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Antifungal activity, phytochemical analysis and chemical composition of Chamomile (*Mtricaria recutita* L.) essential oil against *Rhizoctonia solani* and *Fusarium solani*

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ABSTRACT

Phytochemical analysis revealed that flavonoids, alkaloids, tritrepenes, steroid and resins components are present in flowers of *Matricaria recutita*. Flowers essential oil (EO) of *M. recutita* prepared by hydrodistillation was identified by (GC/Ms) a total of 37 compounds were identified in the plant oil. α-Bisabolol oxide (32.1%) was the principle constituent, followed by Camphene (9.8%), α-Pinene (9.2%), Isopropyl hexadecanoate (6.6%), Camphor (6.0), 1,8-Cineole (6.1%), Sabinene (3.9), α-Terpinene (3.2%). Essential oil was evaluated for its antifungal activity against some pathogenic fungi, *R. solani* and *F. solani* under laboratory condition, it was tested by food poisoned technique. Result indicated that the growth of fungi *R. solani* and *F. solani* at amount of 0.75, 0.375, 0.1875 and 0.0935 µL/mL compared with negative control (0 µL/mL) and positive control (Dimethomorph 0.5g/L). Essential oil at 0.75 µL/mL had good antifungal activity against the growth of *R. solani* and *F. solani. R. solani* was the more sensitive to essential oil *M. recutita* with the largest inhibition zone 73% followed by *F. solani* with inhibition zone 62% compared with negative control with inhibition zone 0.0% for *R. solani* and *F. solani.*by the use of by Dimethomorph 0.5g/L (fungicidal) for *R. solani* and *F. solani* it gaves the result of 76% and 71 % inhibition zone for *R. solani* and *F. solani,* respectively as a positive control.

***Keywords*:** phytochemical analysis, chemical composition, *Mtricaria recutita* L. essential oil, *Rhizoctonia solani, Fusarium solani*, Dimethomorph.

Introduction

Nowadays, application of chemical compounds is considered as the most inexpensive and the most common method in plant disease control. However, their adverse affects on human health and the environment promoted man to produce natural pesticides (Hayes and Laws 1991). Natural products are used as new agrochemicals for controlling plant diseases (Cardellina, 1988). Biological active compounds found in plants appear to be more adaptable, acceptable and safer than synthetic compounds and display a healthy source of potential pathogens control agents (Tripathi *et al.,* 2008). The composite family contains very useful medicinal genera such as *Matricaria* (Hadaruga *et al*., 2009). *Matricaria recutita* L. (syn. *M. chamomilla* L., *Chamomilla recutita* L. Rauschert) both of the German chamomile, *Chamaemelum nobile* (L.) and (syn. *Anthemis nobilis* L.) are known as Roman chamomile (Shahram and Omid 2011). *M. recutita* is being cultivated commercially as a medicinal herb with several applications in traditional medicine in different parts of Iran especially, it has a stable natural monocyclic sesquiterpene alcohol named α-bisabolol as the main component (Pereira *et al.*, 1997; Rodriguez *et al.,* 1998).

The essential oil of some *Matricaria* species were examined. The major components of the chamomile (*M. recutita*) essential oil were bisoprolol, bisoprolol oxides, chamazulene, farnesene and spiroether (Pino *et al.,* 2002; Sashidhare *et al.,* 2006; Orav *et al.,* 2010).

The biological activity of chamomile is mainly due to the phenolic compounds, flavonoids apigenin, quercetin, patuletin, luteolin and glucosides, the essential oil constituents such as α-bisabolol and its oxides and azulenes (Hadaruga *et al*., 2009).

In recent years, medicinal plants were used as novel antimicrobials, insects, nematodes and vertebrates (Alinezhad, *et al.* 2011). Plants are rich with beneficial secondary metabolites. Their essential oils and extracts were had some widely biological activities, especially antimicrobial effects against different pathogenic organisms (Shams, *et al.* 2006; Bakkali, *et al.* 2008; Tolouee *et al.* 2010).

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This study was carried out to determine the phytochemical analysis, chemical composition and antifungal activities of (*Mtricaria recutita* L.) essential oil against *Rhizoctonia solani* and *Fusarium solani.*

Materials and Methods

1- Plant material

The fresh flowers of *Matricaria recutita* family compositae were collected from Research Station Qanater (Qalyubia), Research Department of medicinal and aromatic plants- Horticulture Research Institute, Agriculture Research Center. The flowers were washed with tap water followed by sterile distilled water, shade dried at room temperature and then powdered by using electrical blender to get fine powder for further use.

2- Phytochemical screening of *Matricaria recutita* flowers

According to the methods adopted by Peach and Tracey (1955), Harborne (1998) the flower powder was subjected to following tests.

2.1- Test for Flavonoids

Few drops of dilute sodium hydroxide were added to one ml of methanolic extract. An intense yellow color indicates the presence of flavonoid compounds.

2.2-Tests for Alkaloids

Dragendorffs reagent

It was prepared by mixing a solution of 0.8 g bismuth nitrate pentahydrate with 40 ml distilled water and 10 ml glacial acetic acid with solution of 8.0 g potassium iodide in 20 ml distilled water.

1ml dilute HC1(1%) were added to 5ml of chloroform extracts then 1ml of with Dragendorffs reagent was added. An orange or red precipitate produced immediately indicated the presence of alkaloids.

2.3- Test for Triterpenoids.

10mg of extract chloroform were added 1 ml of acetic anhydride and 2 ml of conc. H_2SO_4 were added. Formation of reddish violet colour indicated the presence of triterpenoids.

2.4- Test for Steroids

One ml of the extracts was mixed with 10ml of chloroform, then equal volume of concentrated sulphuric acid was added at sides of the test tube. The upper layer turns red and sulphuric acid layer showed a yellow with green fluorescence. This indicated the presence of steroids.

2.5- Test for Saponins

The extract was diluted with 20ml distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam showed the presence of saponins.

2.6- Test for Tannins

Few drops of (1%) lead acetate were added to 5ml of the extract. A yellow precipitate was formed, indicated the presence of tannins.

2.7- Test for 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 conc. sulfuric acid was added. A bright purplish red color was produced which changed to violet and then to brown in the presence of resins.

3- Isolation of the essential oils from *Matricaria recutita*

The fresh flowers of *Matricaria recutita* was extracted by hydro distillation for 6 hours in Clevenger. The aqueous phase was saturated with sodium chloride and extracted with diethyl ether.

The ether was dried over anhydrous sodium sulfate and concentrated at room temperature. The essential oil was stored at 4°C in brown tube till analysis and examined bioassay testing.

4- Identification of the chemical composition by GC/Ms

To detect the chemical composition the mass spectrometer (Shimadzu Qp-2010 plus) scanned over the range of 50-650 m/z set at an ionizing voltage of 70 ev; values were compared with those from the standard mass library of the Micro Analytical Center- Cairo University.

5- Tested Fungicide

Trade name: Agromorf 50% WDG
Common name: Dimethomorph**.**
Chemical name: $C_{21}H_{22}CINO_4$
Recommended rate of application: 50g/100 L.

6-Fungal strains used

Pure cultures of *Rhizoctonia solani* and *Fusarium solani* were supplied from the department of Fungicides, Bactericides and Nematicides, Central Agricultural Pesticides Laboratory, A. R. C.

7- Antifungal assay

Antifungal activity of oil *Matricaria recutita* was determined by food poisoned technique (Mohanty *et al.,* 2012) added to get the required concentration, oil at 0.9375, 0.185, 0.375, 0.75 µl/ml were mixed with 50ml of sterilized PDA medium and transferred equally into three Petri dishes. The media was allowed to solidify. Then seven days old fungal culture disk of 5-mm diameter was taken and inoculated to the center of Petri dishes containing oil *Matricaria recutita* compared with two control, fungicide Dimethomorph 50 % (WDG) at recommended concentration as a positive control and PDA medium without oil served as a control. All dishes were incubated at 27±2°C and radial growth of colony was measured when the mycelia of control had almost filled the Petri dishes. Each test was performed in triplicate.

The fungal growth inhibition which was calculated due to treatment against control using the following formula: (Satya *et al.,* 2014).

$$\text{Inhibition of growth (\%)} = R\text{-}r/R * 100$$

R is the radial growth of fungal mycelia in the control plate.
r is the radial growth of fungal mycelia in the treated plate.

Abbott's formula (1925) was used to correct the percentage of growth inhibition were as Finney (1971) was used to calculate the EC_{50} values. The toxicity lines were drawn for evaluating EC_{50} and slop for every treatment.

Results and Discussion

1- Qualitative screening for phytochemical constituents of *Matricaria recutita* flowers.

Regarding to phytochemical screening of the *Matricaria recutita* flowers data in Table 1 reveled that flavonoid, alkaloid, tritrpenoid, steroid and resins components were present in *Matricaria recutita* flowers according to Newal *et al.,* (1996). *Matricaria recutita* are including terpenoids and flavonoids which are responsible for a wide range of biological activities.

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

The analysis of *Matricaria recutita* oil showed thirty- seven compounds which are given in Table 2. The identified components by GC-Ms. *Matricaria recutita* oil contained α-Bisabolol oxide (32.1%) was the principle constituent followed by Camphene (9.8%), α-Pinene (9.2%), Isopropyl hexadecanoate (6.6%), Camphor (6.0), 1,8-Cineole (6.16%), Sabinene (3.9), α-Terpinene (3.2%).

Other compounds comprised (1.9 %) of total oil. 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: chamazulene (61.3%) was the principal component followed by Isopropyl hexadecanoate (12.7%), Trans-trans-farnesol (6.9%), E-b-farnesol (5.2%), Z, E-farnesol (4.8%), a-bisabolol (2.0%) and a-bisabolol oxide A (1.7%) Jamalian *et al.*, (2012).

Table 1: Phytochemical screening of *Matricaria recutita* flowers

Component	*Matricaria recutita* flowers
Flavonoid	+
Alkaloid	+
Tritrpenoid	+
Steroid	+
Saponins	-
Tannins	-
Resins	+

Table 2: Chemical composition of essential oil of *M. recutita* flowers identified components by GC-Ms.

No.	Compounds	%
1	α-Pinene	9.2
2	Camphene	9.8
3	Sabinene	3.9
4	D-3-Carene	0.21
5	α-Terpinene	3.2
6	ρ-Cymene	0.42
7	β-Phellandrene	0.82
8	1,8-Cineal	6.16
9	γ-Terpinene	0.98
10	Artemisiaketone	0.24
11	Z-Sabinenehydrate	0.59
12	α-Thujone	0.68
13	β-Thujone	0.72
14	E-Sabinol	0.74
15	Camphor	6.0
16	4-Terpineol	0.61
17	α-Terpineol	0.86
18	Isomenthyl acetate	0.8
19	E-piperital	0.47
20	α-Cubebene	0.34
21	α-Terpinyl acetate	0.57
22	α-Isocomene	0.15
23	β-Eemene	0.64
24	α-funebrene	0.39
25	Isocaryophyllene	0.37
26	β-Caryophyllene	0.62
27	E-β-faranesene	0.50
28	Germacrene	0.52
29	E-Nerolidol	0.60
30	Spathulenol	0.43
31	Caryophyllene oxide	0.25
32	α-Bisabolol oxide	32.1
33	α-Bisabolol	1.1
34	Chamazulenel	1.6
35	α-Farnesene	1.5
36	β-Farnesene	1.3
37	Isopropyl hexadecanoate	6.6

3- Antifungal activities of essential oil of *M. recutita*.

Data in Table 3 indicated that there was a positive relationship between tested concentrations and percentages of inhibition of both tested fungi. Results indicated that *R. solani* was the more sensitive fungi to oil *M. recutita* with the largest inhibition zone reached to 73 % and 17 mm linear growth followed by 62 % inhibition zone and 28 mm linear growth for *F. solani*. Dimethomorph fungicide was used as a positive control. Dimethomorph exhibited the highest antifungal effect against both tested fungi with recommended concentration. The fungi *R. solani* was more sensitive to Dimethomorph with 76 % inhibition zone and 14 mm linear growth compared with 0.0 % inhibition zone and 90 mm linear growth for *R. solani* and *F. solani* of control.

Table 3: The linear growth and inhibition percent of the tested pathogenic fungi with essential oil *M. recutita.*

Concentrations of EO*. (µl/ml)	***R. solani***		***F. solani***	
	Linear growth (mm)	**% of inhibition**	**Linear growth (mm)**	**% of inhibition**
0.75	17	73	28	62
0.375	22	68	33	57
0.1875	38	52	42	48
0.0935	43	47	60	30
Positive control**	14	76	19	71
Control	90.0	0.0	90.0	0.0

*EO = Essential Oil **Positive control = Dimethomorph

The effect of flowers essential oil of *Matricaria recutita* under microscopic showed that degenerative changes in hyphal morphology. The control mycelium grown on PDA (without EOs) presented normal morphology in both fungi investigated. However, these normal morphological structures changed in the presence of EOs. Cytoplasmic coagulation or fragmentation in the hyphae was recorded. Cell wall disruption and consequent hyphal lysis or necrosis (Khaledi *et al.*, 2014).

Data in Table 4 showed that the tested oil showed fungicidal activity against both tested fungi. *R. solani* was more sensitive to tested oil than *F. solani* their EC_{50} values were 0.16 and 0.25 respectively according to slope value the tested oil possessed the same mode of action against both tested fungi.

Table 4: The EC_{50}, EC_{90} and slop value for the tested pathogenic fungi with essential oil *M. recutita.*

M. recutita						**Fungicide**		
F. solani			***R. solani***			**Dimethomorph**		
EC_{50}	**EC_{90}**	**slop**	**EC_{50}**	**EC_{90}**	**slop**	**EC_{50}**	**EC_{90}**	**slop**
0.25	9.17	0.8±0.19	0.16	4.06	0.89±0.19	0.15	3.14	0.9±0.19

The above indication may be due to the active component in the tested oil specially chamazulene, α-bisabolol that were found in Table 2 this data was agree with Ckay and Blumbery (2006), Kedzia (1990) and Ahmed*, et al.,* (1994) who showed that chamomile oil at 3000 ppm exhibited the highest inhibition against *Aspergillus flavus, Aspergillus parasiticus* and *Fusarium moniliforme*. Its major components, chamazulene, α-bisabolol, flavonoids and umbelliferone, displayed antifungal properties against *Trichophyton mentagrophytes, T. rubrum* and *Candida albicans*. Also, Shahram and Omid (2011) The biological activity of chamomile is mainly due to the flavonoids apigenin, luteolin, quercetin, patuletin and essential oil constituents such as α-bisabolol and its oxides and azulenes.

Conclusion

This study showed the importance of using essential oil *Matricaria recutita* flowers as antifungal activity as an alternative source for the production of chemical commercial antifungal agents. Qualitative phytochemical analyses were assessed and the results detected a lot of phytochemicals. Thirty-seven compounds were identified in essential oil of *Matricaria recutita* flowers by GC/Ms. Essential oil had a good antifungal activity against the growth of *Rhizoctonia solani* and *Fusarium solani* with the largest inhibition zone compared with negative control. Which could be associated

with α -Bisabolol oxide as its main chemical compound in plant oil, however it needs further studies to prove their safety for environmental application and effectiveness in field.

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