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## Ethnobotany and Antibacterial Activities of Two Endemic Species of *Hypericum* in North-East of Iran

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**Abstract:** This research project was a field observation study and we examined the ethnobotany, phenology and antibacterial effects of two species of *H. perforatum* and *H. androsaemum*. The investigation of phenology showed these plants initiate vegetative stage at the early April to June, with wide dispersal and density in humid temperate to dry-cool climate in heavy texture soil and non saline in 10-2400 m from sea level. The ethanolic extracts of flowering aerial parts of either species exhibited a good antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis*, with the diameters of growth inhibition zone in the rang 25, 26 mm in *H. perforatum* and 16, 20 mm in *H. androsaemum*, respectively. Weak antibacterial effects were seen in the infusion and aqueous extracts. Present data show that Gram-negative bacteria are resistant toward all of different extracts. We conclude that the extract of *Hypericum* species should be used when the etiological agent of infection is a Gram-positive bacteria.

**Key words:** Ethnobotany, *Hypericum androsaemum*, *Hypericum perforatum*, disc diffusion, antibacterial activity

### INTRODUCTION

Investigation for finding new natural antimicrobial agents were increased during the last few years, due to the increasing development of drug resistance of human pathogenic organisms as well as the appearance of undesirable side effects of certain antibiotics and the emergence of previously uncommon infections (Bombardelli and Morozzoni, 1995; Barnes *et al.*, 2001).

The screening of the plant extracts and their products for antimicrobial activity have shown that higher plants represent a potential source of new anti infectious agents (Meng *et al.*, 2000; Ho *et al.*, 2001). *Hypericum* is a well known plant in herbal medicine due to the therapeutic efficacy of its different species.

The *Hypericum* L. is the only genus of *Hypericaceae* and it is a well known herbal medicine due to its therapeutic efficacy. It comprises of more than 450 species in 36 sections, with world wide distribution in warm temperate, subtropical and mountainous tropical regions. Members of this genus grow in a variety of habitats ranging from rocky sea-facing cliffs to damp biotopes around mountainous streams (Barnes *et al.*, 2001). Previous studies have shown that *Hypericum* species are very rich the terpenoids compounds (Bombardelli and

Morozzoni, 1995; Barnes *et al.*, 2001). Among different species of this plant, *H. perforatum* is the most important species, which has been used in herbal medicine externally for the treatment of skin wounds, eczema, burns and internally for diseases of the central nervous system, the alimentary tract (Srinivasan *et al.*, 2001; Greeson *et al.*, 2001). Several studies have been published about the antidepressants, antiviral, wound healing, antioxidant and antimicrobial activities of this plant (Barnes *et al.*, 2001; Greeson, 2001).

In the Golestan province in the north-east of Iran, Two wild endemic species of *Hypericum* (*H. perforatum* and *H. androsaemum*), have been used in folklore medicine as a diuretic, wound healing, sedative and antibacterial agent, however very little information are available on the ethnobotany, ethno pharmacology of these plants in this area. The present study was undertaken to assess the ethno botany, phenology and the *in vitro* antibacterial activity of different extracts of two *Hypericum* species from this region.

### MATERIALS AND METHODS

**Plant material:** The field work was conducted for identification of *Hypericum* endemic species over a period of 18 months from April 2004 to November 2005; in their

endogenous localities from 10-2400 meter above the sea level in south east of Golestan province by transect method, respectively (Akhani, 2005).

Phenology of two endemic species was studied during field observation in 2 years. In their habitat 20 plants of each species with similar morphology conditions, were selected and marked, then in each of 15 days interval all processes of their growth and development such as germination, appearance of primary leaves, flowering, fruiting stage and seed dispersal were recorded.

Leaves, fruits, flowers and Aerial parts in blooming of two species were collected and shade dried for about one week and powdered. Their botanical name identified in the Plant Systematic Laboratory, College of Sciences, Islamic Azad University of Gorgan Branch, Iran, where voucher specimens were deposited.

Ethnobotany or traditional knowledge of plants was obtained using the more traditional means of the ethnobiological and ethnobotanical analysis and was carried out using classical ethnobotanical methods (Pieroni *et al.*, 2004).

In this study, ethnobotanical information was collected using participant observation with 4 famous persons chosen among the elderly population and who still retain traditional knowledge about medicinal plants. Most of them belonged to the female group (average age: 76 years), which still retains the most information concerning the heritage of domestic remedies.

#### Extraction

**Ethanol extract:** The fine powder of plant parts were extracted by 70% ethanol were added to 50-60 g of dried powdered in decanter and obtained in period of 24 h. The extracts were rotary evaporated to yield a dense dark-residue. Each sample was diluted with propylene glycol to obtain 100 mg mL<sup>-1</sup> concentration (Rios *et al.*, 1988).

**Water extract:** One hundred milliliter of hot sterile distilled water, 70-80°C, was added to the 30 g powder samples which were allowed to soak for 24 h in water bath at 45-50°C. The extracts were filtered by using filter-paper and the resultant extracts were transformed to sterile glass dishes. Finally obtained 4 dilutions: 1, 1/2, 1/4, 1/8 (Mashhadian and Rakhshandeh, 2004).

**Infusion:** Thirty gram of dried powdered of different plant parts were added to 200 mL of water and boiled for 30 min, then the solid phase were decanted by filtering and aqueous phase was used for research. After the preparation of extracts they soaked in sterile paper Blank discs.

**Test organism:** The test organisms used in the study were obtained from Persian Type Culture Collection, Tehran, Iran (PTCC), namely: *E. coli* (PTCC No. 1330), *P. aeruginosa* (PTCC No. 1074), *S. aureus* (PTCC No. 1112), *S. typhi* (PTCC No. 1595), *S. dysentria* (PTCC No. 1188), *Y. enterocolitica* (PTCC No. 1151) and *E. faecalis*.

**Antimicrobial activity:** The antibacterial effects were tested by the disc-diffusion method, briefly, Muller Hinton Agar plates were cultured with a standardized inoculums (1-2×10<sup>8</sup> cfu mL<sup>-1</sup> equal to 0.5 McFarland) of each bacterial strain then the blank discs contain specific amount of extracts were carefully placed at the labeled seeded plate.

The plates were incubated aerobically at 37°C and examined for zones of inhibition after 24 h. The inhibition zones were measured with a ruler and compared with the control discs (disc containing only propylene glycol that used as diluents of ethanolic extract and disc containing Gentamycin as positive control). Each test was repeated 3 times and means inhibition zone were recorded. Inhibitory zone = 12 mm used as good inhibitory effect of extract (Nostro *et al.*, 2000).

## RESULTS

In this study we found that *Hypericum* is a perennial medicine genus with 6 endemic species in South east of Golestan province: (*H. perforatum*, *H. androsaemum*, *H. linaroides*, *H. tetrapterum*, *H. scabrum* and *H. elangatum*). Results showed the *H. perforatum* and *H. androceamum* are two main herbaceous and sub shrub species, with Cryptophyte to Chamaephyte life forms. They were more distributed in humid temperature to dry cool climate in heavy texture and none saline soil in north moisture slopes of 10-2400 m from sea level.

*H. perforatum* has very abundant in sunny position near the mountainous road in this province, but *H. androceamum* has less density and often cooperated growth in shady humid forest in sub canopy of *Parrotia persica* and *Carpinus betulus*. In mountain, it is grown in sub canopy of *Fagus orientalis* in cold climate, with mean annual precipitation from 369.9 and 696.8 mm.

Investigation of phenology showed these plants begin vegetative growth stage at the early April to June; flowering stage happens June to late July. Fruiting in August to late October and seed dispersed in November and falls from the October to November and then hibernation occurs.

Ethnobotanical data from this two species obtained from the rural native healers and herbal drug sellers in

south east of this province. Aerial parts and their latex of either of two species in herbal medicine externally for the treatment of skin wounds, eczema, sores, abscesses, warts, skin disorders, anti fungal and internally for central nervous diseases and headache. June and July was reported to be the best time for their collection (Table 1). In this Table, we reported for each species, its preparation, traditional uses and localities for each endemic species in the aforementioned methodology.

The ethanol extracts of flowering aerial parts had antibacterial activity against *S. aureus* and *E. faecalis*, with inhibition zone of 25, 26 mm in *H. perforatum* and 16, 20 mm for *H. androsaemum* (Table 2).

There was neither or little activity was seen against *P. aeruginosa*, *E. coli*, *S. tify*, *S. dysentria* and *Y. entrocolitica*. Weak antibacterial effects were seen in infusion and aqueous extracts (Table 3).

Ethanol extracts of flowering aerial parts of *H. perforatum* was more effective than *H. androsaemum* (Table 2), it should be mentioned that 42.5% of all used bacterial species are sensitive to various type of extracts that prepared from *H. perforatum* and only 24.7% of the above bacteria are sensitive to *H. androsaemum*.

We found that the ethanol extracts was even more effective than aqueous and infusion against tested bacteria (Table 3).

Table 1: Ethnobotany and traditional uses of *Hypericum* endemic species in Golestan province

Endemic species	Part used	Preparation	Traditional uses
<i>Hypericum perforatum</i> 10-2400 m	Flowering aerial parts and leaves	Decoction and topical application	In washes of wounds, warts, skin abscesses, antiseptic gingival for sore throat and bruises, anti-hypertensive, anti-fever with wild <i>Levandula</i> for headache, migraine and back pain. anti-rheumatic, heal menstrual pain and urinary trace infection to mixture with fleshy cone of <i>Juniperus communis</i> , anti-inflammation for bronchitis, cough, chest infection and arthritis with mixture by root of <i>Imula helenium</i> , <i>Malva sylvestris</i> and <i>Echium amoenum</i> .
<i>Hypericum androsaemum</i> 450-1800m	Flowers, leaves and latex	Decoction and oniment	Hepato- dupurative, a good tonic for liver with <i>Silybum marianum</i> and <i>Glycyrrhizia glabra</i> , sour cream with Cow oil for anti- inflammation, insect and snake bites healing, warts, athlete's foot and chickenpox with <i>Melissa officinalis</i>

Table 2: Antibacterial activity of ethanolic extracts of *Hypericum*

Plants	Part used	Antibacterial activity zone of inhibition (mm)*						
		E.c	S.a	P.a	E.f	S.t	Sh. d	Y.e
<i>Hypericum perforatum</i>	FR**	-	13	-	11	8	10	13
	FL	9	12	-	14	8	11	9
	AP	10	26	-	25	9	10	11
	LE	11	12	-	14	10	10	10
<i>Hypericum androsaemum</i>	GE	16	15	12	11	14	17	16
	FR	9	14	-	12	8	10	10
	FL	9	14	-	13	9	9	8
	FA	10	16	-	20	10	11	10
	LE	-	15	-	13	9	9	-
	GE	16	15	12	11	14	17	16

\*Data are the average of three experiments; \*\*FR:fruit; FL: Flower; AP: Aerial Parts in blooming; LE: Leaves; GE: Gentamycine; \*\*\*E.c: *Escherichia coli*; S.a: *Staphylococcus aureus*; P.a: *Pseudomonas aeruginosa*; E.f: *Entrococcus faecalis*; S.t: *Salmonella typhi*; Sh. d: *Shigella dysentria*; Y.e: *Yersinia entrocolitica*

Table 3 Antibacterial activity of ethanolic, aqueous and infusion extracts of *Hypericum perforatum* and *Hypericum androceamum*

Plants	Tested organism	Antibacterial activity*		
		I	A	E**
<i>Hypericum perforatum</i>	E.c	-	-	+
	S.a	+	-	++
	P.a	-	-	-
	E.f	+	+	++
	S.t	-	+	+
	Sh.d	-	-	+
	Y.e	+	-	+
<i>Hypericum androsaemum</i>	E.c	-	-	+
	S.A	+	-	++
	P.a	-	-	-
	E.f	+	-	++
	S.t	-	-	+
	Sh.d	-	-	+
	Y.e	-	-	+
Total sensitivity***		18.2%	6.5%	64%

\*Data are the average of three experiments; -: No inhibition; +: Moderate inhibition; ++: Very inhibition; \*\*E: Ethanol; A: Aqueous; I: Infusion; \*\*\*%: Sensitivity of tested bacteria toward ethanolic, aqueous and infusion extracts obtained from different part of two species of *Hypericum*

As a whole 37.2, 35, 20 and 8% of all tested bacteria are sensitive to the extracts which was prepared from aerial parts in blooming, flower, leaf and fruits, therefore it can be seen that the aerial parts of two species in blooming were more effective than other parts of the plants (Table 1 and 2).

## DISCUSSION

Golestan province has always been a region for practicing folklore medicine. However very little information is available on the ethnobotany and antibacterial effect of herbal medicine in this area.

In the present study, we are reporting the ethno botanical and antibacterial effects of two endemic species of *Hypericum*, which were grown in the mountainous area located in Golestan province.

We found that, *Hypericum* species are widely used in the southern mountainous villages of Golestan province for treating of headache, migraine, cough and systemic infection, it should be mentioned *H. perforatum* more widely used than *H. androsaemum*.

Present results showed a remarkable antibacterial activity of the ethanol extract of *H. perforatum*, against gram-positive bacteria (*S. aureus* and *E. faecalis*) and these extract show wider inhibition zone in compare with Gentamycin.

In other studies researcher found that the antibacterial extracts of the herbal plants is mostly on gram positive bacteria (Herrera *et al.*, 1996; Kelmanson *et al.*, 2000; Ahmad and Beg, 2001).

The inhibitory effect of *Hypericum* on the *S.aureus* can pave the way for the application of this extract for the treatment of infections such as infected wounds, abscesses, arthritis, osteomyelitis, endocarditis, on the other hand the later effect on

*E. faecalis* which is a resistant bacterium can be a proper medication for the Urinary tract infection, wounds and endocarditis. The management of these two bacteria is one of the most problems in medicine.

Our findings ratify the proper use of this plant for the treatment of bacterial infection in the traditional medicine which was practiced in this region.

Our results showed that, the ethanolic extracts of *H. perforatum* and *H. androsaemum*, was more effective than the aqueous and infusion extracts, which are similar to other studies (Pistelli *et al.*, 2000; Sokmen *et al.*, 1999) which confirming that either the ethanolic or methanolic extracts are much better than other solvents.

Phytochemical research revealed the presence of tannin, flavonoids (such as hypericin and hyperphorin)

and anthraquinones in *Hypericum* species could be responsible for the antibacterial properties reported by Rabanal Prado (2002), Dall and Agnoll and Ferraz (2003).

We found that the flowering aerial parts of *Hypericum* species have got the best antibacterial efficacy compared to other parts. The *Hypericum* species have secondary metabolite with antibacterial effects, that increased in blooming stage, because in this process quantity of flavonoids in leaves and aerial parts were increased (Schwob, 2004).

Our data show that Gram-negative bacteria are resistant toward all of different extracts we used in this study, even ethanolic extract that obtained from flowering aerial parts, due to this reason we conclude that application of such a herbal medicine should be used after the identification of the type of bacteria and the extract should be used when the bacteria is Gram-positive.

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