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Original Article

Chemical composition and anxiolytic evaluation of *Achillea Wilhelmsii* C. Koch essential oil in rat

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Abstract

Herbal based remedies are used worldwide to treat psychiatric disorders. The aim of this study was to analyse the essential oil composition of *Achillea Wilhemsii* C. Koch (Asteraceae) and to evaluate its anxiolytic effects in the elevated plus maze (EPM) model of anxiety in rat. Gas chromatography/mass spectrometry (GC/MS) analysis of the essential oil showed that the main compounds of the oil were p-ocimen (23%), 1, 8-cineole (20.8%) and carvone (19.13%). The EPM results showed that 1 mg/kg (i.p.) of the oil significantly ($P<0.05$) increased the percentage of the time spent and the number of entries in the open arms of the maze while it did not change the total number of entries in the maze arms. These effects were not reversed with 2 mg/kg flumazenil and 5 mg/kg naloxone. We concluded that a minimum dose of 1 mg/kg of the oil has anxiolytic effects which are not probably mediated through GABA and opioid receptors.

Keywords: *Achillea Wilhelmsii* C. Koch, Anxiolytic, Essential oil, GC/MS, Elevated plus maze

INTRODUCTION

Anxiety disorders are among the most common psychiatric disorders that have debilitating effects on the quality of life of many people around the world. Pharmaco-therapy has shown good results in many cases but prevalence of side effects such as physical dependence to the main group of used drugs, benzodiazepines, has drawn attention towards developing new drugs or using alternative medicine and especially plant-derived product as substitutes (1). Some of the medicinal plants that are used as sedative or anxiolytic are *Matricaria recutita* (2), *Salvia guaranitica* (3), *Valeriana officinalis* (4), *Passiflora caerulea* (5) and *Stachys lavandulifolia* (6).

Achillea (Asteraceae) is a perennial herb that has around 100 species worldwide from which about 19 species are found in Iran. Different species of *Achillea* are used in folk medicine as sedative, anti-inflammatory, analgesic, anthelmintic and to relieve symptoms in premenstrual syndrome (PMS) (7). Studies show that different *Achillea* spp have a wide range of pharmacologic effects such as antioxidant, antimicrobial, anxiolytic and cytotoxic activities (8-10). *Achillea Wilhelmsii* C. Koch (*A. Wilhelmsii*) is one of the widespread species of *Achillea* in Iran. Numerous studies have been conducted to evaluate the pharmacologic effects of the essential oil as well as different extracts of this herb. It is shown that this plant has antimicrobial (11), antitumoral (12), immuno-modulating (13), antihypertensive (14) and vagolitic (15) properties.

Although *A. Wilhelmsii* is used as an anxiolytic plant in folk medicine, however studies to support anxiolytic properties of the volatile oil have not yet been reported. The aim of the present study was therefore to determine the essential oil components of *A. Wilhelmsii* and to evaluate its anxiolytic effects in a rat model. In this study the volatile oil constituents of *A. Wilhelmsii* from Iran and

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its anxiolytic effects on the elevated plus maze (EPM) model of anxiety in rats were investigated.

MATERIALS AND METHODS

Plant

Aerial parts of *A. wilhelmsii* were collected in Kermanshah (West of Iran) in July 2012 and the voucher specimens were deposited at the herbarium of School of Pharmacy (012 C) at Kermanshah University of Medical Sciences, , of Iran.

The air dried aerial parts of the plant were powdered and subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. Anhydrous sodium sulphate was used to dehydrate the essential oil. The oil was stored at -20˚C until the use.

Gas chromatography/mass spectrometry (GC/MS) analyses of the volatile oil

An HP 6890N GC system, coupled with an HP MSD5973N quadruple mass spectrometer was used. The extracted compounds were separated on an HP-5MS capillary column (30 m length, 0.25 mm internal diameter, 0.25 mm film thickness). Split injection was employed for distillation of the samples with a ratio of 50:1. The column oven temperature was programmed to rise from an initial temperature of 40 ºC to 150 ºC at 4 ºC /min, and then to 240 ºC at 10 ºC /min. The injection temperature and ion source temperature were 240 ºC. Helium was used as the carrier gas with a flow rate of 1.2 ml/min. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 50–550 amu. Compounds were identified using the Wiley 7n.L Mass Spectral Library (Wiley, New York, NY, USA).

Animal studies

Male Wistar rats (KUMS breeding house, Kermanshah) weighing 200 ± 20 g were kept in 12:12 hr light/dark cycle and controlled room temperature of 23-26 °C. The animals had access to tap water and food *ad libitum*. Experiments were done on 8 groups of rats (n=8). Test groups were as follows; Volatile oil (0.5 and 1 mg/kg), Diazepam (1 mg/kg), Vehicle (saline with tween 80 0.1% V/V), combination of diazepam (1mg/kg) and naloxone (5 mg/kg) or flumazenil (2 mg/kg), combination of volatile oil (1 mg/kg) and naloxone (5 mg/kg) or flumazenil (2 mg/kg). All procedures were approved by the Ethical Committee of the Kermanshah University of Medical Sciences. All drugs were obtained from Sigma-Aldrich Corporation and dissolved in saline (with tween 80, 0.1% V/V).

Elevated plus maze (EPM) test

EPM model of anxiety which is a standard model for testing the anxiolytic drugs (16) was used to evaluate the effect of *A. wilhelmsii* essential oil on anxiety and locomotor activity of the rats. The plus shaped apparatus consisted of two open (10 × 50 cm) and two closed arms (10 × 50 × 40 cm) that extended from a common central platform (10 × 10 cm). The maze was elevated to a height of 50 cm above the floor. Testing sessions started 30 min after the intraperitoneal (i.p.) injection of the drugs. Each test session was 5 min. long. The rats were first placed on the open arm of the maze and variables such as the number of entries to open and closed arms and the percentage of time spent in open and closed arms were measured during the test period.

Data analysis

SPSS (version 11.5) was used for the statistical analysis of the data. One way analysis of variance (ANOVA) with the Tukey post test was used to analyze the differences between groups. The significant level was set at $P<0.05$.

RESULTS

EPM results

The results of the EPM showed that diazepam (1 mg/kg), as confirmed in other studies (17,18), has anxiolytic activity. To determine the minimum effective dose of the oil, doses were started from 0.5 mg/kg with 0.5 mg/kg increments and it was found that 1 mg/kg of the oil significantly ($P<0.05$) increased the percentage of time spent and the number of entries in the open arms of the maze compared to the vehicle treated group. This dose of the drug did not change the total number of entries in the maze arms. The effect

of diazepam on open arm entry and time is expectedly abated by $GABA_A$ receptor antagonist; flumazenil (2 mg/kg, i.p.) (Figs. 1 and 2). Total number of entries was decreased significantly in diazepam treated group ($P<0.05$). Neither naloxone (5 mg/kg) nor flumazenil (2 mg/kg) could significantly decrease the number of open arm entries, total number of entries or the percentage of open arm time in the oil treated group (Figs. 1 and 2).

The oil composition of A. wilhelmsii

Mass spectra and IR were used to determine the essential oil composition of *A. wilhelmsii*. Fifty five compounds were identified which represented 98% of the oil constituents. The main compounds of the oil were p-ocimen (23%), 1,8-cineole (20.8%), carvone (19.13%), camphor (6.67%), and verbanol acetate (3.53%). Composition of the essential oil of *A. wilhelmsii* is shown in table 1.

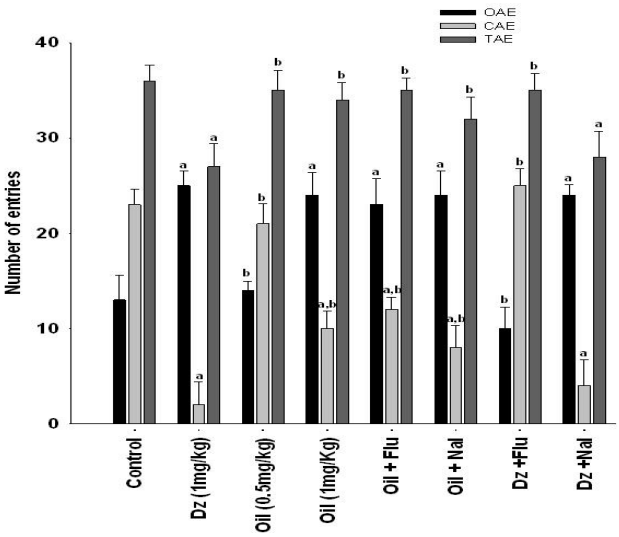

Fig. 1. Effects of intraperitoneal injection of essential oil; Oil 0.5 and 1mg/kg, diazepam; Dz 1mg/kg, flumazenil/oil; Flu 2 mg/kg, Oil 1mg/kg, naloxone/oil; Nal 5 mg/kg, Oil 1mg/kg, flumazenil/diazepam; Flu 2 mg/kg, Dz 1mg/kg, naloxone/diazepam; Nal 5 mg/kg, Dz 1mg/kg, and vehicle control were assessed on the number of entries in open arms OAE, number of entries in close arms CAE and total number of entries in open and close arms TNE of the EPM model of anxiety in rats. (n=8). The letter "a" means a significant difference p<0.05) when compared with control. The letter "b" means a significant difference p<0.05) when compared with diazepam. Data are presented as mean ±SEM.

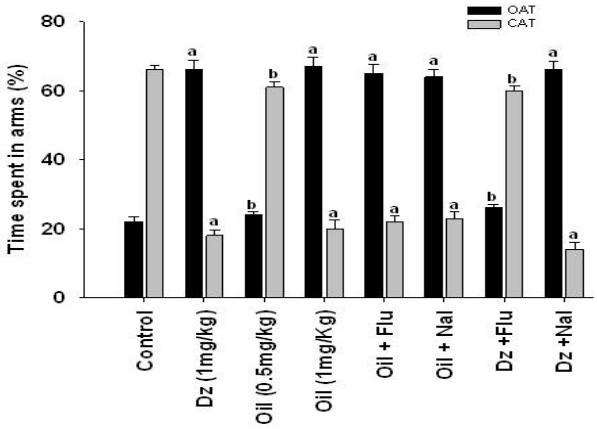

Fig. 2. Effects of intraperitoneal injection of essential oil; Oil 0.5 and 1mg/kg, diazepam; Dz 1 mg/kg, flumazenil/oil; Flu 2 mg/kg, Oil 1 mg/kg, naloxone/oil; Nal 5 mg/kg, Oil 1mg/kg, flumazenil/diazepam; Flu 2 mg/kg, Dz 1mg/kg, naloxone/diazepam; Nal 5 mg/kg, Dz 1mg/kg, and vehicle control were assessed on the percentage of time spent in the open arms OAT and percentage of time spent in the close arms CAT of the EPM model of anxiety in rats n=8. The letter "a" means a significant difference p<0.05) when compared with control. The letter "b" means a significant difference p<0.05) when compared with diazepam. Data are presented as mean ±SEM

Table 1. Volatile oil components of *Achillea wilhelmsii* C. Koch from the west of Iran

Compounds	RI*	Percentage	Identification
α-Thujene	924	0.71	RI, MS
α-Pinene	931	1.81	RI, MS
Camphen	946	0.74	RI, MS
Sabinene	971	0.16	RI, MS
β-Pinene	973	0.55	RI, MS
α –Terpinene	1041	0.14	RI, MS
ρ-Cymene	1025	23.35	RI, MS
1,8 -Cineole	1031	20.83	RI, MS
γ-Terpinene	1056	0.60	RI, MS
Cis-Hydrat-sabinene	1064	0.11	RI, MS
Terpinolene	1086	0.60	RI, MS
α –Pinene oxide	1095	0.13	RI, MS
Linalool	1098	0.74	RI, MS
Cis-Thujone	1103	0.33	RI, MS
Isopentyl isovalerate	1106	0.05	RI, MS
Trans-Thujone	1114	0.07	RI, MS
α –Campholenal	1123	0.48	RI, MS
Allo-Ocimene	1131	0.10	RI, MS
Isopinocarveol	1136	0.15	RI, MS
Trans-Sabinol	1138	0.41	RI, MS
Camphor	1141	6.67	RI, MS
Neo-3-Thjanol	1151	0.05	RI, MS
Pinocarvone	1160	0.08	RI, MS, CoI
Trans-β-Terpineol	1164	0.37	RI, MS
Para-Mentha-1,5-dinen-8 ol	1166	0.11	RI, MS
n-Nonanol	1171	0.04	RI, MS
4-Terpineneol	1174	0.50	RI, MS
Para-Methyl-acetophenone	1180	0.17	RI, MS
Para-Cymen-8-ol	1183	0.94	RI, MS
α –Terpineol	1187	1.26	RI, MS
Myrtenol	1192	0.26	RI, MS
Para–Cymen-9-ol	1205	0.36	RI, MS
Farganol	1214	1.04	RI, MS
Trans-Carveol	1218	0.13	RI, MS
Dihydrocarvone	1239	19.13	RI, MS
Hexyl 3-Methyl butanoate	1244	0.34	RI, MS, CoI
Piperitone	1251	0.39	RI, MS
2-Phenyl ethyl acetate	1254	0.48	RI, MS
Trans-Anethole	1279	0.06	RI, MS
Bornyl acetate	1281	0.59	RI, MS
Para-Cymen-7-ol	1286	0.37	RI, MS
Thymol	1288	0.64	RI, MS
3-Verbenyl acetate	1291	0.09	RI, MS
Carvacrol	1297	0.73	RI, MS
Iso-Verbanol acetate	1303	1.97	RI, MS
Neo-Verbanol acetate	1316	0.08	RI, MS
Verbanol acetate	1340	3.53	RI, MS
Neryl acetate	1363	0.10	RI, MS
Linalool isobutyrate	1374	0.10	RI, MS
α-Copaene	1377	0.53	RI, MS
Cis-Jasmone	1391	0.48	RI, MS
Cis-Caryophyllene	1407	0.05	RI, MS
α –Cis- Bergmotene	1414	0.21	RI, MS
Trans-Caryophyllene	1420	0.07	RI, MS
Linalool butyrate	1425	0.06	RI, MS
Isobornyl isobutyrate	1429	0.09	RI, MS
Allo-Aromadendrene	1462	0.08	RI, MS, CoI
Isobornyl n-butyrate	1469	0.10	RI, MS
Pentyl benzoate	1475	0.07	RI, MS
Neryl isobutyrate	1494	0.59	RI, MS

Table 1. (Continued)

Compounds	RI*	Percentage	Identification
Deha-decalactone	1495	0.76	RI, MS
Menthyl isovalerate	1513	0.21	RI, MS
1-Phenyl heptan-3-one	1523	0.13	RI, MS
Selina-3,7 (11)-diene	1535	0.10	RI, MS
1,10-Decanediol	1547	0.08	RI, MS
Elemol	1549	0.10	RI, MS
Cis-Muurol-5-en-4-α-ol	1554	0.18	RI, MS
Trans-β- Elemenon	1597	0.40	RI, MS, CoI
Epi-Cedrol	1612	0.24	RI, MS
Trans-Isolongifolanone	1621	0.56	RI, MS
Trans-3-Hexenyl phenyl acetate	1631	0.14	RI, MS

*RI, retention indices relative to C6–C24 n-alkanes on the DB-5 column; MS, mass spectrum; CoI, co-injection with an authentic sample.

DISCUSSION

The aim of the present study was to analyze the composition of the volatile oil of *A. Wilhelmsii* and also to evaluate its effects on anxiety state in EPM model of anxiety.

Javidnia and coworkers have previously shown that the main components of the volatile oil of *A. Wilhelmsii* are carvacrol (25.1%), linalool (11.5%) and 1,8 cineol (10.3%) (19) while in our study we detected the main three components as p-ocimen (23%), 1,8-cineole (20.8%) and carvone (19.13%). Another study which has been done by Brunke EJ and coworkers on essential oils of *A. Wilhelmsii* collected from Egypt and Turkey showed that 1, 8 cineol is the main component (20). Studies on other *Achillea* spp have shown camphor, 1,8 cineol, linalool and α-terpineol as the main oil constituents (21-23). The difference in composition and percentage of the constituents of the oil may be attributed to their geographic and bioclimatic distributions (24-27).

The elevated plus maze is an animal model of anxiety which was first introduced by Montgomery in 1955 and is based upon two opposing instincts of exploring a novel environment and fearing of an open environment (28). The results of the volatile oil on EPM test showed that it had anxiolytic effects at the minimum concentration of 1mg/kg. Total number of entries was not changed in oil treated group while it was decreased with diazepam treatment. Total number of entries can be used as an indicator of spontaneous motor activity in EPM (29) so the anxiolytic effect of the oil did not interfere with locomotor activity of the rats. Diazepam, as expected, decreased their locomotor activity.

Monoterpenes are the main components of volatile oils of this plant. It is shown that these compounds have important central nervous system activities such as anticonvulsant (30), analgesic (31) anxiolytic (32-34) and antidepressant (35) effects. Gomes and coworkers showed that 1,4 cineol had anxiolytic effects in mice which was not antagonized with flumazenil (32). Anxiolytic effects of carvone and thujone were also reported in other investigations (36-37). Anxiolytic effects of *A. umbellata* essential oil and *A.millefolium* hydroalcoholic extract are also reported in other studies (8,9)

The EPM model is a valid model for those compounds which have $GABA_A$ agonistic activity like benzodiazepines,. Other compounds such as 5-HT1A agonists, selective serotonin reuptake inhibitors and tricyclic antidepressants did not show consistent results in different studies (16). The anxiolytic effect of diazepam was decreased by the GABA antagonist, flumazenil. Co-administration of the oil with flumazenil did not decrease its anxiolytic effect which suggests that GABA receptors are not involved in its mechanism of action. Since naloxone could not change open arm time and entry compared to diazepam it can be concluded that opioidergic system is also not involved in its anxiolytic effect. It is shown that other receptors such as cannabinoid (38,39), glutamate (40,41), dopamine (42), cholecycto-kinine (43) or adenosine (44) receptors are also involved in anxiety and here may have a role in anxiolytic mechanism of this oil.

CONCLUSION

This study showed that the essential oil of *A. Wilhelmsii* had anxiolytic effects which are probably not mediated through GABA and opioid receptors and unlike diazepam it did not change the locomotor activity of the rats. Its actions may be attributed to its main monoterpenoid compounds including p-ocimen1, 8-cineoleand carvone.

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