

Chemical and Biological Study of Iraqi Kurdistan Chamomile Flower (*Matricaria recutita* L)

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Abstract:

In this study, the chamomile flowers (*Matricaria recutita*L) which grow in Iraqi Kurdistan region during the seasons of (2008) are collected. The percentage of essential oil was determined by using steam distillation and the extraction of flowers performed with petroleum ether (70-80) °C and methanol 70% using ultrasonic extraction. Total phenolic compounds were determined from methanol extracts by using Folin-Ciocalteu method. The extracts were evaluated by thin layer chromatography, ultraviolet absorption and the biological activities were evaluated through their antibacterial action against two types of bacteria using hole method. The flowers showed a composition of 0.071% ash, 0.4% essential oil, 3.2% non oily compounds, 4% oil, 1.9% moisture and 0.19% total phenols. The results showed that the flowers contain biological active compounds and they can be used for treatment of diseases.

Key words: Chamomile, Chromatography, Ultrasonic, Anti-bacterial activity

Introduction:

The use of chamomile (*Matricaria recutita* L.) family *Asteraceae* as a medicinal plant dates back to ancient Greece and Rome. There are two types of chamomile: German chamomile and Roman chamomile. The name "chamomile" comes from two Greek words meaning "ground apple" for its apple like smell. It was used to treat back pain, neuralgia, rheumatism, skin condition and gout [1]. Chamomiles essential oil is a treatment used for malaria and parasitic worm infections, colds and flu [2, 3]. One hundred twenty chemical constituents have been identified in chamomile, including terpenoides, flavonoids and coumarin. It has anti-inflammatory, ant allergic and antispasmodic properties [4], the anti-inflammatory and antiviral effects of chamomile are well documented in animals [5, 6, 7]. Bisabolol comprises 50% of German chamomiles essential oil [8], and it is spasmolytic for

intestinal smooth muscle [9, 10]. It also has anti inflammatory, antibacterial, antipyretic, ulcer protective and antifungal properties [11, 12]. But still no scientific investigation has so far been reported about Iraqi Kurdistan chamomile. Therefore the present investigation has been designed to determine the amount of essential oil, total oils and total phenol content in this plant, and to study their anti-bacterial effects against gram positive and gram negative bacteria.

Materials and Methods:

The flowers of the studied plant were collected during their flowering period in 2008. The flowers were air dried in shade, and preserved in 0°C.

1- Isolation of essential oils [13]

Plant material (200 g) was subjected to steam distillation for 3 hr, in a Clevenger modified apparatus with a water-cooled oil receiver to reduce

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overheating artifacts. The essential oils were collected over water, separated, then dried over anhydrous sodium sulfate and stored at (4-5) °C until they were used. The oil was light yellow and yielded 0.4%v/w.

$R_f = 0.5 \quad 0.72 \quad 0.75 \quad 0.9$ (Benzene: Chloroform 5:5).

2- Ultrasonic extraction of total oil [14]

After isolation of essential oils, the shade-dried powder (20 g) of chamomile flowers soaked with (200) ml of petroleum ether (60-80)°C for(24) hrs and sonicated for(2) hrs in an ultrasonic bath at constant 25°C. The oily extract was filtered, concentrated under reduced pressure and dried in a vacuum at 60° C. The residue (4 g/100 g flower) dark greenish blue oil was obtained.

$R_f = 0.5 \quad 0.76 \quad 0.88 \quad 0.91$ (benzene: chloroform 5:5)

3- Ultra sonic extraction of the flowers with methanol [15]

The fine powdered flowers of chamomile (100 g) was soaked with (200 ml) of methanol for (24) h and sonicated for (2) h in an ultrasonic bath at constant 25°C. The yellow colored extract was evaporated under reduced pressure and dried in a vacuum. The residue (3.0g/100g flower) dark yellow extract was obtained.

$R_f = 0.4 \quad 0.62 \quad 0.75 \quad 0.88$ (ethanol: H₂O 5:5)

4- Determiration of total phenolic contents

The extract of ultrasonic extraction of the chamomile flowers with methanol (200 µl) was added to a (50 ml) volumetric flask containing about (25 ml) of water, at time zero, (2.5 ml) of the Folin-Ciocalteu reagent was added. After three minutes, (5 ml) of saturated sodium carbonate was added with bringing to volume with water. A calibration curve was prepared using Gallic acid and the absorbance of the standards and samples were measured at 765 nm

using a Spectronic Genesys UV/V spectrophotometer. The measurements were recorded as milligrams of Gallic acid equivalents per gram of the flowers (mg/g of flower) [16].

5- Antibacterial activity tests

Antibacterial activity of essential oil, total oil and methanolic extract of the Kurdistan chamomile flowers were measured using pore plate diffusion technique [17]. Muller Hinton agar plates were swabbed with a suspension of each bacterial species using sterile cotton swab. Six plugs were removed from each agar plate using a sterile cork bored to produce 8 mm diameter hole. Different concentrations of the three extracts were added, and allowed to diffuse at room temperature for 20 min. The plates were incubated at 37°C for 24 hrs and the antibacterial activity of the plant extracts were recorded as the mean diameter of the resulting inhibition zones.

Results and Discussion:

Table (1) summarizes the approximate analysis of chamomile flower (*Matricaria recutita* L.) which grown in Iraqi Kurdistan. As it is shown ,these flowers contain ; essential oil (0.4%),total oil (4)%, total phenolic contents (0.19)% and non-oily compounds (3.2)%.The essential oil is obtained during the steam distillation of the powdered flowers ,and total oil by using ultrasonic extraction. Thin layer chromatography of these two oils showed four different spots, while ultrasonic extraction of the flowers with methanol showed presence of many compounds as it is appear in its ultraviolet absorption figure (1).

Figure (2) represents the ultraviolet absorption of the separated total phenolic compounds, which determined by Folin-Ciocalteu reagent. The figure shows absorption at (680,

610, 500, and 540) nm. Since there is no authentic materials and mass spectroscopy, it is not possible to identify these components.

These extracts were also investigated for their antibacterial activity against gram positive and gram negative bacteria. The pore method was used for testing various concentrations of these extracts.

It is obvious from the data in table (2) that minimum concentration of chamomile essential oil has inhibitory effect against *Staphylococcus aureus* (15 mm) and *E.coli* (12mm). However, this oil showed high inhibition action at (30 mg/ml) on *staphylococcus aureus* (25mm).

The petroleum ether (60-80) °C of chamomile flower (5-30 mg/ml) showed no inhibitory effect on the growth of *E.coli*, while minimum concentration (5 mg/ml) of this extract produced (12.2 mm) zone of inhibition against *Staphylococcus aureus*.

Whereas, minimum concentration of the methanolic extract of chamomile flower (5 mg/ml) was effective only against *Staphylococcus aureus*. On the other hand, *E.coli* was not affected by the methanolic extract at (5-30 mg/ml). In general, the results in table (1) showed that these flowers contain high amount of oil, and phenolic compounds. Also it is clear from table (2) that chamomile essential oil has a distinguishable antimicrobial activity against *Staphylococcus aureus* and *E.coli*, that is could be ascribed to the terpenoids such as α -bisabolol, α -bisabolol oxide A and B, and Chamazoline, which are naturally occurring in the plant flowers [18].

The finding that petroleum ether (60-80)°C and methanolic extracts of the chamomile flowers showed inhibitory effect on the growth of *Staphylococcus aureus* is may be due to the Flavonoids, Cumarins, and Spiro ethers contents in these

extracts[19], The resistance of *E.coli* to the petroleum ether and methanolic extracts may be due to the cell membrane permeability[20]. The results of this study supported by the work of some researchers [21] who reported the antimicrobial activity of chamomile (*Matricaria recutita* L.) flowers extracts.

Since chamomile tea has long use in folk medicine, it is used externally and internally to treat an extensive list of conditions, and it is widely cultivated in Iraqi Kurdistan Region. It seems important to candidate for further research to help in saving the lives of many people.

Table (1): The approximate analysis of Iraqi Kurdistan Chamomile Flowers

Analysis	%percent composition
Ash	0.07
Moisture	1.9
Essential oil	0.4
Total oil (petroleum ether extract)	4
Non- oily compounds (methanol extract)	3.2
Total phenolic compounds	0.19

Table (2): Inhibition zones (mm) of Kurdistan chamomile flower extracts at various concentration on two types of bacteria

Type of Extract	Amount of extract mg/ml	Diameter of inhibition zone (mm)	
		<i>Staphylococcus aureus</i>	<i>E. Coli</i>
Essential oil	5	≥ 15	≥ 12
	10	≥ 17.5	≥ 13
	20	≥ 21.5	≥ 15.5
	25	≥ 23	≥ 17.5
	30	≥ 25	≥ 20
Petroleum ether (60 - 80)°C extract	5	≥ 12.2	-
	10	≥ 13.5	-
	20	≥ 14.9	-
	25	≥ 15.5	-
Methanol extract	5	≥ 10	-
	10	≥ 11.5	-
	20	≥ 13	-
	25	≥ 13.5	-
	30	≥ 14.2	-

* All values expressed as mean of three replicates
(-) no activity

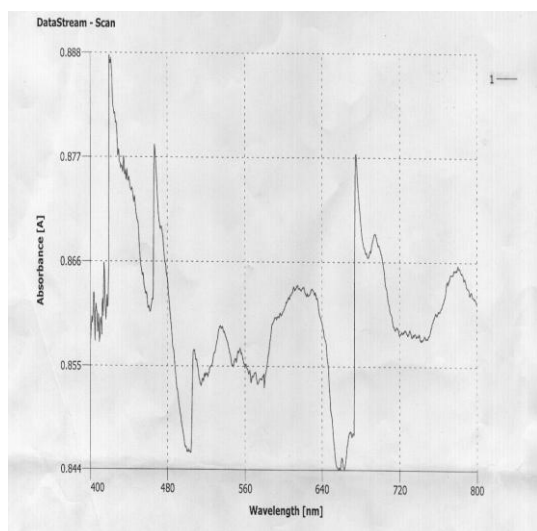


Fig (1): Ultraviolet spectra of methanol extract from Iraqi Kurdistan chamomile flowers

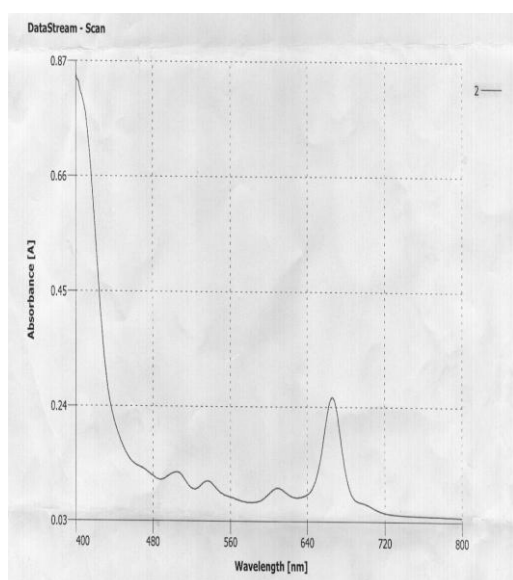


Fig (2): Ultraviolet spectra of total phenolic from Iraqi Kurdistan chamomile flowers

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دراسة كيميائية وبيولوجية لأزهار نبات بابونج كردستان العراق (*Matricaria recutita* L)

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الخلاصة:

تمت في هذه الدراسة جمع أزهار نبات البابونج في كردستان العراق سنة 2008. تم تحديد نسبة الزيوت الأساسية باستخدام طريقة التقدير البخاري، ووجد أن نسبة هذه الزيوت هي (0.4%). وقد تم فصل المواد ذات الخصائص الدهنية باستخدام مذيب الإيثر البترولي (60-80) درجة مئوية، وطريقة الألتراسونيك للإستخلاص، وبلغت النسبة المستخلصة (4%). وتم الحصول على مستخلص الميثانولي (70%) للأزهار باستخدام نفس الجهاز بنسبة (3.2%). أما المواد الفينولية الكلية فقد تم فصلها بطريقة فولين-سيوكالتين وبجهاز سبيكتروفوتوميتر، وبلغت نسبتها (0.19%).

ولغرض التعمق في دراسة تأثير هذه المستخلصات، تم استخدام طريقة كروماتوغرافيا الطبقة الرقيقة وطيف الأشعة فوق البنفسجية، واستخدمت تراكيز مختلفة من هذه المستخلصات ضد نمو نوعين من البكتيريا. وأظهرت النتائج أن هذه الأزهار تحتوي على نسبة (0.07%) رماد و (1.9%) رطوبة وعلى عدد من المواد الفعالة بيولوجياً والتي يمكن استخدامها في المجال الطبي.