Medicinal and Aromatic Plant Science and Biotechnology ©2009 Global Science Books

Fatty Acid Composition and Antimicrobial Activity of Leaf and Flower Extracts of *Hypericum mysorense*

H. P. Shilpashree[1] • V. Ravishankar Rai[2*]

[1] Department of Biotechnology, Manasagangotri, University of Mysore, Mysore - 570 006, Karnataka, India
[2] Department of Microbiology, Manasagangotri, University of Mysore, Mysore - 570 006, Karnataka, India

Corresponding author: * **rrai33@hotmail.com**

ABSTRACT

Hypericum mysorense is an important medicinal source for screening bioactive compounds. The chemical composition of the essential oil (EO) obtained from leaf and flower extracts of *H. mysorense* was analyzed by GC-MS. Linoleic acid was abundant in both samples. The EOs of both extracts showed antimicrobial activity against several microorganisms at 60-80 µg/ml.

Keywords: antibacterial activity, bioactive compound, essential oil, GC-MS, linoleic acid, palmitic acid

INTRODUCTION

Hypericum spp. have been used traditionally for their biochemical characteristics and secondary metabolite production. These species contain a number of biologically active detectable compounds, namely naphtodianthrones, phloroglucinols, flavonoids, procyanidins, tannins, essential oils, amino acids, phenylpropanoids, xanthones and other water-soluble components (Greeson *et al.* 2001; Andrija *et al.* 2007; Cirak *et al.* 2007; Hashida *et al.* 2007, 2008; Sagratini *et al.* 2008). The composition of the essential oils (EOs) of *Hypericum* spp. (Baser *et al.* 2002; Touafek *et al.* 2005; Karim *et al.* 2008; Nogueira *et al.* 2008) and antimicrobial activities of bioactive compounds has been previously reported in different *Hypericum* spp. (Rocha *et al.* 1995; Dall *et al.* 2003; Cecchini *et al.* 2007; Milosevic *et al.* 2007).

Hypericum mysorense is an important medicinal plant found in southern parts of Karnataka at 900-500 m. I*n vitro* cytotoxicity activity of *H. mysorense* extracts have been reported against HEp-2, RD, Vero and DLA cell lines (Vijayan *et al.* 2003). *H. mysorense* extracts exhibited significant antiviral activity against herpes simplex virus type-I (Vijayan *et al.* 2004) and antimicrobial activity against several bacterial and fungal pathogens (Mukherjee *et al.* 2002). To the best of our knowledge, no previous studies have been reported on *H. mysorense* for the presence of fatty acids and its antibacterial properties.

MATERIALS AND METHODS

Plants

H. mysorense plants were collected in July from the Western Ghats of Karnataka, India. Aerial parts of *H. mysorense*, including leaves and flowers, were used. The plant specimen was identified by Professor Gopalakrishna Bhatt, Taxonomist, Purna Pragnya College, Udupi, Karnataka, India. A voucher specimen was deposited at the Department of Applied Botany, University of Mysore, India.

Isolation and transmethylation of fatty acids

The isolation and transmethylation of fatty acids were carried out using the method of Garcés and Mancha (1993). 50 g of fresh plant material was heated with a mixture containing methanol, heptane, tetrahydrofuran, 2,2-dimethoxypropane and sulphuric acid (37: 36: 20: 5: 2). At 80°C simultaneous digestion and lipid transmethylation took place in a single phase. After cooling at room temperature, the upper phase was collected for gas chromatography (GC) and GC-mass spectrometry (GC-MS) analyses.

GC and GC-MS

The fatty acid methyl esters (FAMEs) were analyzed by GC using a Unicam 610 gas chromatograph equipped with an SP-2330 capillary column (30 M × 0.25 mm, 0.2 µm thickness), a flame ionization detector and a Unicam 4815 recording integrator. Separations were conducted with temperature programme from 180 to 200°C at 5°C/min after an initial 2 min hold. FAMEs were identified by comparison of retention times with authentic standards (Sigma, India).

GC-MS analyses were conducted with GC-MS equipment (HP 5890-E Series GC System) with mass selective detection. An Innowax column (30 M × 0.25 mm) was used, and the temperature was programmed from 150 to 230°C at 2°C/min with an initial hold of 4 min and a final hold of 36 min. The carrier gas was helium (1 ml/min) and the split ratio was 50: 1. The injection port was held at 250°C and the detector at 300°C. The mass spectrometer was operated in electron impact ionization mode (70 eV). FAMEs were identified by comparison with retention time and mass obtained from their respective authentic pure standards (Sigma, India). Relative percentage amounts of each fatty acid were reported after three independent experiments. Values were subjected to one-way analysis of variance (ANOVA) using Tukey's B test at P=0.05 significant level. Results are expressed as mean values in **Table 1**.

Tested material for antimicrobial activity

Crude EOs of leaves and flowers of *H. mysorense* were prepared as above and antimicrobial activity conducted by the disc diffusion method (Portillo *et al.* 2001). Microorganisms used were bacterial strains (*Escherichia coli* K12, *E. coli* PBR 322, *E. coli* PUC 9, *Bacillus brevis* ATCC, *B. cereus* DMC65, *Streptococcus pyogenes* DMC41, *Pseudomonas aeruginosa* DMC66, *Staphylococcus aureus* DMC70) collected from the Department of Microbiology, J. S.S. Hospital, Mysore, India. The antibiotics ampicillin and sulbactam (each at 10 µg/disc) were used as positive controls.

Received: 28 August, 2009. Accepted: 14 December, 2009.

Short Communication

Table 1 The composition of fatty acids present in leaf and flower extracts of *H. mysorense*.

Fatty acids	Leaf extract (%)	Flower extract (%)
Capric (10:0)	0.6	0.4
Lauric (12:0)	4.49	2.4
Myristic (14:0)	3.5	4.5
Palmitoleic (16:1, *cis*-9)	3.1	0.9
Palmitic (16:0)	21.98	26.8
Linoleic (18:2, *cis*-9,12)	31.20	28.5
Oleic (18:1, *cis*-9)	16.06	25.69
Stearic (18:0)	7.98	7.2
Arachidic (20:0)	5.3	2.88
Erucic (22:1, *cis*-13)	0.18	0.23
Behenic (22:0)	3.77	1.34
Lignoceric (24:0)	1.90	14.9

RESULTS AND DISCUSSION

Distribution and abundance of fatty acids

The fatty acid composition of total lipids in the aerial parts of *H. mysorense* is presented in **Table 1**. Linoleic acid was the most abundant fatty acid in leaf (31.20%) and flower (28.5%) extracts. Similar results were also observed in previous studies on other *Hypericum* spp. (Stojanovic *et al.* 2003). Also, a comparatively higher amount of palmitic acid was observed in leaf (21.98%) and flower (26.8%) extracts. A previous report in *H. perforatum* showed that palmitic acid (20.3%) was the most abundant fatty acid, and a similar lauric, linoleic and behenic acid composition of the EO obtained in this study (Girzu *et al.* 1995). Oleic acid was more abundant in the flower extract than in the leaf extract. The levels of capric and erucic acids were low in both leaf and flower extracts. Lignoceric acid was more abundant in flower than in leaf extracts.

Antimicrobial activity of fatty acids

The results of antimicrobial activity of leaf and flower extracts are given in **Tables 2** and **3**, respectively. The EOs effectively inhibited the growth of microorganisms at 60-80 µg/ml, i.e. both leaf and flower extracts of *H. mysorense* had antimicrobial activity but only partial activity at 40 µg/ml. Similar results were observed in *H. perforatum* (Toker *et al.* 2006).

ACKNOWLEDGEMENT

The authors re thankful to Department of Microbiology, J.S.S. Hospital, Mysore for providing bacterial strains for antimicrobial studies.

REFERENCES

Andrija S, Michael S, Axel PL, Zaklina S, Nils R (2007) Essential oil composition of *Hypericum* L. species from Southeastern Serbia and their chemotaxonomy. *Biochemical Systematics and Ecology* **35**, 99-113

Baser KH, Cozek T, Nuriddinov HR, Demirci AB (2002) Essential oils of two *Hypericum* species from Uzbekistan. *Chemistry of Natural Compounds* **38**, 54-57

Cecchini C, Cresci A, Coman MM, Ricciutelli M, Sagratini G, Vittori SLD, Maggi F (2007) Antimicrobial activity of seven *Hypericum* entities from Central Italy. *Planta Medica* **73**, 564-566

Cirak C, Radusiëne J, Karabuk B, Janulis V, Ivanauskas L (2007) Variation of bioactive compounds in *Hypericum perforatum* growing in Turkey during its phenological cycle. *Journal of Integrative Plant Biology* **49**, 615-620

Dall AR, Ferraz A, Bernardi AP, Albring D, Nör C, Sarmento L, Lamb L, Hass M, Von Poser G, Schapoval EES (2003) Antimicrobial activity of some *Hypericum* species. *Phytomedicine* **10**, 511-516

Garcés R, Mancha M (1993) One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Analytical Biochemistry* **211**, 139-143

Girzu M, Carnat A, Chabard J (1995) Fatty acid composition of *Hypericum perforatum* flowering tops. *Oleagineux Corps Gras Lipides* **2**, 317-318

Greeson JM, Sanford B, Monti DA (2001) St. John's wort (*Hypericum perforatum*): a review of the current pharmacological, toxicological and clinical literature. *Psychopharmacology* **153**, 402-414

Hashida W, Tanaka N, Takaishi Y (2007) Prenylated xantones from *Hypericum ascyron*. *Journal of Natural Medicines* **61**, 371-374

Hashida W, Tanaka N, Kashiwada Y, Sekiya M, Ikeshiro Y, Takaishi Y (2008) Tomoeones A-H, cytotoxic phloroglucinol derivatives from *Hypericum ascyron*. *Phytochemistry* **69**, 225-230

Karim H, Kamel M, Mouna BT, Olfa O, Monem K, Brahim M (2008) Essential oil composition of *Hypericum perfoliatum* L. and *Hypericum tomentosum* L. growing wild in Tunisia. *Industrial Crops and Products* **27**, 308-314

Milosevic T, Solujic S, Sukdolak S (2007) *In vitro* study of ethanolic extract of *Hypericum perforatum* L. on growth and sporulation of some bacteria and fungi. *Turkish Journal of Biology* **31**, 237-241

Mukherjee PK, Saritha GS, Suresh B (2002) Antimicrobial potential of two different *Hypericum* species available in India. *Phytotherapy Research* **16**, 692-695

Nogueira T, Curto MJC, Figueiredo AC, Barroso JG, Pedro LG, Rubiolo P, Bicchi C (2008) Chemotaxonomy of *Hypericum* genus from Portugal: Geographical distribution and essential oils composition of *Hypericum perfoliatum*, *Hypericum humifusum*, *Hypericum linarifolium* and *Hypericum pulchrum*. *Biochemical Systematics and Ecology* **36**, 40-50

Portillo A, Vila R, Freixa B, Adzet T, Cañigueral S (2001) Antifungal activity of paraguayan plants used in traditional medicine. *Journal of Ethnopharmocology* **16**, 93-98

Rocha L, Marston A, Potterat O, Kaplan MA, Stoecklievans H, Hostett-

Table 2 Antimicrobial activity of leaf extract of *H. mysorense* essential oils.

Bacteria	* DIO 40 µg/disc	* DIO 60 µg/disc	* DIO 80 µg/disc	Antibiotic (SAM)
Escherichia coli K12	12	18	16	20
E. coli PBR 322	14	16	14	20
E. coli PUC 9	-	10	8	16
Bacillus brevis ATCC	14	18	16	22
B. cereus DMC65	-	16	18	12
Streptococcus pyogenes DMC41	8	12	10	14
Pseudomonas aeruginosa DMC66	12	14	14	-
Staphylococcus aureus DMC70	6	10	8	16

(–): Not active; *Diameter of inhibition (mm), SAM: Ampicillin / Sulbactam (10 /10 µg/disc)

Table 3 Antimicrobial activity of flower extract of *H. mysorense* essential oils.

Bacteria	* DIO 40 µg/disc	* DIO 60 µg/disc	* DIO 80 µg/disc	Antibiotic (SAM)
Escherichia coli K12	14	18	18	20
E. coli PBR 322	10	16	14	20
E. coli PUC 9	8	10	10	16
Bacillus brevis ATCC	12	18	12	22
B. cereus DMC65	-	12	10	12
Streptococcus pyogenes DMC41	-	8	10	14
Pseudomonas aeruginosa DMC66	12	14	14	-
Staphylococcus aureus DMC70	10	18	16	16

(–): Not active; *Diameter of inhibition (mm), SAM: Ampicillin / Sulbactam (10 /10 µg/disc)

mann K (1995) Antibacterial phloroglucinols and flavonoids from *Hypericum brasiliense*. *Phytochemistry* **40**, 1447-1452

Sagratini G, Ricciutelli M, Vittori S, Öztürk N, Öztürk Y, Maggi F (2008) Phytochemical and antioxidant analysis of eight *Hypericum* taxa from Central Italy. *Fitoterapia* **79**, 210-213

Stojanovic G, Palic R, Tarr CH, Reddy CM, Olivera M (2003) *n*-Alkanes and fatty acids of *Hypericum perforatum*, *Hypericum maculatum* and *Hypericum olympicum*. *Biochemical Systematics and Ecology* **31**, 223-226

Toker Z, Kvzvi G, Çetin HOZ, Kvzvi M, Ertekin S (2006) Compositions and antimicrobial activities of the essential oils of two *Hypericum* species from Turkey. *Fitoterapia* **77**, 57-60

Touafek O, Nacer, Kabouche A, Kabouche Z (2005) Analysis of the essential oil of Algerian *Hypericum perfoliatum* (L). *Flavour and Fragrance Journal* **20**, 669-670

Vijayan P, Vinod Kumar S, Dhanaraj SA, Mukherjee PK, Suresh B (2003) *In vitro* cytotoxicity and antitumour properties of *Hypericum mysorense* and *Hypericum patulum*. *Phytotherapy Research* **17**, 952-956

Vijayan P, Raghu C, Ashok G, Dhanaraj SA, Suresh B (2004) Antiviral activity of medicinal plants of Nilgiris. *Indian Journal of Medical Research* **120**, 24-29