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Study the factor for extracting the leaves of the wild plant *Melilotus officinal* L. growing under the conditions of Basra Governorate

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I. Abstract:

Given the medicinal importance of the wild plant' melilot' and its widespread distribution in the soils of Basra, this study was conducted to identify the main active components of the essential oil extracted from its leaves using GC-MS using the hexane maceration method. The results revealed the presence of 34 compounds, the most important of which is Coumarin, a highly medicinal compound, which constituted 2.45%.

Keywords: wild fennel, essential oils, GC-MS, active compounds

II. Introduction:

Many wild plant species are found in Basra Governorate, serving medicinal purposes, as they are an important source of active ingredients that offer numerous benefits that synthetic chemical medicines lack (Chevalier, 1996). These substances are known as secondary metabolites, including essential oils obtained from different parts of the plant. They are of great importance in many fields, such as pharmacy, medicine, chemistry, and the perfume industry. They are sources of aromatic chemicals. The biological and pharmacological activities of essential oils and their components have gained prominence in recent years. They are indispensable for combating infectious or parasitic agents that cause diseases (Derwichet *et al*., 2010 ; Zoubiri *et al*., 2014).. Among these plants is the wild one, *Melilotus officinalis* L., an annual winter plant belonging to the legume family Fabaceae. It is erect and branched, reaching a height of about 60 cm. It has compound, pinnate, trifoliate leaves with small, slender petioles. The leaflets are small, oval, and inverted. The edges are serrated, and there is a red line in the midrib. Flowers are small and clustered in racemes with a long stalk, the fruit is a spherical, flattened, wrinkled, and often contains a single, small, spherical, yellow seed. Plant found in many countries around the world, including India, Europe, and Africa grows after rainfall, especially in irrigated farms and home gardens. , blooms in March and August. The plant's secondary metabolites contain terpenes, coumarins, and steroids (Ahmed and Zara, 2012). The plant has antibacterial, anticoagulant, skin moisturizing, expectorant, and treats parasitic diarrhea. It is used as an ointment for inflamed areas of the body, tumors, and pain (Qureshi *et al*., 2008). It also treats allergies and mucosal diseases (Shamim *et al*., 2015), liver diseases, symptoms of bruises, insect bites, and high blood pressure (Menkovia *et al*., 2011). Gudzenko and Vinogradov(2014) studied the active components in the essential oils of the *Melilotus officinalis* L. grown under Ukrainian conditions using the Gc-mass technique and revealed the presence of

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25 compounds, the compound hexahydrofanesy lacetone occupied the highest percentage of 16.64%, followed by B.cudesmol 11.49%, globulol 8.65%, and Bisablon oxide 7.43%. Jasicka-Misiak *et al*. (2017) noted when separating the active components in the wild plant' melilot' using the Gc-mass technique and steam distillation the presence of 26 compounds, benzena cetaldehyde occupied the highest percentage of 5.84%, B-methare 2.33%, B-phellandrene 2.17%, Phenylethyl alcohol 2.03%, and thymol 2.03%, while using Soxhlet extraction the compound Carophyllene appeared. 11.23%, P-eugenol 6.5%, and Phenylacetic acid 5.35%. Using the Ultra sound solution extraction method, the highest concentrations of Phenylacetic acid 25.54%, Pheyl methyl benzenethanol 6.44%, and P-acetoxyanisole 2.89% were obtained. As for the SPE solid phase extraction method, the two most abundant compounds were Phenylacetic acid N-bytylbenzen sulfonamide 13.04% and 10.94%. Al-Boudi *et al*. (2018) obtained when separating the main components of the essential oil extracted from the leaves of the wild plant' melilot' using the GC-MS technique with methanol using the Soxhlet device and the Hexane maceration method. 34 compounds were identified, constituting about 99.91% of the components of the essential oil. The origin of the compound n-Docosane 39.82% and Hydrocoumarin 15.39% and methyl 3-(2-hydroxyphenyle) propionate 14.29%. The essential oil was also extracted using the hexane maceration method, with Benzenedicarboxylic acid-1,2 accounting for 17.77% and octadecatrienoic acid-9,12,15 accounting for 12.85%. Ragab *et al*. (2021) found that when screening for active ingredients in the *Melilotus indicus* under Egyptian conditions using GC-MS, 32 compounds appeared. Dodecano,2-octyl-1,2 had the highest percentage (36.34%), gamma-sitosterol (12.26%), and heptaecosane (5.63%). This study aimed to conduct a chemical analysis of the essential oil extracted from the leaves of the wild plant' melilot' growing in Basra using GC-MS using hexane extract.

III. Materials and Methods:

The biologically active compounds in the oil of the leaves of wild plant' melilot' were identified by extracting the oil from the leaves using hexane in the laboratories of the College of Agriculture, University of Basra, Iraq. Gas chromatography-mass spectrometry (GC-MS) model QP210 Ultra.Shamadzu.APAN was performed, equipped with a DB-MAS5 capillary column (95% methylpolysiloxane, 5% vinyl) as the stationary phase, in addition to the use of helium gas (99.9%). The following conditions were used: Injection mode: split, column oven temperature: 50°C, injection temperature: 250°C, column flow: 1.53 ml/min, pressure: 90.0 kPa, purge flow: 6.0 ml/min, split ratio: 46.9, total flow: 79.2 ml/min, linear velocity: 44.8 cm/s, interface temperature: 250°C, mass spectrometer: ion source temperature: 200°C, Solvent cut-off time: 4.00 min, Detector gain: 0.84 kV + 0.40 kV, Start time: 4.00 min, End time: 41.71 min, ACQ mode: Scan, Event time: 0.40 s, Scan speed: 2000, Start speed: 35.00, End speed: 800 (Al-jabir *et al*.,2020).

IV. Results and Discussion:

The main components of the essential oil extracted from the leaves of the wild plant' melilot' with hexane (Table 1) and Figure (1) were identified using GC-MS. 34 compounds were identified as the main components of this essential oil, arranged in order of their percentages, as follows: Tricosene-9 (35.5%), (Z,Z,Z) octadecatrienoic acid -15-12-9 (14.39%), Tetracosanal (12.39%), gamma-sitosterol (4.85%), Heptadecane (4.75%), n-Hexadecanoic acid (3.42%), phytol (2.88%), and Coumarin (2.45%). The latter has important medical roles, as it is an antifungal, anticoagulant, and antidiabetic foot

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ulcer (Jasicka-Mislak *et al*. 2017). It also has an inhibitory effect on the growth of cancer cells. Directly, it works to inhibit the incorporation of nucleoside type Thymidine and Uridine (H3) into DNA and RNA respectively, as well as inhibiting the incorporation of the amino acid Leucine (H3) involved in the process of protein synthesis (Marshall *et al*., 1994). Gawron and Glowiak (1982) confirmed that the Coumarin compound has a clear effect on the growth of cancer cells of the Hela cell type, according to its ability to bind with the cell components and then affect the metabolic activities of the cell. We conclude from this study that the wild chicory plant has a good content of active components, especially Coumarin, which has medical and pharmaceutical importance for the possibility of accepting it as a suitable application for treatment or an auxiliary treatment.

Table:Components Indentified in *Melilotus officinal* L.

No.	Name of the compound	RT	Peak Area %
1	Tetradecane	16.749	0.377
2	Coumarin	17.513	2.452
3	Hexadecane	19.207	0.245
4	Diethyl Phthalate	19.291	0.207
5	3-Eicosene, (E)-	21.346	0.486
6	n-Hexadecanoic acid	23.108	3.427
7	Eicosane	23.41	0.259
8	1-Naphthalenepropanol, .alpha.-ethenyldecahydro-.alpha.,5,5,8a-tetramethyl-2-methylene-, [1S-[1.alpha.(R*),4a.beta.,8a.alpha.]]-	24.249	0.279
9	Phytol	24.534	2.886
10	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	24.869	14.396
11	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	24.978	1.989
12	Docosane	25.23	0.308
13	Phytyl decanoate	25.44	0.687
14	Phytol	26.874	0.981
15	Pentacosane	27.688	0.719
16	Bis(2-ethylhexyl) phthalate	28.191	0.596
17	Tetracosanal	28.745	0.501
18	Heptadecane	29.19	4.729
19	Pentacosanal	29.483	0.335
20	13-Docosenamide, (Z)-	29.886	1.496
21	Tetracosanal	30.221	12.493

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22	**9-Tricosene, (Z)-**	**30.691**	**35.519**
23	**Nonacos-1-ene**	**31.345**	**0.464**
24	**Hexacosanal**	**31.664**	**1.766**
25	**Hentriacontane**	**32.084**	**1.409**
26	**1-Tricosene**	**32.176**	**0.736**
27	**dl-.alpha.-Tocopherol**	**32.704**	**0.247**
28	**p,.beta.-Dinitrostyrene**	**32.981**	**0.23**
29	**Campesterol**	**33.954**	**0.605**
30	**Tetracosane**	**34.063**	**0.728**
31	**1-Tetracosene**	**34.248**	**2.95**
32	**.gamma.-Sitosterol**	**35.062**	**4.853**
33	**Stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z)-**	**35.263**	**0.383**
34	**N-Methyl-1-adamantaneacetamide**	**36.337**	**0.259**

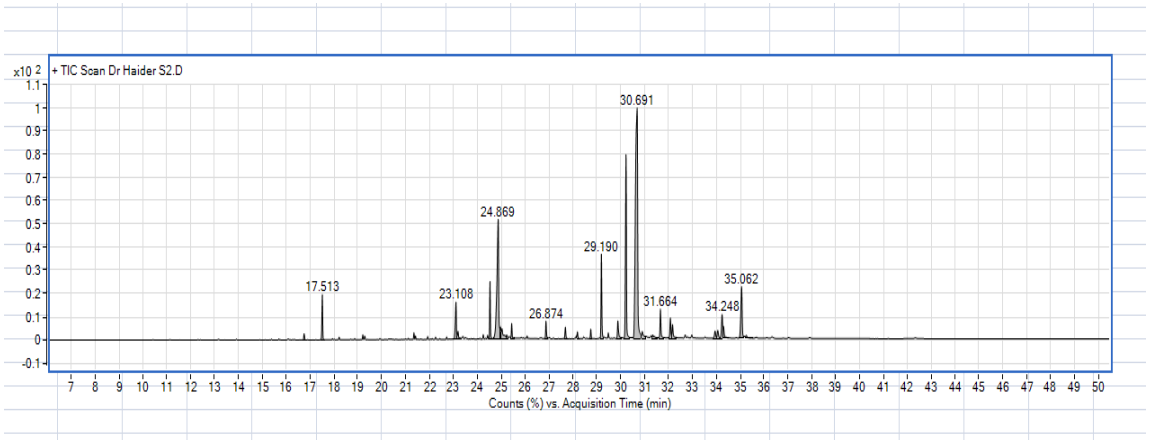

Fig.1:

A bioactive compound in ***Melilotus officinal*** **L**. leaves that indentified by GC-Mass device

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