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RESEARCH ARTICLE

NEW PHENOLIC GLYCOSIDES FROM ROOTS OF *ACTAEA SPICATA* LINNEAUS

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***Actaea spicata* Linn. (Ranunculaceae) has been traditionally used for the treatment of various ailments such as rheumatism, inflammation, nerve diseases, lumbago, scrofula and chorea. Despite a long tradition of use, no systematic phytochemical work has been carried out on this potential plant. The present investigation was undertaken to isolate and characterize phenolic compounds from ethyl acetate fraction of methanol extract of *A. spicata* roots. Column chromatography of polyphenol rich ethyl acetate fraction of methanol extract of *A. spicata* yielded two new phenolic constituents characterized by various spectroscopic techniques such as FT-IR, ^{1}H NMR and ^{13}C NMR, and identified as 4 C-glucosyl-3, 5-dihydroxy-2-methoxy benzoic acid and its acetyl derivative 5-acetoxy-4 C-glucosyl-3-hydroxy-2-methoxy benzoic acid.**

Key words: *Actaea spicata*, Column chromatography, Polyphenols, Benzoic acids, Spectroscopy.

INTRODUCTION

Actaea spicata Linn., commonly known as Baneberry and Grapewort, belongs to family Ranunculaceae (**Figure 1**). A survey of ethnopharmacologic records reveals that the plant has been traditionally used in the treatment of rheumatism, inflammation, rheumatic fever, lumbago, scrofula, nervous disorders, chorea, and as emetic, expectorant, laxative, stomachic and purgative (Chopra *et al* 1956; Khare, 2007; Duke *et al* 2008). The plant has also been used in traditional systems of medicines of various countries for the treatment of snake bite, asthma, and externally for skin complaints. In some parts of Europe the powdered leaves, stems and flowers are used as an insecticide (Kirtikar and Basu, 1975). *A. spicata* has been reported to contain isoquinoline alkaloids magnoflorine, corytubrine; triterpene glycosides including actein and trans-aconitic acid (Fleming and Gruenwald, 2000). Trans aconitic acid, isolated from ethanolic fractions of *A. spicata*, was found to exhibit cytostatic action against Ehrlich's ascites tumour (Nikonov and Syrkina-Krugliak, 1963). An exhausted literature survey on *A. spicata* revealed that sporadic phytochemical and pharmacological reports are available on this plant. As *A. spicata* has been used traditionally for the treatment of various ailments, this plant holds great potential for in depth phytochemical and pharmacological evaluations. The present investigation was aimed at isolation of novel phytoconstituents from ethyl acetate fraction of methanol extract of *A. spicata* roots and their characterization by spectroscopic techniques.

MATERIALS AND METHODS

Plant material

Dried roots of *A. spicata* were procured from K. R. Indo German American Trading company, Kurukshetra (Haryana), India in the month of November 2008. Identity of the plant was

confirmed through Dr. H.B. Singh, Scientist F, Head of Raw material Herbarium and Museum (RHMD), National Institute of Science and Information Resources (NISCAIR), New Delhi, India.

Figure 1. *Actaea spicata* Linn.

Solvents

All the solvents used in the present investigation were, of LR grade, procured from Central Drug House Pvt. Ltd., Mumbai.

Chemical

Diphenyl boric acid-*β*-ethyl amino ester (SIGMA, USA) was used for the preparation of natural product reagent.

Preparation of methanol extract

Dried, coarsely powdered roots of *A. spicata* (500 g) were extracted with petroleum ether using a Soxhlet apparatus. The marc was air dried and extracted with methanol using a Soxhlet apparatus for 18 h. The methanol extract was dried under reduced pressure using rotary vacuum evaporator (Perfit, Ambala), and screened for different classes of phyto-constituents (Farnsworth, 1966).

Preparation of ethyl acetate fraction (polyphenol rich fraction)

The methanol extract (25 g) of *A. spicata* roots was suspended uniformly in water, placed in three-necked round bottom flask connected with magnetic stirrer, and partitioned with ethyl acetate by heating for 30 min at 50°C with continuous stirring. This procedure was repeated five more times. All the shakings of ethyl acetate were pooled and concentrated under reduced pressure. While concentrating ethyl acetate fraction, a compound (**GB_1**: 1.426 g) was separated as white amorphous powder. The ethyl acetate fraction (6.982 g) obtained was rich in polyphenols.

Column chromatography of ethyl acetate fraction

The ethyl acetate fraction (4.5 g) of methanol extract of *A. spicata* roots was loaded onto a column packed with silica gel (60-120; E-Merck, Mumbai), and eluted using hexane, hexane-ethyl acetate, ethyl acetate or ethyl acetate-methanol as the mobile phases. A total of 86 fractions, 250 ml each, were collected. These were pooled, based on similar thin layer chromatograms, to get 6 fractions ranging from F_1 to F_6. Thin layer chromatography (TLC) chromatograms were taken on silica gel G pre-coated aluminium based plates (E-Merck, Mumbai) using solvent system Toluene : Ethyl acetate : Glacial acetic acid :: 5 : 4 :1, and visualized under UV chamber (254/366 nm; Gupta Scientific Store, Ambala) after spraying with natural product reagent.

Characterization of isolated constituents

GB_1 and GB_2 were subjected to FT-IR, ^{1}H NMR (400 MHz) and ^{13}C NMR (400 MHz) spectroscopy, and the characterization data are given below.

GB_1: M.p. 213 -216°C; IR (KBr): *v* 3382, 2939, 2889, 1706, 1612, 1463, 1234, 1127, 1013, 900-500 cm^{-1}; ^{1}H-NMR (DMSO): 3.46 (1H, t, H-3'), 3.66 (2H, m, H-4' and H-5'), 3.84 (1H, t, H-2'), 3.92 (3H, s, ph-OCH_3), 4.00 (1H, d, *J* = 9.9 Hz, H-6'), 4.09 (1H, t, H-6'), 4.86 (1H, d, *J* = 10.4 Hz, H-1'), 7.15 (1H, s, H-6), 7.62 (s, OH groups of C-glucose), 8.31 (1H, s, OH-5), 9.07 (1H, s, OH-3) ppm; ^{13}C NMR (DMSO): 59.8 (ph-OCH_3), 61.2 (C-6'), 70.4 (C-5'), 72.8 (C-2'), 73.9 (C-1'), 79.4 (C-3'), 81.5 (C-4'), 109.8 (C-6), 115.1 (C-1), 117.6 (C-4), 140.4 (C-2), 147.7 (C-3), 150.7 (C-5), 163.1 (C=O) ppm.

GB_2: M.p. 145-148°C; IR (KBr): *v* 3382, 2939, 2889, 1706, 1612, 1463, 1234, 1127, 1013, 900-500 cm^{-1}; ^{1}H-NMR (DMSO): 2.17 (3H, s, ph-$OCOCH_3$), 3.51 (1H, t, H-3'), 3.67-3.73 (2H, m, H-4' and H-5'), 3.86 (1H, t, H-2'), 3.94 (3H, s, ph-OCH_3), 4.00 (1H, d, *J* = 12 Hz, H-6'), 4.12 (1H, t, H-6'), 4.86 (1H, d, *J* = 10.4 Hz, H-1'), 7.18 (1H, s, H-6), 7.49 (s, OH groups of C-glucose), 9.08 (1H, s, OH-3); ^{13}C NMR (DMSO): 20.8 (ph-$OCOCH_3$), 59.9 (ph-OCH_3), 61.5 (C-6'), 70.6 (C-5'), 72.8 (C-2'), 74.0 (C-1'), 78.9 (C-3'), 81.5 (C-4'), 110.7 (C-6), 116.5 (C-1), 118.2 (C-4), 140.9 (C-2), 141.5 (C-5), 147.1 (C-3), 165.1 (C=O), 169.1 (ph-$OCOCH_3$).

RESULTS AND DISCUSSION

A. spicata is a plant with centuries old history of use as a traditional medicine with potential anti-inflammatory, anti-spasmodic, anti-bacterial and anti-rheumatic activities. An exhausted literature survey on *A. spicata* revealed that sporadic phytochemical and pharmacological reports are available on this plant. Thus, this plant holds great potential for in depth phytochemical evaluation. Keeping in view the fact that the plants or foodstuffs such as fruits and vegetables containing phenols possess excellent antioxidant activity (Kiselova *et al* 2006; Kedage *et al* 2007; Klimczak *et al* 2007; Jayaprakasha *et al* 2008; Dai and Mumper, 2010; Patel *et al* 2010; Gowri *et al* 2011), the present investigation was envisaged to isolate phenolic compounds from polyphenol rich ethyl acetate fraction obtained from methanol extract of *A. spicata* roots. *A. spicata* roots were defatted by extracting with petroleum ether (60-80°C) in a soxhlet apparatus. The marc was air dried and extracted for 18 h with methanol using soxhlet apparatus. The methanol extract was dried and employed for present investigations. Preliminary phytochemical screening of methanol extract of *A. spicata* roots showed presence of phenols and flavonoids. The ethyl acetate fraction was obtained by partitioning methanol extract with ethyl acetate by heating for 30 min at 50°C with continuous stirring.

While concentrating ethyl acetate fraction, a compound (**GB_1**: 1.426 g) was separated as the white amorphous powder. Column chromatography of polyphenol rich ethyl acetate fraction was carried out by using hexane, hexane-ethyl acetate, ethyl acetate or ethyl acetate-methanol as the mobile phases in increasing order of polarity. **Table 1** shows fractionation of ethyl acetate fraction of methanol extract of *A. spicata* roots using column chromatography. Fraction 4 (F_4) yielded a pure compound (yellow star shaped crystals) designated as GB_2 (102 mg).

Table 1. Fractionation of ethyl acetate fraction of methanol extract of *A. spicata* roots using column chromatography.

Fraction	Eluent	Yield (g)	Constituent(s) isolated
F_1	hexane	0.02	–
F_2	hexane + ethyl acetate (1 : 1)	0.58	–
F_3	hexane + ethyl acetate (2 : 3) hexane + ethyl acetate (3 : 7)	0.72	–
F_4	hexane + ethyl acetate (1 : 4)	1.02	Yellow star shaped crystals (**GB_2**: 102 mg)
F_5	hexane + ethyl acetate (1 : 9) ethyl acetate	1.62	–
F_6	ethyl acetate + methanol (99 : 1) ethyl acetate + methanol (98 : 2) ethyl acetate + methanol (19 : 1)	0.32	–

GB_1 and GB_2 were characterized by various spectroscopic techniques such as FT-IR, ^{1}H NMR (400 MHz) and ^{13}C NMR (400 MHz), and identified as 4 C-glucosyl-3, 5-dihydroxy-2-methoxy benzoic acid and its acetyl derivative, *i.e.* 5-acetoxy-4 C-glucosyl-3-hydroxy-2-methoxy benzoic acid. These phenolic compounds were first time reported in *A. spicata*. **Figure 2** and **Figure 3** show structures of isolated phenolic compounds on the basis of their FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy data. In the future, the detailed phytochemical and pharmacological studies will be carried out on *A. spicata* Linn. with a view to isolate the biological markers, and to determine markers in the crude plant material quantitatively so that the plant can be standardized on the basis of biological markers.

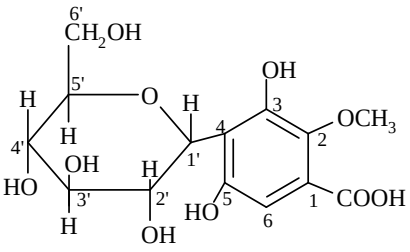

Fig. 2. Structure of isolated phenolic component GB_1

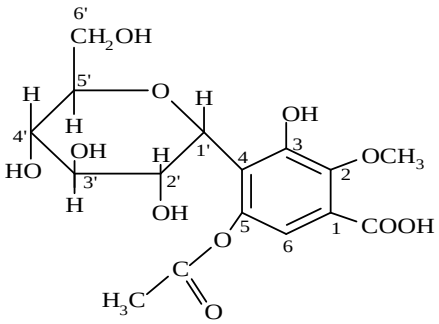

Fig. 3. Structure of isolated phenolic component GB_2

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