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

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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 naphthodianthrones, 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. In 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-1 (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 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, 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 hold was 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. Hospital, Mysore, India. The antibiotics ampicillin and sulbactam (each at 10 μg/disc) were used as positive controls.
The authors re thankful to Department of Microbiology, J.S.S. Hospital, Mysore for providing bacterial strains for antimicrobial studies.

REFERENCES


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


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

RESULTS AND DISCUSSION

Distribution and abundance of fatty acids

The fatty acid composition of total lipids in the aerial parts of H. myroserene 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 EOs 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. myroserene had antimicrobial activity but only partial activity at 40 μg/ml. Similar results were observed in H. perforatum (Toker et al. 2006).

Table 2 Antimicrobial activity of leaf extract of H. myroserene essential oils.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>* DIO 40 μg/disc</th>
<th>* DIO 60 μg/disc</th>
<th>* DIO 80 μg/disc</th>
<th>Antibiotic (SAM)</th>
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<tbody>
<tr>
<td>Escherichia coli K12</td>
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<td>E. coli BPR 322</td>
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<tr>
<td>Staphylococcus aureus DMC70</td>
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(*) Not active; *Diameter of inhibition (mm), SAM: Ampicillin / Sulbactam (10 / 10 μg/disc)

Table 3 Antimicrobial activity of flower extract of H. myroserene essential oils.

<table>
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<tr>
<th>Bacteria</th>
<th>* DIO 40 μg/disc</th>
<th>* DIO 60 μg/disc</th>
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(*) Not active; *Diameter of inhibition (mm), SAM: Ampicillin / Sulbactam (10 / 10 μg/disc)


