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Ethnobotany, chemical composition and antibacterial activity of the essential oil from *Achillea micranta* L. in Golestan province

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Abstract

Achillea micranta L. is a wild endemic medicine herb with important therapeutic effects has been used in traditional north of Iran. GC-MS analysis of the isolated essential oil from flowering aerial parts of *A. micranta* resulted to identification of 23 constituents, that eucalyptol (1,8-cineol) with 19.9%, borneol (11.9%), camphor(11.1%) and thujene (5.1%) were the major constituent of essential oil studied, respectively. The antibacterial activity was individually evaluated against 2 tested bacteria. We concluded that the essential oil showed a good antibacterial activity against tested bacteria but the Gram-positive bacteria *staphylococcus aureus* was more sensitive. This study confirms that the essential oil of *A. micranta* L. possesses antibacterial properties in vitro. 1,8-cineol, borneol and camphor can be considered as the main antibacterial constituents of the oil studied.

Key words: *Achillea micranta* L., Antibacterial activity, Essential oil, Ethnobotany.

Introduction

The genus *Achillea* (*Asteraceae*) comprises about 85 species, most indigenous to Europe and Asia. This genus those used traditionally to health wounds and skin infection (Mackuet et al., 2003). There are 40 *Achillea* species have ethno pharmacologic importance as known to be used in folk remedies for various purpose (Cavalcanti et al., 2006).

The antimicrobial and anti inflammation activities of the essential oil of various extracts from *Achillea* species have been reported before (Barel et al., 1999; Simic et al., 2000; Fillippi et al., 2006). Neither the antimicrobial activities of the essential oils of *Achillea setacea* and *Achillea micranta* nor the composition of the oils of *A. teretifolia* have been studied before (Maffei et al., 1993).

Chemical composition of the essential oil of various *Achillea* species have been reported before, that the 1,8-cineol, camphor, borneol, thujene and α-terpineol were the major constituents (Cavalcanti et al., 2006).

Popular indications of this plant include treatment of the wounds, hemorrhages, headaches, inflammation, pain, spasmodic diseases, flatulence and dyspepsia (Blumenthal et al., 2000, Mackuet et al., 2003). Previous reported that extracts from *Achillea* species had an anti oxidant capacity, which is constituent with their total faloonoid and phenol contents (Konyalioglu, 2005). Essential oils of *Achillea* genus had rich bioactive compounds. Recently there are some published reports and invitro studies about their antimicrobial properties, antispasmodic, anti inflammation, antibacterial for wound healing (Benzic, et al., 2003; Fillippi, et al., 2006; Agnihotri, et al., 2005; Baser, et al., 2002; Senatore, et al., 2004).

Ecological study and phonological process were affected not only on the rates of yield, but also on quality and quantity of secondary metabolites, therefore obtaining the best medicinal products, ecological effects and phonological stages is necessary (Van Wyk, et al., 1997). *Achillea* species make up the list of the most important indigenous economic plants of Golestan

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mountainous that traditionally used by the rural healers as diuretic, emmenagogu agents, in wound healing, for abdominal pain, diarrhea and flatulence in North of Iran. Present study was designed to evaluate the phenology, ethno pharmacology and chemo type and invitro antibacterial activity of the essential oil of *A. micranta* L. that wild growing in south east of Golestan province in North of Iran.

Material and method

Collection of plant material

The aerial parts (leaves and flowers) of *Achillea micranta* L. was collected in Charbagh, 75km south east of Goletan province, when flowering, mid-June 2003 and late-July 2003, respectively. The voucher specimen of the department of biology Islamic Azad University-Gorgan branch, Golestan province.

Ethnobotany

In our study, all information was gathered through ethno botanical questionnaire, using participant observation with two famous elderly members of selected communities and who still retain traditional knowledge about medicinal plants in small mountainous village *Charbagh* in south east of Golestan province which most of them belonged to the female group (average age: 76 years), who**m** still retain considerable knowledge about these practices. We used a similar methodology and a comparable approach in all local regions. Our work conducted in such way to be able to compare the collected information. During the first phase of this field study, the required information about identified species, such as locally name, plant part used, the culinary process and other medicinal application was asked and tape- recorded.

Extraction

About 200 g of the dried powders of top flowering species were separately subjected to steam distillation for 2h, in full glass apparatus. The oils were isolated using a Clevenger type apparatus. The oil was stored frozen in dark glass bottles until they were used.

Oil analysis

The oils were analyzed by GC (9-A-shimadzu) and GC/MS (Varian-3400) column (DB-1,60mm-0.25mm fused silica capillary column film thickness 0.25μm using a temperature program of 50-250°C at a rate of 4°C/min, injector temperature 260°C, carrier gas: Helium, the constituents were identified by comparison of their mass spectra with those in the computer library and with authentic compounds. The identifications were confirmed by comparison of their retention indices with those of authentic compounds or with literature data. The components of the oils were identified by matching their mass spectra and retention indices with those of the Wiley 275 library in the computer library and literature .The yield of each component was calculated per Kg of the plant material, while its percentage composition was determined from the peak areas of the total oil composition.

Oil dilution solvent

Microbial strains were streaked on Muller Hinton agar plates using sterile cotton swabs. 5 micro liter of dimethylsulphoxide (DMSO) loaded on sterile blank disks were placed on the agar plates and were incubated at appropriate temperatures for 24-48h. There was any antimicrobial activity on the plates and hence DMSO was selected as a safe diluting agent for the oil. 5 micro liters from each sterile oil dilutions was added to sterile blank discs. The solvent also served as control (25, 50, 100, 200).

Test organism

The test organisms used in the study were obtained from Persian Type Culture Collection, Tehran, Iran (PTCC), namely: *Staphylococcus aureus* (PTCC N. 1112) and *Pseudomonas aeroginosa* (PTCC N. 3142).

Antimicrobial activity

The antibacterial effects were tested by the disc-diffusion method, briefly, Muller Hinton Agar plates were cultured with a standardized inoculums (1.5×10^8cfu/ml equal to 0.5 McFarland) of each bacterial strains, then the saturated discs with different concentration of essential oils were carefully placed on the plates, the plates then were incubated aerobically at 37°C and inhibition zones were measured after 24 hr. The inhibition zones were compared with the control disc containing Gentamycin as positive control. Each test was repeated 3 times and means inhibition zone were recorded. Inhibitory zone ≥12mm used as good inhibitory effect of extract. Disc diffusion method was employed for the

determination of antimicrobial activities of the essential oil .A broth micro dilution broth susceptibility assay was used (Nostro, 2000**)**.

Result and discussion

Phenology and autecology

This study in many field observations, we found that *Achillea micranta* L. is one of the most important indigenous endemic medicine herbs in Golestan province, North of Iran. It seems to prefer growing with wide density in sunny position, western aspect of grassland of mountain to 2100 meter above the sea level in dry-cool climate and prefer soils with organic carbon (3.2%), nutrient material (10.8%), EC (0.4) and pH (7.5) from in Charbagh small village in south east of Golestan province.

Phenology showed that it is begin vegetative growth stage at the early of May, flowering in mid-June, fruiting in August and falls in September.

Ethnobotany

Ethnobotanical date showed the *Achillea micranta* L. with Persian name "Bumadaran" and locally name "Zarde maramboo" is wide used traditionally for healing wounds, internal bleeding, dysmenorrheal and stomachache, spasm, anti in flammation, headache, gastro intestinal, fever and sedative.

Authors have been reported to effect of *Achillea* species to treat wounds, sore, inflammation and their essential oil compositions in quality and quantity were different , similar identified in due chemo type and their medicinal effects in dependence on different climatic and ecological conditions in various regions of the world (Maockute, et al., 2003; Suleimenov, et al., 2004).

Chemical composition of the essential oil

Steam distillation of dried flowering aerial parts of *A. micranta* L. was pale yellow about 23 constituents was identified by means of GC-MS analysis of the essential oil from *A. micranta* respectively. In Table- 1, Eucalyptol (1,8-cineol), borneole, camphor and thujene were the major constituent of the essential oil, representing 19.9, 11.9, 11.1 and 5.1% of the total oil, respectively.

1,8-cineole , camphor and borneole were found to be the major constituent of *A. micranta* L. oil in our research (Table -1).

Table 1. Chemical composition of essential oil from *A. micranta* L.

Compounds	Rt	%
α-pinene	9.7	1.2
camphene	10.35	1.5
sabinene	11.66	4.7
α-phellandrene	13.04	1.1
α-terpinene	13.59	1.2
eucalyptol(1,8-cineole)	14.50	19.9
γ-terpinene	15.70	4.2
thujene	18.15	5.1
iso thujene	18.66	2.3
cis-mentha-2-en-1-ol	18.95	2.3
camphor	20.07	11.1
pinocarvone	20.91	0.3
borneol	21.15	11.9
terpinene-4-ol	21.81	5.4
α-terpineol	22.46	3.5
trans-piperitol	23.28	3.8
7-menthyl-3-menthylene-6-octan	23.78	2.0
3,7-dimethyl-3-6-octadinene	24.50	1.0
bornyl acetate	26.93	1.5
eugenol	30.25	1.6
caryophyllene	33.06	2.4
nerodiol	39.60	2.6
caryophyllene-oxide		2.1
others not identified(6)	40.71	7.3%
total		100%

It can be concluded that camphore type compounds as observes in some *Achillea* oil studied, such as the 1,8-cineole, camphor and α-terpineole have been found as major compounds in many other *Achillea* species (Rustayian, et al., 1998; Simic, et al., 2000).

Antibacterial activity

The in vitro antibacterial tested of the essential oil of *A. micranta* L. against *Staphylococcus aureus* and *Pseudomonas aeroginosa* in Table -2.

Table 2. Antibacterial activity of the essential oil of *A. micranta* using agar disc diffusion method

Tested bacteria	200mg/ml	**Gentamycine
S. aureus	*21	22
P. aeroginosa	12.5	14

s.a: Staphylococcus aureus; p.a: Pseudomonas aeroginosa; * Inhibition zone (mm); **Positive control

Results obtained from disc diffusion method, followed by the measurement of zone inhibition, is the most sensitive bacteria tested in the presence of the oil extracted from *A. micranta* L. respectively.

Staphylococcus aureus was more sensitive test microorganism against the oil applied (Table-2). The above mentioned oil was also found to posse's antibacterial activity against *S. aureus* and *P. aeroginosa* to the best of our knowledge, the in

vitro antibacterial activities of the essential oil of *A. micranta* L. has not been reported before. Based on a reported concerning the antimicrobial activities of the essential oil from *A. fragrantissima*, terpinen-4-ol was concluded as one of the compounds responsible for bacteriosidic effect against several microorganism (Barel et al., 1991).

In our similar works, the antimicrobial of *Achillea* species oils were more pronounced against Gram-positive than Gram negative bacteria as fact previously observed with essential oils from other species. This general resistance among Gram-negative bacteria has been ascribed to the presence of their phospholipidic membrane. (Senatore. et al., 2004; Bezic, et al., 2003; Fillippi, et al., 2006; Baser, et al., 2002).

Different antimicrobial activities of examined essential oils seem to be due to differences in oil composition of *Achillea* species belongs to various habitats. 1,8-cineole and camphor are well known chemicals with their pronounced antimicrobial potential in *Achillea* species , these similar works showed by Benzic, et al., 2003; Fillippi, et al., 2006; Agnihotri, et al., 2005; Baser, et al., 2002.

In similar our work, we found that camphor, 1,8-cineol, borneol can be considered as the antibacterial constituents of the oils of *A. micranta* . In conclusion *A. micranta* L. essential oils gave interesting results, as in their traditional usage for the wound healing, antibacterial activity.

Ethnobotany, phytochemistery, and antibacterial activity of this oil may lead to extraction and production of active compounds in single or combined forms with useful medicinal application.

References:

Angihotri, V.K., Lattoo, S.K., Thappa, R.K. Kaul, P., Oazi, G.N., (2005) Chemical variability in the essential oil components of *Achillea millefolium* agg in India. Plantamed, 71(3): 280-3.

Baser, K.H., Demirci, B., Kocak, S., Akinci, C., Malyer, H., Gulerus, G., (2002) Composition and antimicrobial activity essential oils of *A. multifida*. Planta Med. 10: 941-3.

Benzic, N., Skocibusic, M., Dunkic, V., Radonic. A., (2003) Composition and antimicrobial activity of *A.clavennae* L. J.Essential, Phytother Res, 17(9): 1037-40.

Blumenthal, M., Busse, W.R., Goldberg, A, Grueuwald, J., Hall, T., (2000) In herbal medicine expanden commission E, 11, 282-285

Caralcantis, A.M., Baggio, C.H., Freitas, C.S., Reik, L., (2006) Safety and antinuclear efficacy studies of *A. millefolium* L. after chronic treatment in Wistar ratsg. Ethnopharm, 107,277-284

Fillippi, J, Lanfranchi, D.A., Prado, S., Baldovini, N., Melerhenrich, U.J. (2006) Composition, ethnobotanical and antibacterial of *A.ligustica*. Agric Food Chem, 54(17):6308-13.

Mockute, D., Judzeatieni, A., (2002) Chemical composition of the essential oils of *Achillea millefolium* L.Ssp.millefolium growing wild in Vilnuse. F.J. 13, 97-102.

Rustaiyan, A., Komeilizadeh, H.M.S., Shariatpanahi, Jassbi, A., Masoudi., S., (1998) Comparative study of the essential oils of three Achillea species from Iran. J. Essential Oil Res.10, pp.207-209.

Senatore, F., Napolianto, F., Arnold, A.N., Bruno, M., Herz, W., (2003) Composition and antimicrobial activity of the essential oil of *Achillea falcata* L. Flavour and Fragrance Journal.20, 3, 291-294.

Simic, N., Andjelkonvic, S., Palic, R., (2000) Composition and antibacterial activity of *A. chrisochoma* essential oil , Journal of essential oil, Res 12, pp. 784- 787.

Suleimenov, Y.M., Atazhanova, G.A., Ozek, T., Demirci, B., Kulijasov, A.T., Adekenov, S.M. and Baser, K.H.C., (2004) Essential oil composition of three species of *Achillea* from Kazakhestan. Chemistery natural compounds , 37, 447-450.

Van Wyk, B., Gericke, N., (2000) *People's plants*. Pretoria: Briza Publications, pp. 128.

Konyaliogla, S., karamenderes, C., (2005) The protective effects of *Achilla* L.species native in Turkey against H_2O_2-induced oxidative damage in human erythrocytes and leucocytes .g.of Ethnopharma 102-221-227.

اتنو بوتانی ، بررسی ترکیبات شیمیایی و اثر ضد باکتریال اسانس گیاه دارویی بومادران زرد (*Achillea micranta* L.) در جنوب شرق استان گلستان

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چکیده

گیاه دارویی ***A. micranta* L.** یکی از مهمترین گونه‌های دارویی و بومی است که سال‌هاست در طب سنتی مردم شمال ایران مصارف درمانی فراوان دارد. این تحقیق با هدف اتنوبوتانی، شناسایی ترکیبات ثانوی اسانس و بررسی اثر ضد باکتریال گیاه در جنوب شرق استان گلستان انجام گرفت. نتایج گاز کروماتوگرافی اسانسی حاکی از وجود ۲۳ ترکیب شیمیایی در روغن اسانس گیاه بود که مهمترین آنها شامل اوکالیپتول (۱۹/۹ درصد)، بورنتول (۱۱/۹ درصد)، کامفور (۱۱/۱ درصد) و توجن (۵/۱ درصد) بود. بررسی اثرضدباکتریال اسانس علیه دو باکتری گرم مثبت و منفی نشان داد که گیاه تاثیر مطلوبی علیه رشد باکتری گرم مثبت ***Staphylococcus aureus*** دارد که طبعاً به علت مواد موثره اوکالیپتول، بورنئول و کامفور است که از ترکیبات مهم با اثر ضدباکتریایی می‌باشند. نتایج این تحقیق در تاثیر استفاده سنتی آن در رفع عفونت، التیام زخم و رفع التهابات اندام های داخلی و خارجی بدن قابل توجیه است.

واژه‌های کلیدی: اتنوبوتانی، اثر ضدباکتریال، بومادران زرد (*Achillea micranta* L.)، مواد موثره اسانس.