported

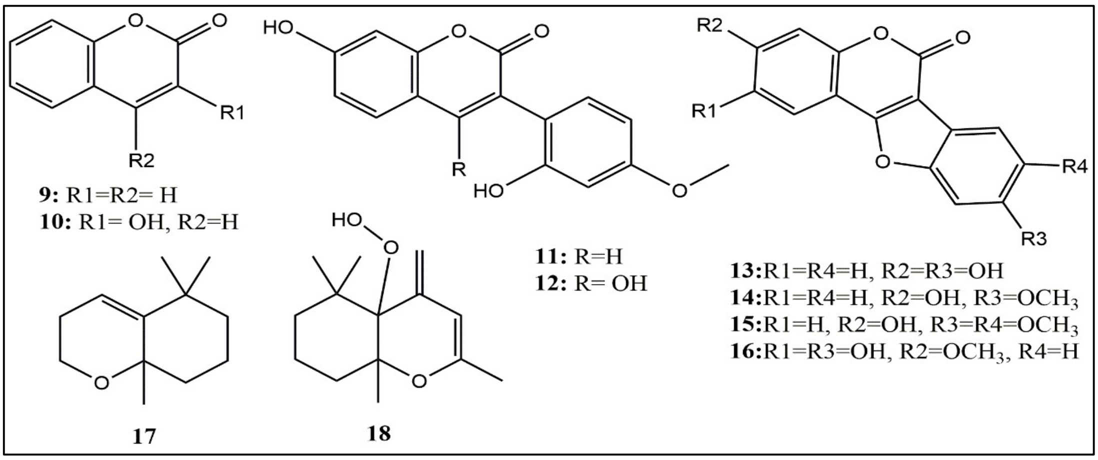

Figure 2. The reported coumarins and chromene derivatives from *M. indicus* and *M. messanensis*

Table 3. Flavonoids obtained from *M. indicus* and *M. messanensis*

Compound	Organ	Species	Ref.
Flavones			
6,3′-dimethoxy luteolin -7-*O*-*α*-arabinopyranosyl(1→6)-*O*-*β*-galactopyranoside (**19**)	S	*M. indicus*	[8]
Flavonols			
Kaempferol (**20**)	Ap	*M. indicus*	[15]
Kaempferol 7-*O*-rhamnoside (**21**)	Wp	*M. indicus*	[15]
		M. messanensis	[16]
Kaempferol 7-*O*-glucoside (**22**), Kaempferol 7-glucronoide (**23**)	Wp	*M. messanensis*	[16]
Kaempferol 3-*O*-rhamnoside (**24**)	Wp	*M. indicus*	[15]
		M. messanensis	[16]
Kaempferol 3-*O*-glucoside (**25**)	Wp	*M. indicus*	[15]
Kaempferol 3-*O*-galactoside (**26**)	Ap	*M. indicus*	[14]
Kaempferol 3-*O*-rutinoside (**27**)	Wp	*M. indicus*	[15]
Kaempferol 3-*O*-(2″-6″-*α*-L-dirhamnopyranosyl)-*β*-galactopyranoside (**28**)	Wp	*M. indicus*	[15]
		M. messanensis	[16]
Kaempferol-3-*O*-(6″-α -L-rhamnopyranosyl)-β-D-galactopyranoside-7-*O*-*α*-L-rhamnopyranoside (**29**)	Ap	*M. indicus*	[14]
Kaempferol 3-*O*-(2″-6″-*α*-L-dirhamnopyranosyl)-*β*-galactopyranoside-7-*O*-*α*- L-rhamnopyranoside (**30**)	Ap	*M. indicus*	[15]
		M. messanensis	[16]
Quercetin (**31**)	Ap, Wp	*M. indicus*	[15, 21]
Quercetin 3-*O*-rhamnoside (**32**)	Ap	*M. indicus*	[15, 21]
	Wp	*M. messanensis*	[16]
Quercetin 3-*O*-glucoside (**33**)	Ap, Wp	*M. indicus*	[15, 21]
Quercetin 3-*O*-rutinoside (**34**)	Wp	*M. indicus*	[15]
Quercetin 3-*O*-(6″-α -L-rhamnopyranosyl)-β-D-galactopyranoside-7-*O*-*α*- L-rhamnopyranoside (**35**)	Wp	*M. indicus*	[15]
		M. messanensis	[16]
Isorhamnetin (**36**)	Wp	*M. indicus*	[15]
Isorhamnetin-3-*O*-glucoside (**37**), Isorhamnetin-3-*O*-rutioside (**38**)	Ap, Wp	*M. indicus*	[15, 21]
Isorhamnetin 3-*O*-(2″-6″-*α*-L-dirhamnopyranosyl)-*β*-D-galactopyranoside (**39**)	Wp	*M. indicus*	[15]
		M. messanensis	[16]
C-glycosyl flavones			
Orientin (**40**)	Wp	*M. messanensis*	[16]
Isoorientin (**41**)			
Isoflavones			
Formononetin (7-Hydroxy-4′-methoxyisoflavone) (**42**)	Wp	*M. messanensis*	[12]
Cyclosin (3′-hydroxyl-formononetin) (**43**)			
Isoflavanones			
Vestitone (7,2′-Dihydroxy-4′-methoxyisoflavanone) (**44**)	Wp	*M. messanensis*	[12]
Medicarpin (l-3-Hydroxy-9-methoxypterocarpan) (**45**)	Wp	*M. indicus*	[15]
		M. messanensis	[12]
7,4′-dimethoxy-8- hydropterocarpan (**46**)	Wp	*M. messanensis*	[12]
5,4′ dihydroxy-6-(3-methlbut-2enyl)-7-methoxpterocarpan (**47**)	S	*M. indicus*	[22]
4′,5′ methylene dioxypterocarpan-7-*O*-*α*-L-rhamnopyranoside (**48**)	St	*M. indicus*	[23]
3-hydroxymedicarpin (**49**), Melilotocarpane B (**50**)	Wp	*M. messanensis*	[12]
Flavanones			
Liquiritigenin (**51**)	Wp	*M. messanensis*	[12]
Dihydroflavonols (Flavanonol)			
Taxifolin (dihydroquercetin) (**52**)	Ap	*M. indicus*	[21]

Ap; Aerial parts, Wp; Whole plant, S; Seeds, St; Stem

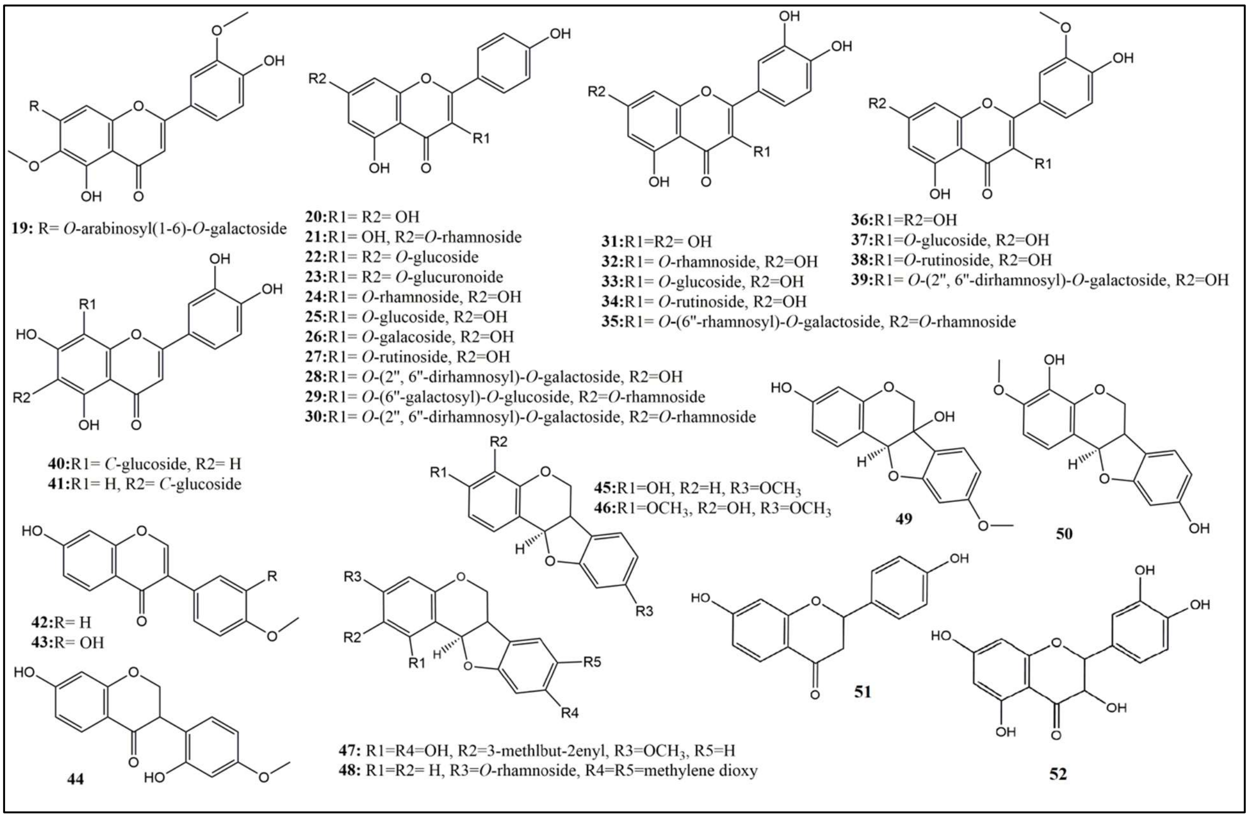

Figure 3. The reported flavonoids from *M. indicus* and *M. messanensis*

Terpenoids and saponins

Terpenoids and saponins are also diffusely distributed in the genus *Melilotus*. The most prominent moiety in terpenoids constituents of *M. indicus* and *M. messanensis* are triterpenes which could be recognized as the chemotaxonomic characters of family Fabaceae [24]. Two saponin components are characterized for *M. messanensis*. The described terpenoid and saponin compounds are noted in Table 4 and Figure 4.

Table 4. Terpenoids and saponins obtained from *M. indicus* and *M. messanensis*

Compound	Organ	Species	Ref.
Terpenoids			
Phytol (**53**)	Wp	*M. indicus*	[15]
Geranyl isovalerate (**54**) Squalene (**55**) Thymol (**56**)	Nr	*M. indicus*	[19]
Stigmasterol (**57**) Campesterol (**58**)	Wp	*M. indicus*	[15]
β-sitosterol (**59**)	Nr	*M. indicus*	[19]
	Wp	*M. indicus*	[15]
	Ap	*M. messanensis*	[25]
7*β*-Hydroxysitosterol (**60**) 7*α*-Hydroxysitosterol (**61**) 7-Oxositosterol (**62**) Ergosterol peroxide (**63**) Gammacer-16-en-3-one (**64**)	Ap	*M. messanensis*	[25]
α-amyrin (**65**)	Wp	*M. indicus*	[15]
Soyasapogenol B (**66**)	Ap	*M. messanensis*	[25]
Soyasapogenol G (**67**) Messagenolide (**68**)	Wp	*M. messanensis*	[26]

Platanic acid (**69**)	Ap	*M. messanensis*	[25]
3-Oxoplatanic acid (**70**)			
Messagenic acid A (**71**)			
Messagenic acid B (**72**)			
Messagenic acid C (**73**)			
Messagenic acid D (**74**)			
Messagenic acid E (**75**)			
Messagenic acid F (**76**)	Wp	*M. messanensis*	[27]
Messagenic acid G (**77**)			
Messagenic acid H (**78**)			
Messagenic acid I (**79**)			
Messagenin (**80**)	Ap	*M. messanensis*	[28]
Botulin (**81**)			
Betulin aldehyde (**82**)			
Betulinic acid (**83**)	Ap	*M. messanensis*	[28]
	Wp	*M. messanensis*	[27]
Lupeol (**84**)	Wp	*M. indicus*	[15]
	Ap	*M. messanensis*	[28]
Saponins			
Azukisaponin V (**85**)	Wp	*M. messanensis*	[12]
Sitosterol 3-*O*-*β*–gluco-pyranoside (**86**)			

Ap; Aerial parts, Wp; Whole plant, Nr; Not reported

Other phytochemicals

Analysis of previous reports revealed a range of other minor phytochemicals that have been reported for *M. indicus* and *M. messanensis*. These include fatty acid esters, alcohols, aromatic compounds, etc (Table 5). Both species contain behenyl alcohol (1-Docosanol) and 2-octyl-1-dodecanol that used traditionally as emollient, emulsifier, and thickener in cosmetics and nutritional supplement. Moreover, the former has been approved by FDA (Food and Drug Administration) as a pharmaceutical antiviral agent [29, 30]. Some common fatty acid esters were also described for the two species.

Table 5. Fatty acid esters and other phytochemicals obtained from *M. indicus* and *M. messanensis*

Compound	Organ	Species	Ref.
Fatty acids			
Palmitic acid	Ap	*M. indicus*	[14]
Esters of fatty acid			
Undecanoic acid, 10-methyl, methyl ester; tetradecanoic acid, methyl ester; pentadecanoic acid, methyl ester; 9-hexadecenoic acid, methyl ester; (Z)-, 2-hexadecenoic acid, methyl ester; (E)-, hexadecanoic acid, ethyl ester; hexadecanoic acid, 15-methyl-, methyl ester; octadecanoic acid, methyl ester; heneicosanoic acid, methyl ester; docosanoic acid, methyl ester; tricosanoic acid, methyl ester;	Wp	*M. indicus*	[15]
9,12-Octadecadienoic acid, methyl ester, (E,E)-, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Wp	*M. indicus* *M. messanensis*	[15, 16]
Aromatics			
Phenol, 2,4-bis (1, 1-dimethyl)	Nr	*M. indicus*	[19]
1-butylhexyl- benzene, 1-propylheptyl-benzene, 1-ethyloctyl-benzene, 1-methylnonyl-benzene, 1-pentylhexyl-benzene, 1-butylheptyl-benzene, 1-propyloctyl-benzene, 1-ethylnonyl-benzene, 1-methyldecyl-benzene, 1-pentylheptyl-benzene, 1-butyloctylbenzene, 1-propylnonyl-benzene, 1-ethyldecylbenzene, 1-methylundecyl-benzene, 1-pentyloctyl-benzene, 1-butylnonyl-benzene, 1-propyldecyl-benzene, 1-ethylundecyl-benzene, 1-methyldodecyl- benzene	Wp	*M. indicus* *M. messanensis*	[15, 16]
Benzofuran			
Loliolide	Wp	*M. messanensis*	[12]
Isololiolide			

Alcohols			
Behenic alcohol (1-Docosanol), 1-Dodecanol, 2-octyl-	Wp	*M. indicus* *M. messanensis*	[15, 16]
7,11,15-trimethyl-3-methylenhexadecan-1,2-diol	Wp	*M. messanensis*	[12]
Cyclohexane-1-methanol, 3,3-dimethyl-2-(3-methyl-1,3-butadienyl), 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl), 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	Nr	*M. indicus*	[19]
Ketones			
Cyclohexane, 1,5,5-trimethyl-6-acetylmethyl, 2-cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl), 3-buten-2-one, 4-(4-hydroxy-2, 6 ,6-trimethyl-7-oxabicyclo[4.1.0] hept-1-yl), 3-(1-Methylhept-1-enyl)-5-methyl-2, 5-dihydrofuran-2-one	Nr	*M. indicus*	[19]
Aldehydes			
2-[4-Methyl-6-(2,2,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclo- hex-1-en-carboxaldehyde	Nr	*M. indicus*	[19]
Carbohydrates			
D-Galacto-D-mannan polysaccharside	S	*M. indicus*	[6]
D-Erythro-pentose, 2-deoxy 3-O-Methyl-D-glucose	Nr	*M. indicus*	[19]
Amino acids			
Asparagine, asparagine N-glucoside	Wp	*M. indicus* *M. messanensis*	[15, 16]

Ap; Aerial parts, Wp; Whole plant, S; Seed, Nr; Not reported

5. Biological evaluations

The genus *Melilotus is* reported to have a wide array of biological activities. Many *Melilotus* species are recognized for their medicinal and pharmacological activities including antioxidant, anti-inflammatory, hypoglycaemic, antimicrobial, anticancer, and hepatoprotective activities. Herein, the review presents the reported biological activities of *M. indicus* and *M. messanensis* which are presented alongside the extractives and the model used in the studies and they both showed significant biological effects [15, 16, 31-35] (Table 6).

The *in vitro* antioxidant potential of different solvent extracts of *M. indicus* were evaluated using DPPH (1, 1-diphenyl-2-picrylhydrazyl) and H_2O_2 radical scavenging capacity assay [32, 33], while the *in vivo* antioxidant prospective of both species were evaluated using serum reduced glutathione content assay [15, 16] (Table 6). Different solvent extracts and some isolated compounds of many plant parts of some *Melilotus* species were tested as *in vitro* and *in vivo* anti-inflammatory agents. The ethanol extract and some isolated coumarins of *M. indicus* were employed to explore the anti-inflammatory potential using four acute oedema *in vivo* models (carrageenan, serotonin, histamine-induced paw oedema and xylene-induced ear oedema and chronic formaldehyde-induced paw oedema). TNF-alpha ELISA kit was used to determine serum levels of TNF-*α*. The tested extract (750 mg/kg) induced comparable anti-inflammatory effects to that of standard-treated groups and significant ($p > 0.05$) downregulation of TNF-α in serum samples. Additionally, the isolated coumarins showed better anti-inflammatory effects in the carrageenan-induced paw oedema model than in other models [31]. Also, different solvent extracts of *M. indicus* showed *in vitro* antidiabetic effect [34, 35], while *in vivo* approaches were reported for the both species [15, 16]. Moreover, it can be suggested that *M. indicus and M. messanensis* could be beneficial in reducing chromosomal and male reproductive defects associated with oxidative stress conditions such as diabetics [15, 16]. Furthermore, some *Melilotus* species have been shown to exert *in vitro* antimicrobial activity against various microorganisms. Antimicrobial potential of aerial parts or leaves different solvent extracts of *M. indicus* were evaluated, presenting efficient antibacterial activities against gram-positive (*Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa, Klebsiella pneumonia, Acinetobacter baumannii* and/or *Escherichia coli*) bacteria. As well, they showed significant antifungal activities against *Aspergillus fumigatus* [33] (Table 6).

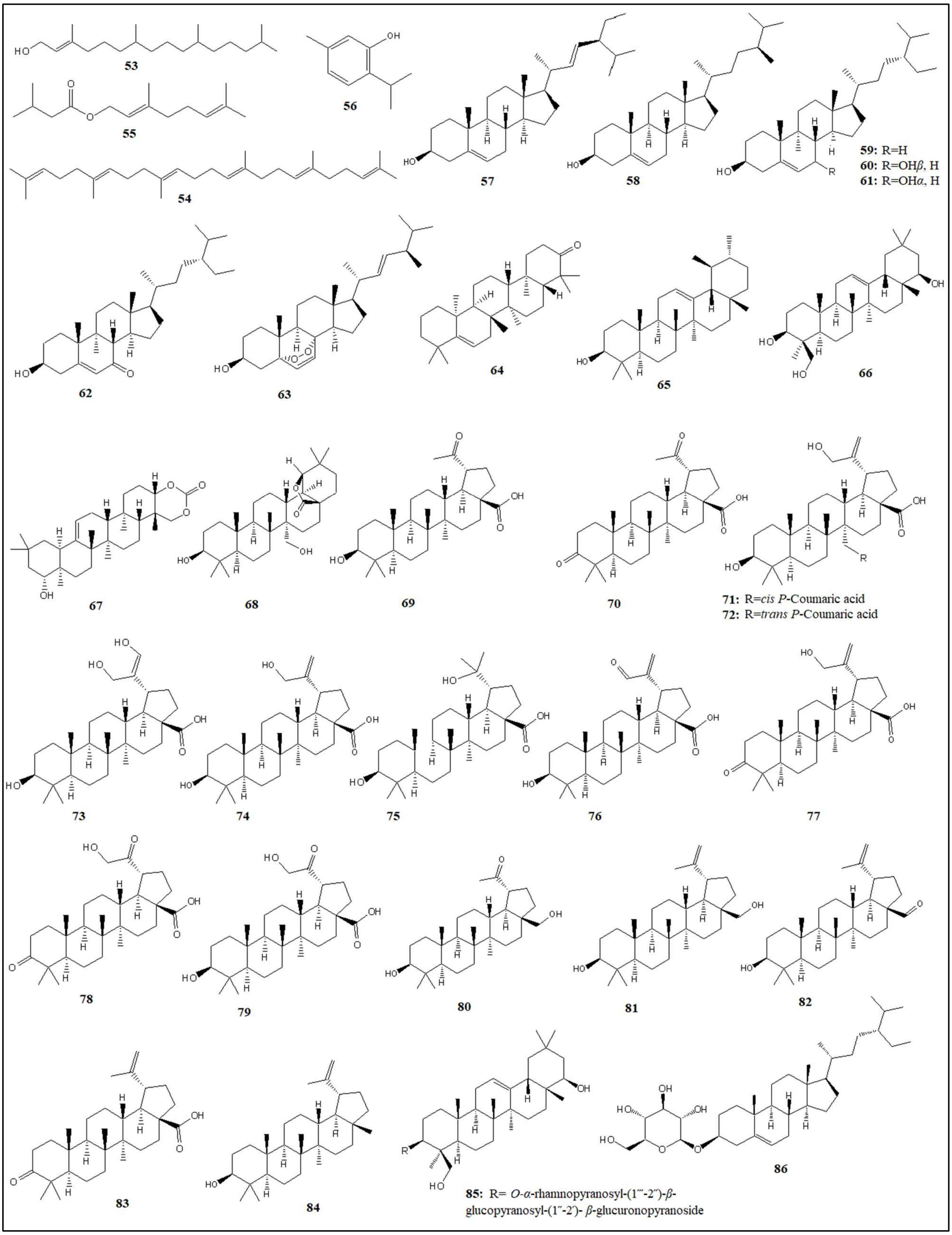

Figure 4. Terpenoids and saponins obtained from *M. indicus* and *M. messanensis*

6. Chemosystematic significances

On the basis of the macromorphological characters, *M. indicus* and *M. messanensis* seem to be similar before the fruiting stage, whereas, they have various common leaves and floral characters [36]. However, on the fruiting stage *M. indicus* and *M. messanensis* are distinct having different pod and seed (shape and surface) characters. *M. indicus* is characterized by the pods that are ovoid to subglobular, papery, clearly flexuous with wrinkled, reticulate, pitted surface sculpture and rounded apex or sometimes mucronate. As well as seeds are sub-orbicular with verrucate surface pattern. While, *M. messanensis* have pod (obliquely-ovoid, valves with looping, tapering at both ends and long sharp beak) along with obliquely oblong seed shape with colliculate surface pattern [36]. Among the some *Melilotus* species from Pakistan, *M. indicus* and *M. messanensis* are grouped together on the basis of seed morphology, both having brown or greenish brown seeds with colliculate or verrucate surface patterns [37]. In the same study, they could be differentiated by the elemental analysis data, where *M. messanensis* characterized by the presence of P and Cu while *M. indicus* has Na and Si.

Herein, the reported phytochemical variation could be helpful to describe the chemosystematic relationship among *M. indicus* and *M. messanensis.* Both species are comparable in the capability to biosynthesis of a wide range of compound groups, i.e., benzoic acids, hydroxyl cinnamic acids, flavonols and isoflavones. These types of compounds were also reported for *M. officinalis*; a very important medicinal species [38], which predict the medicinal outcomes of *M. indicus* and *M. messanensis*. Additionally, the chemical profile of both species can be distinguished, as compound **19** (flavone derivative) and **52** (dihydroflavonol derivative) were restricted to *M. indicus* nevertheless *C*-glycosyl flavonoids (compounds **40** and **41**) were characterized in *M. messanensis.* Also, triterpenes and coumestan-type coumarins are abundantly present in *M. messanensis.* To the best of our knowledge, the chemosystematic relationship among the two species has not been discussed before.

Table 6. Biological effects of *M. indicus* and *M. messanensis* extracts

Species	Organ	Tested extract	Assay	Activity	Ref.
Antioxidant activities					
M. indicus	Leaves	Methanol, ethanol, aqueous and chloroform	DPPH radical scavenging	All extracts showed dose dependent scavenging of DPPH radicals in a way like that of the reference antioxidant (ascorbic acid)	[32]
	Whole plant	Methanol	DPPH and H_2O_2 radical scavenging	The extract exhibited a significant antioxidant activity	[33]
	Whole plant	Aqueous methanol (AM), petroleum ether (PE) and defatted aqueous methanol (DAM) extracts	Serum reduced glutathione content	AM and PE extracts significantly increased serum GSH content in rats compared to diabetic rats	[15]
M. messanensis	Whole plant	AM, PE, and DAM extracts	Serum reduced glutathione content	AM, PE, and DAM extracts exhibited significant activities along with % of change 10, 11 and 13, respectively	[16]
Antidiabetic aspect					
M. indicus	Aerial parts	Methanol, chloroform, ethyl acetate, n-butanol, and water	*α*-amylase inhibitory and urease inhibitory assays	The chloroform fraction showed the highest anti-urease activity. All extracts showed enzyme inhibitory activity in a dose-dependent manner. Moreover, they were manifold more effective against urease than α-amylase. The combination of the extract with acarbose considerably increased the potency of the latter.	[34]
	Whole plant	Methanol	*α*-glucosidase inhibitory activity	Inhibition of *α*-glucosidase (25%)	[35]

	Whole plant	AM, PE, and DAM extracts	Blood glucose level (BGL) at zero, 2 and 4 weeks after oral administration	AM and DAM extracts revealed a significant acute anti-hyperglycaemic effect potentiated after 4 weeks of treatment	[15]
M. messanensis	Whole plant	AM, PE, and DAM extracts	BGL at zero, 2 and 4 weeks after oral administration	All extracts showed anti-diabetic activities after four weeks in comparison with metformin	[16]
Antimutagenic effects					
M. indicus	Whole plant	AM, PE, and DAM extracts	Abnormalities in somatic and sperm cells	AM extract revealed a significant inhibitory effect on the irregularity of bone marrow cells and sperm abnormalities.	[15]
M. messanensis	Whole plant	AM, PE, and DAM extracts	Abnormalities in somatic and sperm cells	All extracts showed high inhibitory indices of bone marrow and sperm cells aberrations in diabetic rats.	[16]
Antipathogenic activities					
M. indicus	Leaves	Methanol, ethanol, aqueous and chloroform	Disc diffusion method against *S. aureus* and *E. coli*	The antimicrobial screening of methanol extract shows sensitivity *S. aureus* but shows resistance *E. coli*.	[32]
	Leaves, stems, fruits	Methanol	Agar well diffusion method against *S. aureus, P. aeruginosa, K. pneumonia* and *A. baumannii*	The leaves extract exhibited considerable antibacterial activity against all the selected strains of microorganisms tested but not in highest ranking compared to other plants.	[33]
	Leaves, stems, fruits		Agar tube dilution method against *A. fumigatus*	The leaves extract exhibited an antifungal potential against *A. fumigatus*	[33]

Conclusion

The genus *Melilotus* offers a better liability for folk uses, phytochemical, and biological data. While less attention has been paid to some species of the genus, this review is focusing on the interesting *Melilotus* species (*M. messanensis*) comparative with the medicinal one (*M. indicus*). Meanwhile, *M. indicus* has been found to have some valuable folk medicine and more pharmacological properties, therefore more intensive research is required to confirm the biological potential of *M. messanensis.* Both species provide a wide range of searchable potential and are comparable in the ability to synthesis the same phytochemical constituents which were also previously described for *M. officinalis* (a commercially medicinal *Melilotus* species). In the future, reliable standardization of their natural products is required for their acceptance as therapeutic agents and the chemical fingerprint must be fully characterized using the most recent available analytical techniques such as HPLC/MS^n. Their phytochemical variation should be studied with regards to their taxonomic ranking and preferably complemented with molecular studies to investigate their chemophenetic significance.

Author Contributions

N.A. Ragab and M.M. Marzouk: conceptualization, experimental (literature survey), formal analysis, writing-review and editing. All authors helped to prepare the manuscript, revised, and approved the final version.

Conflict of interest

The authors declare no conflict of interest.

Reference

[1] Grossberg G.T., Fox B., The Essential Herb-Drug-Vitamin Interaction Guide. New York: Broadway Books(2007).

[2] Asres K., Gibbons S., Hana E., Bucar F: Anti-inflammatory activity of extracts and a saponin isolated from *Melilotus elegans*. *Pharmazie,* **60**(4),310–312 (2005).

[3] Földi M., Zoltán O.T. Unconditioned reflex activity in experimental lymphogenic encephalopathy and therapeutic effects thereon by coumarin from *Melilotus officinalis*. *Arzneimittelforschung,* **20**(11),1623- 1624(1970).

[4] Liu Y.T., Gong P.H., Xiao F.Q., Shao S., Zhao D.Q., Yan M.M., Yang X.W. Chemical constituents and antioxidant, anti-inflammatory and anti-tumor activities of *Melilotus officinalis* (linn.) pall. *Molecules,* **23**(2),1–12(2018). Doi: 10.3390/molecules23020271

[5] The Plant List. Available at: http://www.theplantlist.org/

[6] Gupta A.K., Bose S. Structure of the D-galacto-D-mannan isolated from the seeds of *Melilotus indica* All. *Carbohydr Res,* **153**(1), 69–67(1986).

[7] Qureshi S.J., Khan M.A., Ahmad M. A survey of useful medicinal plants of Abbottabad in northern Pakistan. *Trakia J Sci,* **6**(4),39–51(2008).

[8] Yadava R.N., Jain S. A new bioactive flavone glycoside from the seeds of *Melilotus indica* All. *J Asian Nat Prod Res,* **7**(4),595–599(2005). Doi: 10.1080/10286020310001608949

[9] Khan S., Shinwari M.I., Haq A., Ali K.W., Rana T., Badshah M., Khan S.A. Fourier-transform infrared spectroscopy analysis and antifungal activity of methanolic extracts of *Medicago parviflora*, *Solanum Nigrum*, *Melilotus alba* and *Melilotus indicus* on soil-borne phytopathogenic fungi. *Pakistan J Bot,* **50**(4),1591–1598(2018).

[10] Xu F., Zeng W., Mao X., Fan G.K. The efficacy of *Melilotus* extract in the management of postoperative ecchymosis and edema after simultaneous rhinoplasty and blepharoplasty. *Aesthetic Plast Surg,* **32**(4),599–603(2008). Doi: 10.1007/s00266-008-9149-3

[11] Bhati D., Jain S. Micro nutrient composition of unconventional wild fruits and vegetables **11**(4),2405–2409(2016).

[12] Macías F.A., Simonet A.M., Galindo J.C.G., Castellano D: Bioactive phenolics and polar compounds from *Melilotus messanensis*. *Phytochemistry,* **50**(1),35–46(1999). Doi: 10.1016/S0031-9422(98)00453-1

[13] Raza A., Kamran M., Safdar M.E., Ali H.H., Abbas T., Asif M., Ali L., Rehman A. Management tactics for handling *Parthenium hysterophorus* in non-native environment through phytotoxic compounds of local species. *Int J Agric Biol,* **21**(1),215–222(2019). Doi: 10.17957/IJAB/15.0884

[14] Mahmoud Y.S.Y. A Pharmacognostical Study of *Melilotus indicus (L.) All. Growing in Egypt*. Faculty of Pharmacy, Cairo ,Egypt, (2019).

[15] Ragab N.A., Aboutabl E.A., Marzouk M.M., ElHalawany A.M., Farghaly A.A., Sleem A.A., ElSawi S.A. Chemical Profiling *In Vivo* Antioxidant Effect and Antimutagenic Evaluation of *Melilotus indicus* (L.) All. Extracts. *Trop J Nat Prod Res,* **5**(3),534–542(2021).

[16] Ragab N.A., El Sawi S.A., Marzouk M.M., El Halawany A.M., Sleem A.A., Farghaly A.A., Aboutabl EA: Chemical characterization of *Melilotus messanensis* (L.) all.: Antioxidant, antidiabetic and antimutagenic effects in alloxan induced diabetic rats. *Biocatal Agric Biotechnol,* **33**,101976(2021). Doi: 10.1016/j.bcab.2021.101976

[17] Zhang X.Y, Tao J.Y, Zhao L., Huang Z.J., Xiong F.L., Zhang S.L., Li C.M., Xiao F. *In vitro* anti-inflammatory effects of different solution fractions of ethanol extract from *Melilotus suaveolens* Ledeb. *Chin Med J (Engl,)* **120**(22),1992–1998(2007). Doi: 10.1097/00029330-200711020-00010

[18] Pastura G., Mesiti M., Saitta M., Romeo D., Settineri N., Maisano R., Petix M., Giudice A. Lymphedema of the upper extremity in patients operated for carcinoma of the breast: clinical experience with coumarinic extract from *Melilotus officinalis*. *Clin Ter,* **150**(6),403–408(1999).

[19] Iftikhar H., Ahmed D., Qamar M.T. Study of Phytochemicals of *Melilotus indicus* and Alpha-Amylase and Lipase Inhibitory Activities of Its Methanolic Extract and Fractions in Different Solvents. *ChemistrySelect,* **4**(26),7679–7685(2019). Doi: 10.1002/slct.201901120

[20] Harborne J.B., Mabry TJ: The flavonoids. London: Chapman & Hall Ltd. (1975)

[21] Ahmed SA-K., Al Refai M. Chemical constituents and cytotoxic activities of the extracts of *Melilotus indicus*. *Eur J Chem,* **5**(3),503–506(2014). Doi: 10.5155/eurjchem.5.3.503-506.1070

[22] Saxena V. k., Nigam S: A novel prenylated pterocarpan from *Melilotus indica*. *Fitoterapia,* **68**,403(1997)

[23] Saxena V k., Nigam S: A methylenedioxypterocarpan from *Melilotus indica*. *Fitoterapia,* **68**,343(1997).

[24] Oleszek W., Stochmal A. Triterpene saponins and flavonoids in the seeds of *Trifolium* species. *Phytochemistry,* **61**(2),165–170(2002). Doi: 10.1016/S0031-9422(02)00230-3

[25] Macías F.A., Simonet A.M., Galindo J.C.G. Bioactive steroids and triterpenes from *Melilotus messanensis* and their allelopathic potential. *J Chem Ecol,* **23**(7),1781–1803(1997). Doi: 10.1023/B:JOEC.0000006451.19649.a0

[26] Macías FA:, Simonet AM:, Esteban MD:, Galindo JCG: Triterpenoids from *Melilotus messanensis*; soyasapogenol G, the first natural carbonate derivative. *Phytochemistry,* **41**(6),1573–1577(1996).

[27] Macías F.A., Simonet A.M., Galindo J.C.G., Pacheco P.C., Sanchez J.A. Bioactive Polar Triterpenoids From *Melilotus messanensis*. *Phytochemistry,* **49**(3),709–17(1998).

[28] Macías F.A., Simonet A.M., Esteban M.D. Potential allelopathic lupane triterpenes from bioactive fractions of *Melilotus messanensis*. *Phytochemistry*, 36(6),1369–1379(1994). Doi: 10.1016/S0031-9422(00)89726-5.

[29] Katz D.H., Marcelletti J.F., Khalil M.H., Pope L.E., Katz L.R. Antiviral activity of 1-docosanol , an inhibitor of lipid-enveloped viruses including herpes simplex. *Proc Natl Acad Sci,* **88**(23),10825–10829(1991).

[30] Williams S.D. Chemistry and Technology of the Cosmetics and Toiletries Industry. Springer Science & Business Media(1996).

[31] Saleem M., Asif M., Parveen A., Yaseen H.S., Saadullah M., Bashir A., Asif J., Arif M., Khan I.U., Khan R.U. Investigation of *in vivo* anti-inflammatory and anti-angiogenic attributes of coumarin-rich ethanolic extract of *Melilotus indicus*. *Inflammopharmacology*,1–13(2020). Doi: 10.1007/s10787-020-00703-9

[32] Sonju J.J. Analysis of Phytochemical, Antioxidant and Microbial Property of Various Extracts of the Plant *Melilotus Indica*. *World J Pharm Res,* **6**(4),129–147(2017). Doi: 10.20959/wjpr20174-8050

[33] Naz R., Ayub H., Nawaz S., Islam Z.U, Yasmin T., Bano A., Wakeel A., Zia S., Roberts T.H. Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. *BMC Complement Altern Med,* **17**(1),1–13(2017). Doi: 10.1186/s12906-017-1815-z

[34] Ahmed D., Younas S., Mumtaz Q., Mughal A., Mughal Q.M.A. Study of alpha-amylase and urease inhibitory activities of *Melilotus*. *Pak J Pharm Sci,* **27**(1),57–61(2014)

[35] El-Manawaty M.A,, Gohar L, *In vitro* alpha-glucosidase inhibitory activity of egyptian plant extracts as an indication for their antidiabetic activity. *Asian J Pharm Clin Res,* **11**(7),360–367(2018). Doi: 10.22159/ajpcr.2018.v11i7.25856

[36] Bolus L. Flora of Egypt checklist. Cairo, Egypt: Al Hadara Publishing

[37] Abid R., Ali S.A., Saeed E., Munir I., Hashmi S.U, Ather A., Qaiser M: Chemotaxonomy and seed morphological studies of the genus *Melilotus* Mill. From Pakistan. *Pakistan J Bot,* **50**(2),575–578(2018)

[38] Ilhan M., Ali Z., Khan I.A., Küpeli Akkol E. A new isoflavane-4-ol derivative from *Melilotus officinalis* (L.) Pall. *Nat Prod Res,* **33**(13),1856–1861(2019). Doi: 10.1080/14786419.2018.1477152.