



Antioxidant Activity and Ethno pharmacological Survey of *Achillea biebersteinii* Afan. in the Treatment of Dysmenorrhoea in Traditional Medicine of Golestan Province, Iran

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Abstract

Objectives: *Achillea biebersteinii* Afan. has been used as antispasmodic and sedative agent to treat dysmenorrhoea in traditional medicine of Golestan province. The present study was conducted to evaluate the antioxidant activity. It is also an ethno pharmacological survey of *Achillea biebersteinii* Afan., as antispasmodic and sedative agent in treating dysmenorrhoea in traditional medicine of Golestan province.

Materials and Methods: Ethno pharmacological data was obtained as field observation among well-known indigenous rural healers (68-82 ages) in southwest of Golestan province in August 2011. The data gathered evaluated the potential of *Achillea biebersteinii* in preventing or treating dysmenorrhoea. Flowers of the plant were collected in July 2012 at an altitude of 2600 meters, ethanol extract was obtained by Maceration and antioxidant capacity was obtained by 1,1-diphenyl-2-picryl hydrazyl radical scavenging (DPPH).

Results: Results showed that in traditional medicine of Golestan province, the flowers of *Achillea biebersteinii* has been used in combination with *Peganum harmala*, *Mentha aquatica*, *Cuminum cyminum*, *Foeniculum vulgare* and *Nigella sativa* as antispasmodic, anti-inflammation and sedative agent to treat stomach ache, dysmenorrhoea, facial flushing, cramps and menopause. Also the ethanol extract of the plant is a very good free radical scavenging with the highest antioxidant activity (IC₅₀ = 1.27±0.1 µg/ml in dry weight).

Conclusion: These finding not only confirm the use of the plant in the traditional medicine of Golestan province, but also can serve as a basis for phytochemical and pharmaceutical researches to identify and produce effective herbal drugs in prevention or treatment of common dysmenorrhoea.

Keywords: *Achillea biebersteinii* Afan., Antioxidant, Dysmenorrhoea, Ethno pharmacology

Introduction

In the past two decades, increasing consumption of synthetic drugs and its associated side effects has led World Health Organization (WHO) to study the antioxidant activity of this plant. It also conducted an ethno botanical survey of endemic medicinal plants studying its uses by the rural populations in order to prevent and treat many current diseases (1).

Achillea species such as *Achillea millefolium* and *Achillea biebersteinii* Afan. are antispasmodic, anti-inflammatory, antiulcer and antioxidant agents mainly used for gastrointestinal disorders, estrogen-related effects, menstrual irregularities, wound care and skin inflammation (2,3). *Achillea biebersteinii* Afan. locally named as "Yellow marabou" in Asteraceae family, is a wide spread medicinal plant, found mainly in Europe, Asia and the USA. Its flowers appear from June to September and are widely used as antiulcer, antispasmodic, anti-inflammatory and

anti hemorrhagic agent treating numerous illnesses, and especially used as sedative in treating dysmenorrhoea (4). Rural communities in the mountainous southwest region of Golestan province (namely Charbagh and Deraznoo areas) in north of Iran have a long original tradition of using plants as an antispasmodic, antiseptic, sedative and anti-inflammatory agent to treat gastrointestinal disorders, dyspeptic complaints, stomach ache, dysmenorrhoea and dysmenorrhoea (5). Thus this study was carried out and extensive data on the antioxidant activity of *Achillea biebersteinii* Afan. was collected from the proficient local practitioners of ethnic traditional medicine in Golestan province.

Materials and Methods

Ecological requirements and ethno pharmacology

In the present field observation, we analyzed the ecological requirements and traditional pharmaceutical knowl-

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edge on *Achillea biebersteinii* Afan. in two small mountainous villages isolated in steppe areas within latitudes of 55° 57' 55" to 52° 57' 55" and longitudes of 25° 46' 37" to 15° 42' 37", covering an area of 2800 hectares in southwest of Golestan province (Charbagh and Deraznoo), having several steppe and semi steppe ranges reaching 1800 to 2600 meters respectively, with semi-dry and cold climate. For traditional interviews, all data on plant uses, its local name, plant part and the preparation methods of traditional drugs were obtained through interviews with the practitioners and housewives (56-68 years), analyzed and compared with other findings in similar reports.

Plant material

The aerial parts of blooming plant were collected in late July 2012 from the Charbagh Mountain in southeast of Golestan province (north of Iran). The plant was identified in RCMP (Research Center of Medicine Plant) with voucher specimen (No. HRCMP:451), preserved and deposited in the herbarium of Islamic Azad University of Gorgan branch, Gorgan, Iran.

Extraction and isolation

Sixty grams of air-dried plant flowers were exhaustively extracted with 470 ml methanol by maceration extraction and concentrated by using a rotary evaporator.

Antioxidant activity tests

2,2-Diphenyl-1-picrylhydrazyl radical scavenging capacity assay

The ability of the extract for free radical scavenging was assessed by Arabshahi et al. method (6). The aliquots of plant extract (20, 40, 60, 80, 100 µl) were mixed with a methanol solution of DPPH- (1 mm, 600 µl) and brought to 6 ml with solvent. After incubating in dark and at room temperature the absorbance was measured at 517 nm. A DPPH- blank sample (containing 5.4 ml of methanol and 600 µl of DPPH-solution) was prepared. The percent decrease in absorbance was recorded for each concentration and percentage inhibition was calculated according to the following formula: % inhibition = [(ADPPH - A plant extract)/ADPPH] × 100. ADPPH is the absorbance value of the DPPH- blank sample and extract is the absorbance value of the test solution. The plots of the 'percentage inhibitions amounts of dried plants (mg) in the extract' were used to find the concentration at which 50% radical scavenging occurred (IC50).

Results

Ecological data showed that *Achillea biebersteinii* Afan. with local name of "Yellow Marambou" is a perennial wild herb (10-40 cm), growing in cool dry climate and sandy clay loam soils (1200-2600 m), requiring an average rainfall of 305.9-414.8 mm, Ec = 0.73-2.5. The yellow inflorescences of the plant appear from June to September. Laboratory test showed that the flowers extract had very good antioxidant activity and high potency in scavenging of free radicals (IC50 = 1.27 ± 0.1 µg/ml in dry weight). Ethno

pharmacological data showed that the flowers of *Achillea biebersteinii* Afan. have been considered as safe medicinal plants as a tonic, antispasmodic, sedative, anti-inflammatory and anticholinergic agent in treating dysmenorrhoea. The dosages for various conditions are as below:

Stomach ache and pain relief: The tea of *Achillea biebersteinii* Afan., 5-7 seeds of *Peganum harmala* and aerial parts of *Mentha aquatica*.

Dysmenorrhoea: The tea of *Achillea biebersteinii* Afan., *Matricaria* species, *Cuminum cyminum*, *Foeniculum vulgare*, *Stachys lavandulifolia*, *Ziziphus vulgaris* and the seeds of *Nigella sativa*.

Amenorrhoea: Every night 1 cup of *Achillea biebersteinii* Afan. and *Foeniculum vulgare* distilled with 1 tea spoon of honey.

Internal bleeding: The decoction of *Achillea biebersteinii* Afan., *Myrtus communis*, *Plantago psyllium*, *Malva neglecta*, *Cydonia* seeds and honey, peptic ulcer (infusion of *Achillea biebersteinii* Afan., *Teucrium polium*, flowers and the root of *Malva neglecta* in combination with *Amygdalus* oil and *Ferula asafoetida*), dryness in the mouth and facial flushing (*Urtica dioica*, *Achillea biebersteinii* Afan. or *Achillea millefolium* and *Foeniculum vulgare*) and in palpitation *Achillea micranta*, *Salix aegyptiaca*, *Citrus aurantium* and *Crataegus oxyantha* have been used.

Leishmaniasis: The poultice of tincture of *Perovskia abrotanoides*, *Artemisia annua* and *Achillea biebersteinii* Afan.

Discussion

Dysmenorrhoea (painful periods) is a medical condition of pain during menstruation that interferes with daily activities. It has long been treated by medicinal plant species in traditional medicine of countries (7).

Achillea biebersteinii Afan. is one of the most common herbs used as a tonic, sedative, antispasmodic and anticholinergic agent to treat various cold, wounds, hay fever and dysmenorrhoea. Additionally, it has also been used to help to regulate the menstrual cycle, promote menstruation while reducing heavy bleeding, and relieving dysmenorrhoea. All these medicinal effects have also been reported by other researchers worldwide (8,9).

In similar studies it was reported that the binopacryle (63.82% to 83.63%), 1-8 cineol (14.97% to 3.76%), α-selinene (4.81% to 4.49%), phenols (18.44 ± 0.085 mgGAE g-1) and flavonoid contents (80.30 ± 5.793 mgQUE g-1) were the main chemical composition of *Achillea biebersteinii* Afan. which are responsible for the antioxidant and antispasmodic activity of *Achillea* species (10).

To confirm our ethno pharmacological results, other researchers showed that alkaloids (harmine, β-carboline, peganine, harmalol, harmaline and vasicine) present in the seeds of *Peganum harmala* L. (11) and camphor, myrcene, cineole, caryophyllene, linalool and sesquiterpene lactones were the most prevalent secondary metabolites in the flower extracts of *Achillea* species, being effective as antispasmodic and sedative agent treating dysmenorrhoea and stomach ache (5,8,9).

Chamazulene, eucalyptol, camphor, alpha-terpineol, be-

ta-pinene and borneol which are present in many volatile oils of *Achillea* species, are well-known antioxidant, anti-inflammatory, anti allergic agents with antispasmodic action on rat duodenum against a variety of antitumor cell lines in mouse (8). Flavonoids (apigenin, rutin, luteolin and campherol), and monoterpenes (alpha-pinene, 1,8-cineole and camphor) are believed to be the source of the yarrow's antispasmodic benefits, while the alkaloid, achilleine, is believed to help control both internal and external bleeding. Meanwhile their amounts vary within different species based on ecological factors and climatic condition in different geographical regions (10,12,13).

In Traditional Chinese Medicine (TCM), the flowers of *Achillea* species has been used for a variety of conditions such as bleeding, wounds, haemorrhoids, varicose veins, dysmenorrhoea and tuberculosis condition (3). In the entire American continent, the most important uses were for skin problems and injuries, bleeding conditions, respiratory illnesses, digestive problems, toothaches and eye problems (14). Karamenderes and Apaydin demonstrated that ethanol extract *Achillea micrantha* exhibits an inhibitory effect on the dose-response curves induced by acetylcholine and CaCl₂ on rat duodenum (13).

Some researchers have also reported that *Teucrium polium* L., *Peganum harmala* L. and *Achillea millefolium* L. , contain harmine, harmaline, vasicinone, deoxyvasicinone, achillin, limonene, borneol, α -cadinol, caryophyllene oxide and terpinen-4-ol alkaloids (15,16). According to other reports the terpenoids (eucalyptol, camphor, α -terpinol, β -pinene, and borneol) are the principal components of *Achillea millefolium* L. oil with the strongly radical scavenging (IC₅₀=1.56 μ g/ml), antispasmodic, anti cholinergic and antibacterial activity against *Streptococcus pneumoniae*, *Clostridium perfringens*, *Candida albicans*, *Mycobacterium smegmatis*, *Acinetobacter lwoffii* and *Candida krusei* (17-19). Thus these results confirmed that the oil and extract of *Achillea* species possess antioxidant, antispasmodic and antimicrobial properties in vitro and thus in the treatment of dysmenorrhoea.

Conclusion

These data are the first reports on antioxidant activity and ethno pharmacological information of *Achillea biebersteinii* Afan. It is used as antispasmodic and anti-inflammatory agent in the traditional medicine of Golestan province to treat wounds, skin infections, abdominal pain, leishmaniosis infection and dysmenorrhoea. It is notable that it can serve as a basis for phytochemical and pharmaceutical studies to identify and produce effective herbal drugs in prevention and treatment of common regional diseases.

Ethical issues

The local ethics committee approved the study.

Conflict of interests

Authors declare that there is no any conflict of interest.

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