Preliminary phytochemical, chemical composition and antibacterial activities of chamomile (*Matricaria recutita* L.) essential oil

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Abstract: Preliminary phytochemical, chemical composition and antibacterial activities of chamomile)*Matricaria recutita* L.) essential oil were studied. Preliminary phytochemical showed that percentages of sterols or tritrepenes, alkalids, glycosides, phenols and resins in flower of *M. recutita.* The essential oil from chamomile flowers was extracted by hydrodistillation. The components of essential oil were identified by GC–MS. A total of forty-two compounds were identified. α-Bisabolol oxide was the main compound, represented by 30.8%, followed by Camphene (10.7%), α-Pinene (9.2%), Isopropyl hexadecanoate (7.3%), Camphor (6.3), 1,8-Cineole (6.16%), Sabinene (4.13), and α-Terpinene 3.01 . Essential oil of chamomile was evaluated for its antibacterial activities against three bacterial subspecies, *Pectobacterium carotovorum sub sp carotovorum, Ralstonia solanacearum*, and *Streptomyces scabies*. Results showed that the essential oil had antibacterial activities against the three tested bacteria. Streptrol was used as a positive control. Bacteria *Streptomyces scabies* was more sensitive to essential oil.

Keywords: phytochemical - chemical composition - antibacterial activities - *Matricaria recutita L.* essential oil.

1.Introduction

Most synthetic chemicals as herbicides are halogenated hydrocarbons with relatively long environmental half-lives and have toxic properties than most natural compounds. Thus, natural compounds have increasingly become the focus of those interested in discovery of pesticides **(Duke,1990).** Natural products are used as new agrochemicals for controlling plant diseases **(Cardellina, 1988).**

The genus Matricaria, consists of about 40 species throughout the world **(Su *et al.,* 2012).** This genus is widely used as medicinal plant in the world **(Dragland *et al*., 2003).** *Matricaria recutita* L. (syn. :*M. chamomilla; M. suaveolens*; *Hungarian chamomile; German chamomile; Chamomilla chamomilla; Chamomilla recutita*) resides in the Asteraceae)Compositae) family **(Salamon, 1992).**

The essential oils of some *Matricaria* species were examined. The major components of the chamomile (*M. recutita*) essential oil were bisabolol, bisabolol oxides, chamazulene, farnesene and spiroether **(Matos *et al*., 1993; Pino *et al*., 2002; Sashidhara *et al*., ;2006 Karami *et al*., 2009; Gosztola *et al*., 2010; Orav *et al*., 2010).** Active compounds of chamomile are terpenoids: α-bisabolol, α-bisabolol oxide A ,and B-chamazulene, sesquiterpenes; coumarins: umbelliferone; flavonoids: luteolin, apigenin, quercetin; spiroethers: en-yn dicycloether and other components such as anthemic acid, choline, tannins, polysaccharides and phytoestrogens **(Newall *et al*., 1996; Bagchi *et al*., 2001; McKay and Blumberg, 2006; Karbalay-Doust *et al*., 2010)**

M. recutita is contains many active compounds. *M. recutita* is used as a medicinal herb with many applications in traditional medicine. The active components of *M. recutita* such as terpenoids and flavonoids are responsible for a wide range of biological activities **(Newall *et al.,* 1996).**

The biological activities of chamomile are due to the phenolic compounds, flavonoids, apigenin, quercetin, patuletin, luteolin and their glucosides, but also to the main components of the essential oil from the flowers like α-bisabolol and its oxides and azulenes, including camazulene **(Hadaruga *et al*., 2009).**

In recent years, natural products were used as novel antimicrobials, which are including higher plants, microorganisms, insects, nematodes and vertebrates **(Alinezhad 2011)** .Plants are rich with beneficial secondary metabolites. Plant extracts and essential oils (EOs) were had some widely biological activities, especially antimicrobial effects against different pathogenic organisms **(Bakkali 2008, Shams-Ghahfarokhi *et al*., 2006, Tolouee 2010 and Jamalian *et al.,* 2012).**

Plant pathogenic bacteria cause many diseases of plants in the world **(Vidhyasekaran, 2002)** There are some bacteria diseases such as soft rot caused by *Pectobacterium carotovorum carotovorum* on carrot, potato, tomato, leafy greens, squash and other cucurbits, onion, green peppers, etc,. Brown rot was caused by *Ralstonia solanacearum* (*Pseudomonas solanacearum*) on potato, bacterial wilt or southern wilt of tomato, tobacco, eggplant, and some ornamentals, and Moko disease of banana .Common scabies was cused by *Streptomyces scabies* on potato tubers.

The objectives of the current work were:

-1Phytochemical analysis.

-2Chemical composition of essential oil of *M. recutita* flowers.

-3Determine the bacterial activities.

2.Materials and methods

2.1.Plant material

M. recutita flowers were bought from local grocery store. Plant flowers were identified by Flora and Phytotaxonomy Unit, Horticultural Research Institute, Agricultural Research Centre. Flowers were washed and air dried at room temperature, then in an oven at 50 °C. Air-dried flowers were ground in an electric blender to become fine powder and kept at room temperature.

2.2. Preliminary phytochemical Investigation of *M. recutita*

According to the methods adopted by **Peach and Tracey (1955), Niazi (1972), Balbaa *et al.*, (1976) and Abu-**

Shaweish (1981) the flower powder was subjected to the following tests:

2.2.1. Tests for sterols and/or triterpenes

About 10 gm of flower powder was mixed with 20 ml of $CHCl_3$ and filtered. The filtrate was subjected to the following tests:

2.2.1.1. Liebermann - Burchard test

About 5 ml of $CHCI_3$ filtrate was evaporated to a small volume, 1 ml of acetic anhydride was added, and then 2 ml of H_2SO_4 were poured on the walls of the test tube to form a lower layer below the $CHCI_3$ solution. Formation of reddish-brown ring at the junction of the two layers indicates the presence of unsaturated sterols and/or triterpenes.

2.2.1.2. Salkowski test

About 5ml of concentrate H_2S0_4 was added to about 5 ml of $CHCI_3$ filtrate. Development of a red brown color indicates the presence of unsaturated sterols and/or triterpenes.

2.2.2. Tests of Alkaloids

About 10 g of the flowers powdered was mixed with 50 ml of dilute HCl acid. The acidic extract was filtered, rendered alkaline with dilute NH_4OH, and then extracted with $CHC1_3$. The chloroform extracts were evaporated to dryness. The residue was dissolved in 1 ml of dilute HC1. If this solute gave white turbidity with Mayer's reagent (Pot. Mercuric Iodide), brown precipitate with Wagner's reagent (Iodine-Pot. Iodide solution) and orange color with Dragendorffs reagent (Pot. Bismuth Iodide), alkaloids are positively present.

2.2.3. Test of flavonoids

About 5 g of the flowers powder was macerated overnight at 150 ml of cold 1% solution of HC1 acid, filtered; the filtrate was tested for flavonoids as follows:

a) NaOH was add to about 10 ml of the filtrate to make alkaline, a formation of a yellow color, indicates the presence of flavonoid compounds.

b) On shaking about 5 ml of the filtrate with 5 ml amyl alcohol, the absence of color from the alcohol layer, indicates the absence of free flavonoids.

2.2.4. Tests of glycosides

The reduction tests with Fehling solution on flowers powder, before and after hydrolysis with mineral acid are compared as follows:

The 1st test: About 1 gm of powdered drug with about 10 ml of alcohol was boiled for 5 minutes, then filtered through cotton in an evaporating dish, nearly to dryness on a water bath. Extract the residue with 5 ml of hot water and filter through filter paper and divide the filtrate into 2 equal portions

Portion (1) mixed with 0.5 ml NaOH and 1ml of Fehling's solution, heated on a water bath. Reduction was observed in the test tube. Portion (2) mixed with 1ml of dilute HC1, heat for 10 minutes in a boiling water bath, then neutralize with dilute solution NaOH and heated in a boiling water bath.

Compared the two test tubes, before and after hydrolysis, excessive reductions indicated the presence of glycosides. The 2nd test: An amount of 0.5 ml NaOH was added to extract and 0.5 ml excess Fehling's solution and heated on a water bath for 30 minutes, then filtered (the filtrate should show the blue color of Fehling's solution) acidified with dilute H_2SO_4, hydrolyze and heated on a water bath for 10 minutes then rendered alkaline by NaOH, then heated on a water bath. Red precipitation or even change of the color of the feeling's solution to green, indicates that the plant extract may contains glycosides.

2.2.5. Test of Saponins

Sample was dissolved in normal saline solution. An aliquot (5.0 ml) of the solution was added to 10 ml of 1: 40 suspension of red blood corpuscles in normal saline solution and the solution was then added for 5 minutes to detect the presence of saponine through the observation of heamolysis.

2.2.6.Test of Tannins

Two ml of bromine water were added to 5.0 ml of the ethanol flowers powder extract, tannins was detected by buffer colored precipitate.

2.2.7. Test of Resins

In a porcelain dish sample was dissolved in 10.0 ml acetic acid anhydride by the aid of gentle heat, then cooled and a drop of concentrated sulfuric acid was added. A bright purplish red color was produced which changed to violet and then to brown in the presence of resins.

2.2.8. Test of Phenols

The Folin-Denis reagent was used to detect the phenol compounds. Flowers powder was dissolved in distilled water then one ml. of Folin-Denis reagent was added. A blue color was produced in the presence of phenols. Folin -Denis reagent: 100-gram sodium tangistate, 20-gram phosphomolbdic acid, and 50.0 ml. concentrated Phosphoric acid, then 750 ml distilled water was added. The mixture was boiled under reflex condenser for two hours. After cooling the solution was completed to one liter (the color of the solution was green-yellow).

2.3.Extraction of essential oils from *M.recutita* flowers:

The essential oil from flowers was extracted by hydro distillation using an apparatus of Clevenger. About 500 g of flowers of *M. recutita* were used with 1600 ml of distilled water. The extraction took 6 hours. The solvent was filtrated and eliminated by reduced pressure distillation in rotary evaporator. The essential oil was stored at 4°C in brown tube till analysis and examined for antibacterial activities.

2.4.Identification by GC/MS:

Agilent 6890 gas chromatography equipped with an Aglient mass spectrometric detector, with a direct capillary interface and fused silica capillary column (30 m x 0.32 mm x 0.25 um film thickness). Essential oil was injected under the following conditions:

Helium was used as carrier gas at approximately 1 ml/min., pulsed split less mode. The solvent delay was 3 min. and the injection size were 1.0 ul. The mass spectrophotometric detector was operated in electron impact ionization mode ionizing energy of 70 e. v. scanning from m/z 50/500 the ion source temperature was 230 ◦C. the electron multiplier voltage (EM voltage) was maintained 1050 v above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was start at 60 ◦C (3 min.) then elevated to 280 and 250 ◦C, respectively. Wiley and Nist 05 mass spectral

data base was used in the identification of the separated peaks.

2.5.Bacteria:

The bacteria used in the present study were three subspecies *Pectobacterium carotovorum sub sp carotovorum* (*P.c.carotovorum*), *Ralstonia solanacearum* (*R. solanicerium) and Streptomyces scabies* (*S. scabies*) was obtained from Faculty of Agriculture, El-Shatby Alexandria University.

2.6. Antibacterial test

Concentrations 1, 2,3,4,5 and 6 mg/ml of essential oil of *M. recutita* flowers and bactericide (streptrol, 0.1 mg/ml) were screened for their antibacterial activities against tested bacteria using same technique for Antibacterial test. filter paper disc was steeped in sterile water and inoculated plates as control. Inhibition zone around disc was measured after 72h incubation at 30°C. Three replications were done for each one.

2.7.Statistical analysis

The obtained data in all parts of this study were statistically analyses, using the stander mothed of completely randomized design (CRD). Data were tested using the general linear model (GLM) procedure of the statistical analysis system (SAS) (version 9.3). The difference among the means were tested and compared using the least significant difference (LSD) at P<0.05 **Steel and Torrie (1960).**

3.Results and Discusstion

3.1. Preliminary phytochemical investigation of the *M. recutita* flowers:

General features of preliminary phytochemical investigation of the *M. recutita* flowers were presented in Table (1). Data showed that all of sterols or tritrepenes, alkalids, glycosides, phenols and resins were presented in flowers powder of *M. recutita.* The presence of preliminary phytochemical in *M. recutita* was supported by **(Murti *et al.*,2011**). Also *M. recutita* are including terpenoids and flavonoids which are responsible for a wide range of biological activities **(Newall *et al.,* 1996).**

3.2. Chemical composition of essential oil of *M. recutita* flowers:

The major components of essential oil of *M. recutita* flowers were identified by GC/MS are indicated in Table (2). Forty-two compounds were found in essential oil of *M. resutita*, which α-Bisabolol oxide (30.8%) was the principal component followed by Camphene (10.7%), α-Pinene (9.2%), Isopropyl hexadecanoate (7.3%), Camphor (6.3), 1,8-Cineole (6.16%), Sabinene (4.13), α-Terpinene (3.01).

Table (1): Preliminary phytochemical investigation of *M. recutita* flowers

Test	M. *recutita*
Sterols or tritrepenes	+
Alkalids	+
Glycosides	+
Phenols	+
Tanins	-
Saponin	-
Resins	+

+present, -absent

Other compounds comprised 1.86% of total oil. Essential oils in aerial parts were depended sample origin, environmental and climatic conditions **(Kazemi, 2015).** The essential oil of *M. recutita* was isolated and identified of thirty-seven components. The major components were bisabolol oxide A (20–33%) and B (8– 12%), bisabolon oxide A (7–14%), (E)-farnesene (4–13%), α- bisabolol (8–14%), chamazulene (5–7%), and en-yn-dicycloether (17–22%) **(Orav *et al*., 2001).** Another study indicated that the major components of the essential oils were as follows: bisabolol oxide A (39.4%), bisabolone oxide A (13.9%), (*Z*)-en-yne-dicycloether (11.5%), bisabolol oxide B (9.9%), α-bisabolol (5.6%), and chamazulene (4.7%) **(Raal *et al*., 2011).** Another study showed that the major components in the chamomile essential oil were camazulene (19.9%), α bisabolol (20.9%), A and B bisabolol-oxides (21.6% and 1.2% respectively) and β-farnesen (3.1%). At lower concen-

Table (2): Chemical composition of essential oil of *M. recutita* flowers

Number	Components	%
1	α-Pinene	9.2
2	Camphene	10.7
3	Sabinene	4.13
4	D-3-Carene	0.21
5	α-Terpinene	3.01
6	P-Cymene	0.40
7	β-Phellandrene	0.85
8	Limonene	0.26
9	1,8-Cineole	6.16
10	Benzeneacetaldehyde	0.32
11	γ-Terpinene	0.98
12	Artemisiaketone	0.24
13	Z-Sabinenehydrate	0.59
14	α-Linalool	0.13
15	α-Thujone	0.68
16	β-Thujone	0.72
17	E-Sabinol	0.74
18	Camphor	6.3
19	Borneol	0.58
20	4-Terpineol	0.61
21	α-Terpineol	0.86
22	Isomenthyl acetate	0.8
23	E-Piperitol	0.47
24	α-Cubebene	0.34
25	α-Terpinylacetate	0.57
26	α-Isocomene	0.15
27	β-Eemene	0.64
28	α-Funebrene	0.39
29	Isocaryophyllene	0.37
30	β-Caryophyllene	0.62
31	E-β-farnesene	0.51
32	Germacrene	0.51
33	Bicyclogermacrene	0.32
34	E-Nerolidol	0.61
35	Spathulenol	0.42
36	Caryophyllene oxide	0.25
37	α-Bisabolol oxide	30.8
38	α-Bisabolol	1.1
39	Chamazulene1	1.6
40	α-Farnesene	1.5
41	β-Farnesene	1.2
42	Isopropyl hexadecanoate	7.3

trations of essential oil were identified caryophyllene-oxide, α- and β-caryophyllene and spathulenol, and some monoterpenes like β-phellandrene (0.8%), limonene (0.8%), β-ocymene (0.4%) and γ-terpinen (0.2%) **(Costescu *et al*., 2008).**

3.3.Antibacterial activities of essential oil of *M. recutita* flowers*:*

Antibacterial activities of the *M. recutita* were presented in Table (3). Data indicate that the oil exhibited had antibacterial activities against the tested bacteria at different concentrations. Also, data showed the inhibition zone increased with increasing concentration of oil. Result indicated that *S. scabies* was the most sensitive bacteria to oil *M. recutita* with the largest inhibition zone (3.46 cm) followed by *P. c. carotovorum* and *R. solanicerium* with inhibition zone (3.15 and 3.14 cm), respectively. Streptrol was used as a positive control. Streptrol exhibited the highest antibacterial effect against the three tested bacteria with concentration (0.1 mg/ml). The bacteria *S. scabies* was more sensitive to streptrol with inhibition zone (4.62 cm). The antibacterial activities of chamomile had reported by **Aggag and Yousef (1972).** Essential oil of chamomile components, α-bisabolol had strong activity against Gram-positive and Gram-negative bacteria. The active components of *M. recutita* are including terpenoids and flavonoids which are responsible for a wide range of biological activities **(Newall *et al.,* 1996).** The biological activities of chamomile oil are due to the phenolic compounds, such as; flavonoids quercetin apigenin, luteolin, patuletin and their glucosides, but also to the main components of the essential oil extracted from the flowers like α-bisabolol and its oxides and azulenes, including camazulene **(Hadaruga *et al*., 2009).**

Conclusions

The essential oil of *M. recutita* had antibacterial properties. Oil may be used in conjunction with synthetic compounds. Sterols or tritrepenes, alkalids, glycosides, phenols and resins are present in *M. recutita* flowers. Data show forty-two compounds were identified in essential oil. Essential oil of *M. recutita* had antibacterial activity against the three tested bacterial subspecies*, P. c. carotovorum, R. solanicerium,* and *S. scabies***.**

Table (3):Antibacterial activities of essential oil of *M.recutita*

Treatment	Conc. (mg/mL)	A average inhibition zone (cm)		
		P.c. carotovorum*	***R. solanicerium***	***S. scabies***
Control		0.00 ± 0.00	0.00±0.00	0.00±0.00
M. recutita	1	1.41±0.18	1.49±0.14	1.63±0.14
	2	1.44±0.01	1.61±0.19	1.70±0.06
	3	1.73±0.25	2.07±0.12	2.18±0.17
	4	2.22±0.19	2.46±0.24	2.73±0.04
	5	2.71±0.04	2.86±0.27	3.06±0.08
	6	3.15±0.13	3.14±0.16	3.46±0.22
Streptrol	0.1	4.01±0.12	4.13±0.17	4.62±0.20
	LSD0.05	0.041	0.037	0.039

Pectobacterium carotovorum sub sp carotovorum* (*P.c. carotovorum*), *Ralstonia solanacearum* (*R. solanicerium) and *Streptomyces scabies* (*S. scabies*).*

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الاختبارات الإبتدائية الفيتوكميائية والتركيب الكيماوي والنشاط البكتيري لزيت البابونج

سناء عبداللة مسعود

مركز البحوث الزراعية-محطة بحوث ايتاى البارود-محافظة البحيرة-مصر

الملخص العربى

تم دراسة كلا من الاختبارات الفيتوكميائية الأولية والتركيب الكيماوي لزيت ازهار البابونج (*Matricaria recutita* L)وتأثيره على ثلاث سلالات بكتيريا. فأظهرت الاختبارات الفايتوكميائية الأولية أن الأزهار تحتوي على كلا من المركبات الآتية:

الإستيرول أو التراى تريبسين، القلويدات، الجليكوسيدات، الفينولات والراتنجات. وتم فصل الزيت والتعرف على مكوناته بواسطة جهاز GC/MS فوجد أنه يحتوي على 42 مركب وكانت أكثر المركبات نسبه في الزيت هم كتالي:

أوكسيد الألفا-بيزابلول (30.8%) والذي كان المكون الرئيسي تلاه مركب الكامفين (10.7%)، الألفا بَينين (9.2%)، الأيزوبروبايل هيكساديكانوات (7.3%)، كامفور (6.3%)، 8,1-كينيول (6.16%)، سابينين (4.13%) وألفا-تيربينين (3.01%).

وعند دارسه تأثير زيت البابونج على البكتيريا فوجد ان الزيت له تأثير على البكتيريا المسببة للجرب والعفن البنى والعفن الطري، كما ان البكتريا المسببة للجرب هي اكثرهم حساسيه للزيت. كما استخدم المبيد البكتيري استربترول كمقارنه مع الزيت البابونج الذي كان له تأثير قوى وفعال ضد البكتيريا.