PHYTOCHEMICAL INVESTIGATION OF SOME PLANTS OF THE GENUS MELILOTUS, FAMILY LEGUMINOSAE, GROWING IN EGYPT

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By

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TO
MY PARENTS
AND
MY BROTHER

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بسم الله الرحمن الرحيم

INTRODUCTION

Family Fabaceae (Leguminosae)

Fabaceae (Leguminosae) is the second largest well known and widely distributed family in the world [1-3]. It comprises 600 genera and about 12000 species [4]. Fabaceae is classified into three subfamilies: Papilionoideae, Mimosoideae and Caesalpinoideae [2, 4]. Papilionoideae is one of the important subfamilies, which include 377 genera [4] and at least 10000 species [5].

Subfamily Papilionoideae

Plants of this subfamily are usually herbs, shrubs or trees with simple or compound leaves. The flowers are zygomorphic, their sepals are usually five and free, the two lateral (wings) are parallel with each other while the lower two are interior.

The stamens of these plants are often ten, monadelphous or diadelphous and they are usually inserted with the petals.

Fruits is usually a legume or pod (dehisces along both sutures sometimes fruit may be transformed into lomentum by formation of false T.S septa into one seeded indehiscent parts. Seeds may or may not have scanty endosperm [5, 6].

Genus *Melilotus*

Genus *Melilotus* is a member of the wild promising economical medicinal plants; it belongs to family Fabaceae, subfamily papilionoideae [1, 4, 7-9]. it includes 25 species [4], distributed in temperate and subtropical regions especially Europe, Asia, Mediterranean region, North and Eastern Africa and Ethiopia [10-13].

Melilotus is known as "coumarin smelling herb," sweet clover (English), mélilot (French) and in Arabic as Handaquq, Iklil el-malek, Reqraq and Eshb el-malek [14-17].

Melilotus species are annual or short-lived, perennial herbs; flowers are white or yellow carried in elongated, often narrow raceme; leaves are pinnately trifoliate; fruits are globosed or ovoid, smooth or veined and 1 to 4-seeded [12, 13, 18].

Melilotus is represented in Egypt by seven species *M. albus* (*argutus*), *M. sulcatus*, *M. segetalis*, *M. messanensis* (*siculus*), *M. elegans*, *M. indicus* (*indica*) and *M. serratifolius* [12].

The main botanical differences between *M. indicus* (L.) All. and *M. messanensis* (L.) All. are listed in table (1) [12, 13].

	M. indicus	***M. messanensis***

Table 1 - Botanical differences between *M. indicus* (L.) All. and *M. messanensis* (L.) All.

Height	15-50 cm	20-60 cm
Stems	Erect and branched	Erect and much branched from the base
Leaflets	0.8-2.4x0.3-1.2 cm obovate to oblong -cuneate	1-2.5x 0.6-1.5 cm Obovate-orbicular to Lanceolate-cuneate
Margins	sharply serrate-dentate margin	sharply serrulate margin
Apex	Truncate- apiculate apex	Round or emarginated-apiculate apex
Racemes	1-2.5cm 20-30-flowered, pale yellow	2-3mm 6-16 flowered, yellow
Seeds	1.5-2mm Minutely tuberculate	2.5-3.5 mm Irregular subglobose
pods	1.5-3mm Subglobose with reticulate ridges	6-8mm Oblique ovoid with concentric ridges

Biological activities of genus *Melilotus*

Several biological activities have been reported for genus *Melilotus*, which can be summarized as follows:

1. Folkloric and medicinal uses of genus *Melilotus*:

Genus *Melilotus* has a great history of folkloric medicinal uses in *Mexico* [11, 19], *Ethiopia* [20, 21], *North India*, South *Europe* [21], *America* [7] and *Tasmania* [18].
Melilotus was used in medicine as an emollient; it is employed occasionally as lotions and enemas [7]. Also, a decoction of *Melilotus* can be applied to the sores of the breast of new mothers [19].

Melilotus was recommended for both external and internal uses in cases including ulcers, abdominal and rheumatic pains as either infusions or liquid extracts [22].

M. elegans is widely used for the treatment of haemorrhoids, varicose vein and lacerated wounds [20, 21]. *Melilotus* has been used as a poultice or plaster on swelling or on inflamed areas because it is antiseptic [11, 19, 21].

Melilotus is favored in neuralgia affecting many areas (headaches) [20- 22]. The leaf tea of *Melilotus* can be used as a relaxing tea; it has the reputation of being sedative and headaches relief [19]. *Melilotus* species have been used in making wine and vodka [19].

An infusion of *Melilotus* species is reported to be used for catarrhs of respiratory tract [20-23], therefore a decoction of *M. officinalis* is used internally for chest complaints [7, 23].

Different *Melilotus* species are used as aromatic ingredient in tobacco [23]. *Melilotus* species have the reputation of reducing flatulence [19], being a digestive, relieving colics and curing diarrhoea [7, 19].

The scent of *Melilotus* becomes stronger when the plant is dried, so it is used as a flavouring agent that is very much like vanilla [11, 18, 19, 23].

In *Egypt*, an infusion of the flowering branches of *M. indicus* is used as an emollient and antispasmodic [16, 17], while the seeds are used as anthelmintic, antipyretic, for leprosy, bowl complaints, infantile diarrhoea, astringent, narcotic [21] and for diseases of genital organs of both sexes [16, 17].

2. Antispasmodic activity:

An extract of *M. officinalis* (flowers and leaves) decreased both spontaneous and bradykinin-induced contraction of the smooth muscle of isolated bovine mesenteric lymphatics. This was attributed to the presence of 0.2% coumarins [24].

3. Antibacterial activity:

The ethyl acetate fraction of 95% of ethanolic extract of *M. indicus* has shown a good antibacterial activity against both gram positive bacteria e.g., *Bacellus anthracis, B. cereus* and gram negative bacteria e.g. *Proteus vulgaris* and *Salmonella Newport* [21].
This antibacterial activity seems to be related to the presence of phenolic acids [25].

4. Antifungal activity:

The extracts of the seeds and stems of *M. indicus* have shown good antifungal activity against *Epidermophyton floccosum* [26], *Penicillium notatum, P. purpurogenus* and *Aspergillus fumigatus* [21].
This antifungal activity is attributed to the presence of pterocarpanoids [26].

5. Antiviral activity:

The defatted methanolic extract of leaves of *M. elegans* showed mild antiviral activity against influenza A virus. Flavonoids as well as sesquiterpenes were reported to have such antiviral activity [27].

A Russian patent aqueous extract was prepared from *Melilotus* herb, which is indicated for the treatment of chlamydiosis, herpes, viral hepatitis A, and B, erysipelas and acute enteric infection [28].

6. CNS activity:

Therapy with coumarin isolated from *M. officinalis* antagonized the convulsion effect of isoniazid and pentylenetetrazole in both rats and guinea pigs [29-31].
It also reduced the spontaneous motility of rats (experimental lymphogenic encephalopathy) [32, 33].

Furthermore, the methanolic extract from *M. suaveolens* showed a strong inhibitory effect on monoaminoxidase activity (MAO) [34].

7. Body thinning effect:

A Japanese patent preparation, which contained coumarins or their derivatives from *M. officinalis*, improved the circulation and inhibited fat cell increase [35].

8. Immune enhancing activity:

The immunostimulant [22, 36], antianemic, and adaptogenic activities of polysaccharides from *M. officinalis* were evaluated in experiments on mice and rats [36].

9. Anthelmintic activity:

The anthelmintic efficacy of Kamala® (a pharmaceutical preparation from *Melilotus philippinensis* fruit) against poultry cestodes, such as *Raillietina tetragona* and *R. echinobothrida*, in chickens was found to be fairly good [37].

10. Radical scavenging activity (RSA):

Several toxic effects accompanied the use of synthetic antioxidants, therefore studies were carried out on large number of aromatic, spicy, and medicinal plants especially those containing phenolic compounds to measure RSA.

It was found that the honey of *M. officinalis* obtained during the plant flowering period possessed quite high antioxidant activity as it distinctly reduced polyphenol oxidase [38].

The antioxidant activity of the methanolic extract of blossoms of *M. officinalis* has been measured using 2, 2 diphenyl-2-picryl hydrazyl hydrate (DPPH). This test showed an absorption inhibition of DPPH color by 75.9% [38].

The RSA was attributed to the presence of flavonoids, which act as the primary antioxidants; such property is especially distinct for flavonols [38].

Therefore, *Melilotus* can be considered as a potential source of antioxidants of a natural origin [39].

11. Anticancer activity:

Some of the laboratory data suggested that coumarins of *Melilotus* have a direct effect on tumor cells as well as on immune system [22].

A reputable report on 45 patients with metastatic renal cell carcinoma showed an objective tumor response rate (about 50% or more reduction in tumor size) in 33% of patients and surprisingly three patients had a complete response after using the therapy of coumarin [40].

Moreover, the antinoeplastic action of coumarin was detected to be effective in metastatic prostatic carcinoma patients, whereas those with small tumor volumes are the most likely to respond [22, 40].

12. Cardiovascular activity:

Several clinical trials have been carried out on dogs using intravenous injection of *Melilotus extract* ® (linnea co.), *Lymphaselect*® (indena co.) and extract of *M. officinalis*.

The results showed an increase of arterial systolic pressure which led to an increase in coronary blood flow which is a favorable effect on myocardial ischemia induced by temporary clamping of the coronary artery[41]. This cardiovascular activity could be due to coumarins content [22, 41].

A patent French pharmaceutical preparation containing *Melilotus* extracts was used for preventing ischemia and/or pathologies associated with ischemia [42].

13. Oral anticoagulant therapy:

Dicoumarol was the first used oral anticoagulant. This compound is formed by bacterial action in damaged hay of *Melilotus* [19].The discovery of this compound led to the preparation of synthetic analogues, the most widely used of which is warfarin [8, 22].

The study of the potential anticoagulant activity of total ethanolic extracts of *M. messanensis* and *M. indicus* has been carried out orally on adult normal rabbits; the results showed a prolongation in plasma prothrombin time (PPT) [43].

14. Anti-inflammatory and Antioedema activities:

Melilotus possesses a unique anti-inflammatory activity which makes it effective for treatment of high protein oedemas including burns and lymphoedema [22] as reported in both commission E monograph [22, 44] and the British herbal pharmacopoeia (1996) [9].

The proposed mechanism responsible for this activity is that *Melilotus* enhances the breakdown of accumulated protein by stimulation of macrophage activity and number [22, 40], so it improves the blood and lymph flow, accelerates wound healing and reduces postoperative oedema [22].

The anti-inflammatory activity of different extracts and different parts of *Melilotus* species has been carried out. Several *in-vivo* experiments on both animals and human have been reported.

The principle of these studies on the experimental animals, (rats [20, 45] and male rabbits [46]), is based on induction of oedemas by different methods such as: thermal injury [45], the oil of turpentine injection [46] and carageenin induced rat paw oedema [20].

After inducing oedema in the experimental animals, *Melilotus* extracts are given intraperitoneally [45, 46] or orally [20].

Results showed that *Melilotus* extracts exerted an oedema inhibitory activity [20, 45], it had a stimulatory effect on epidermal regeneration, shortened the time of wound healing [47] and it showed potent inhibitory activity on the migration of leukocytes [48]. Similar results were obtained from standards; such as hydrocortisone sodium hemisuccinate [46] and sodium salt of indomethacin [20].

These effects were attributed to the presence of robinin [20], coumarins [45] and saponin (azukisaponin V) [48].

Some combinations of *Melilotus* have been investigated such as *Esberiven*® ointment (*Melilotus* extract and heparin) which showed a synergistic wound healing effects [47]. Furthermore, *Troxerutin*® (*Melilotus* and rutin) was received by 25 pregnant women, symptoms such as heavy legs disappeared completely in 68% [22].

Clinical trials showed that *Melilotus* extracts induced a very significant improvement in lymphoedema at the second stage [49]. Also, it was effective in treatment of 79% of patients with chronic lymphoedema of upper arm, which is resulted from the lymphadenectomy of breast cancer [50].

Interestingly, "Nivea"company invented a patent pediatric anti-inflammatory ointment containing *M. officinalis* [51].

In addition, two Russian liposomal topical preparations contained chloroform extract of *Melilotus* for treating burns and inflammations [52, 53].

15. Treatment of Chronic venous insufficiency:

Diseases of venous system are characterized by thrombotic and inflammatory processes namely thrombophlebitis and varicosis. Chronic venous insufficiency is a common disease especially in civilized countries; its typical symptoms include calf cramps, pain, sensation of fatigue, leg ulcers, heaviness and itching [44].

Melilotus is used for symptomatic treatment of venous insufficiency, therefore, *Melilotus* has been used as an important ingredient in many herbal drug formulae alone or in combination, such as FLEBS CREMA (dermo-cosmotic compound include *Melilotus* and *Ruscus* extract) [54], a combination of alphatocopherol, rutin, *M. officinalis*, and *Centella asiatica*[55], *Melilotus extract* ®(Linnea co.), *lymphaselect*® (Indena co.), *Esberiven*® (France)[25], *Venalot*® (Germany)[25], *Troxerutin*® (Germany)[22,56].

Many clinical studies have been carried out using some of these *Melilotus* preparations which were given orally [22] or topically [54, 55].

These studies showed a significant improvement of the clinical parameters of chronic venous insufficiency (pain, cramps, heaviness) [22, 54] in 90% of patients especially those who did not wear elastocompressive stockings [55].

A patent German preparation concerns the use of thrombolytics in tablet form for the treatment of thromboembolytic disposition; it includes a combination of acetylsalicylic acid and the dried extract of *M. officinalis* [57].

16. Estrogenic activity:

Phytoestrogens are plant estrogen, where their structures are similar to estrogen, such as isoflavones and lignans. Isoflavone can bind and activate the estrogen receptor like true estrogen. *Melilotus* is an important source of isoflavones [58], the most important of which is coumestrol (3, 9-dihydroxypterocarpone) isolated from the seeds of *M. indica*. This isoflavone was found to possess a good estrogenic activity [59].

17. Genus *Melilotus* as a component in cosmetic preparations:

Some patent Japanese cosmetic preparations containing *Melilotus* extracts as the main ingredient are available, such as skin-lightening preparations [60] and skin-moisturizing preparations [61].

Furthermore, ethanolic extract of *M. officinalis* has the effect of potentiating the production of laminin 5 in epidermal cells. That is why Japan intended to provide a wrinkle-preventing cream containing *Melilotus* extract [62]. Also, *Melilotus* extract is used for prevention of skin staining, where it acts as a macrophage activator and a melanin production inhibitor [63].

A patent Korean cosmetic composition containing dried mixture extracts of *M. officinalis* and *Trichosanthes kirilowii Maxim* provided anti-inflammatory and moisturizing effects [64].

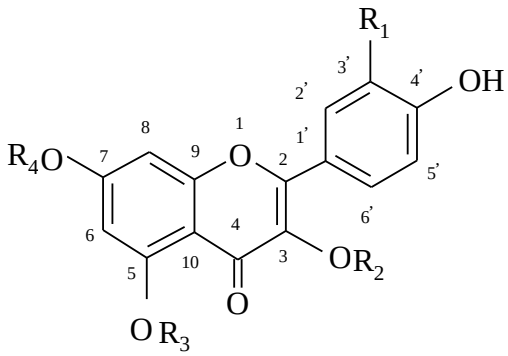
Previous Phytochemical Investigation of Genus

Melilotus

Literature survey of genus *Melilotus* revealed the presence of different types of flavonoidal constituents, saponins, phenolic acids, coumarins, sterols and miscellaneous compounds.

A) Flavonoidal constituents of genus *Melilotus*:

Table 2 - Flavonoids previously isolated from genus *Melilotus*

NAME	STRUCTURE				SOURCE
FLAVONOL					
	R_1	**R_2**	**R_3**	**R_4**	
Kaempferol	H	H	H	H	*M. siculus*[43] *M. officinalis*[65]
kaempferol 3-*O*-galactoside-7-*O*-rhamnoside	H	-gal	H	-rha	*M. elegans*[20, 66, 67]
kaempferol 3-*O*-rhamnosyl-galactoside-7-*O*-rhamnoside (robinin)	H	-gal-rha	H	-rha	*M. elegans*[22, 66, 67] *M. officinalis*[68, 69]
kaempferol 3-*O*-galacto-gluco-arabino-rhamnoside	H	-rha-ara-glu-gal	H	H	*M. officinalis*[68]
kaempferol 3-*O*-galacto-glucoside-7-rhamno-rhamnoside (melitin)	H	- glu-gal	H	–rha-rha	*M. alba*[70]
Quercetin	OH	H	H	H	*M. siculus*[43] *M. officinalis*[69]
Quercetin-3-*O*-galactoside	OH	-gal	H	H	*M. siculus*[43]