



Phytochemical Studies from the Roots of *Girardinia heterophylla*

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ABSTRACT

Girardinia heterophylla (Family: Urticaceae) roots has not been studied so far. The swollen base of roots were collected from and extracted with petroleum ether. The dried petroleum extract was subjected to column chromatography and TLC. Three compounds were isolated from the roots of *Girardinia heterophylla*. On the basis of spectral analysis they were identified as β -sitosterol, γ -sitosterol and ursolic acid. In this study the presence of γ -sitosterol and Ursolic acid in roots of *Girardinia heterophylla* has been reported for the first time.

Keywords: *Girardinia heterophylla*, β -sitosterol, γ -sitosterol, ursolic acid.

INTRODUCTION

Girardinia heterophylla, commonly known as '*DansKandali*', is abundantly found in the Himalayas. It can be seen growing extensively as an underutilized biomass in the forest areas situated in outskirts of the villages. Swollen base of roots of *Girardinia heterophylla* is used as soap/shampoo for washing. The leaves of this plant are boiled and cooked for vegetables. The whole plant is also used as cattle fodder to improve milk production. The stem portion of the plant yields the valuable fiber, which has been traditionally used by the tribal for making rope based products used for packing grains and

transportation purpose. After extracting the fiber from the stem, residue of the bark portion is used as fuel wood. Traditionally, bark powder is also used as a bandage material for faster healing of wounds and setting of broken bones.

Girardinia heterophylla is among those plant species which have not been systematically studied for their biological active constituents in order to establish their potential use through its different parts viz. leaves, stem and roots. The availability, different uses and phytochemical examination of *Girardinia heterophylla* (*Danskandali*) have not been studied in

Uttarakhand and scarce literature is available on the plant.

It is of immense importance to screen the medicinal plants growing in nature to find the additional source of bioactive compounds to be used for human welfare. The increasing global interest towards this interface between chemistry and biology has gained more importance and the public demand is continuously rising for the cost effective medications and biological agents from sustainable and natural resources.

The study mainly focuses on the phytochemical examination of species of *Girardinia heterophylla* grown in Mussoorie hills of Dehradun district of Uttarakhand state.

MATERIAL AND METHODS

General experimental procedures

TLC Plates coated with silica gel G to a thickness of 0.25 mm were used. SD-fine silica gel (100-200 mesh) was used for column chromatography. Melting point was determined on a melting point apparatus in a sealed glass capillary tube and mixed melting point (m.m.p.) by authentic sample. GC-MS conditions for analyzing the compounds were TRAC-MS (Finnigan company).

Plant material

The plant samples were collected from the Middle Himalayas at an altitudinal range of 22,00m to 25,00m in Mussoorie and Dhanaulti areas of Dehradun District. The plant samples of leaves, stem and roots were collected in the month of November 2010.

Extraction and Isolation

The roots (600g) of *Girardinia heterophylla* were milled after air drying and were extracted with the solvent petroleum ether (60-80°C) and methanol. The petroleum ether extract (2.57%) was obtained and further examined. Petroleum ether extract is column chromatographed over silica gel and elution of the column with varying amount of ethyl acetate in petroleum ether afforded three compounds having code of GHRPTA, GHRPTB and GHRPTC.

Weight of petroleum ether extract	=	15.42gm
Weight of silica gel used for adsorption of extract	=	75gm
Weight of silica gel used for building of column	=	322gm
Solvent used for packing column	=	
Petroleum ether		
Retention volume	=	435
Volume of each fraction	=	100 ml

The further details are given in table-1.

RESULTS AND DISCUSSION

Three compounds were isolated from roots of *Girardinia heterophylla* having code numbers GHRPTA(β -sitosterol), GHRPTB (γ -sitosterol) and GHRPTC (Ursolic acid) are described hereunder.

Compound GHRPTA (β -sitosterol)

It was eluted with petroleum ether: ethyl acetate (98:2) on concentrating yielded white silky needles (45mg, 0.008%, m.p. 136-137°C), $[\alpha]_D^{25}$ 100°C (0.05 in CHCl_3). It gave Liebermann Burchard test for terpenoids and steroids, having molecular formula $\text{C}_{29}\text{H}_{50}\text{O}$. (M^+ , m/z 414). The GC-MS spectrum

Table-1: Compounds of Petroleum Ether Extract of Root and their TLC system with Eluents

Compounds	Eluents	Fraction no.	Volume	Solvent system for TLC
GHRPTA	Petroleum ether: Ethyl acetate (98:2)	1-132	132x100	Petroleum ether: Ethyl acetate (95:5)
GHRPTB	Petroleum ether: Ethyl acetate (97:3)	133-283	150x100	Petroleum ether: Ethyl acetate (93:7)
GHRPTC	Petroleum ether: Ethyl acetate (90:10)	428-551	123x100	Petroleum ether: Ethyl acetate (1:1)

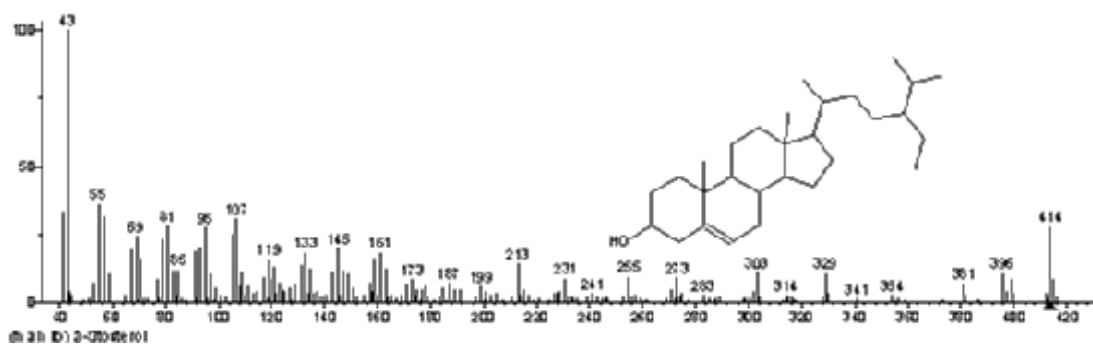


Fig. 1: Mass Spectra of β -Sitosterol

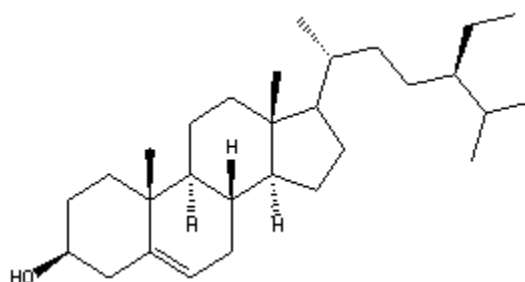


Fig. 2: Structure of GHLPA (β -Sitosterol)

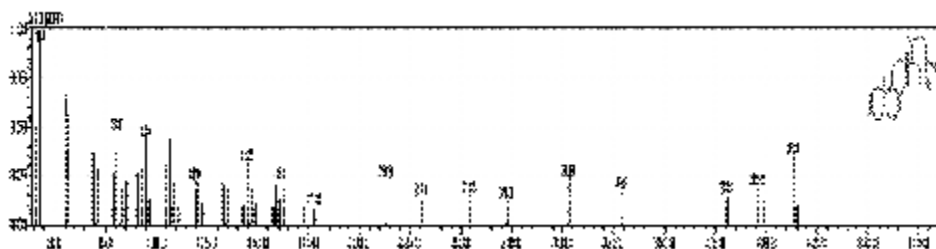


Fig. 5(a): Mass Spectra of Compound γ -Sitosterol

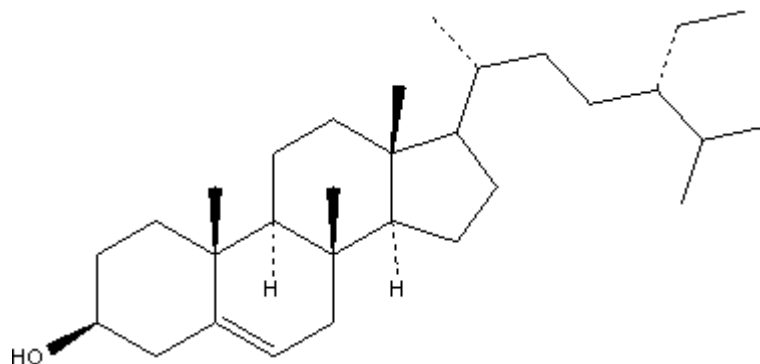


Fig. 5(b): Structure of Compound GHLPB (γ -sitosterol)

revealed the compound to be the β -sitosterol [Fig. 1,2]. It was identified as β -sitosterol by direct comparison with an authentic sample (m.m.p., Co-TLC and superimposable IR) and by comparison of its mass spectrum (Figure-4A) with that provided in the NIST standard chart library.

β -sitosterol has antidiabetic activity¹. It reduces the symptoms of Benign Prostatic Hyperplasia (BPH) and also found as an anti-inflammatory agent²⁻³.

Compound GHRPTB (γ -sitosterol)

It was eluted with petroleum ether: ethyl acetate (97:3) on concentrating yielded as white crystals (30mg), m.p. 147-148°C, $[\alpha]_D - 45$ (CHCl₃). The yield of the γ -sitosterol was found to be 0.010%.

It gave Liebermann Burchard test having molecular formula C₂₉H₅₀O, (M⁺, m/z 414). The compound GHLPB was analyzed by GC-MS. It was identified as γ -sitosterol by comparison of its mass spectra given in figure-3 with that of γ -sitosterol provided in the NIST standard chart library. The mass spectral values correspond to literature⁴. γ -sitosterol has been shown to be an epimer of β -sitosterol. The only difference of these two epimers was the 24-ethyl substituent. The 24-ethyl is present in the side chain of γ -sitosterol [Figure-4]. GC-MS assisted the characterization of γ -sitosterol has been reported in the literature⁵⁻⁷ has beta-chirality which was indicated as 24S in the NIST standard chart library. This is the first report of isolation of γ -sitosterol in the leaves of *Girardinia heterophylla*.

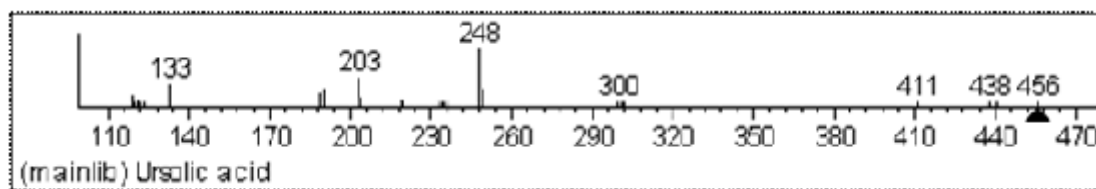


Fig. 11(a): Mass Spectra of Ursolic acid

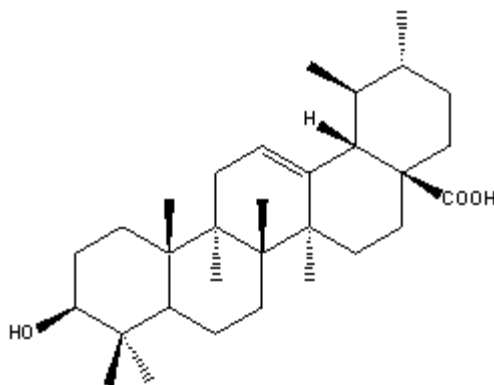


Fig. 11 (c): Structure of compound GHRPTC (Ursolic acid)

List of compounds isolated from the root of *Girardinia heterophylla*

Plantpart	Type of Extract	Class of Compound	Name of Compound	Yield(%)
Root	Petroleum ether extract	Phytosterol	β -sitosterol	0.008
		Phytosterol	γ -sitosterol	0.010
		Pentacyclitriterpene acid	Ursolic acid	0.001

γ -sitosterol reduces the hyperglycemia in STZ- induced diabetic rats due to increased insulin secretion and inhibition of glucogenesis. It can be used in *Diabetes mellitus*⁸. Docking studies of the ligand γ -sitosterol with four different target proteins showed that this is a good molecule which docks well with various targets related to *Diabetes mellitus*, thus γ -sitosterol can be considered for developing into a protein antidiabetic drug⁹.

Compound GHRPTC (Ursolic acid)

The fractions were eluted with petroleum ether: ethyl acetate (90:10) on concentrating a white silky needles (8.0mg) was obtained having m.p. 286°C with 0.001% yield. It was identified as ursolic acid given in [Figure-5,6] and by direct comparison with an authentic sample (m.m.p., Co-TLC and superimposable IR). This is the first report of isolation of ursolic acid [Figure 11(C)] in the plant of *Girardinia heterophylla*.

It possesses diverse pharmacological actions like anticancerous^{10, 11} antiulcer¹² hypoglycaemic¹⁴ antihyperlipidemic¹⁵ Antimicrobial¹⁶⁻¹⁷, antiviral¹⁸, anti-inflammatory¹⁹, antihistaminic²⁰⁻²² CNS depressant²³, Hepatoprotective²⁴, cardiotoxic sedative and tonic effects²⁵.

CONCLUSION

The phytochemical constituents were

isolated from swollen base of root of *Girardinia heterophylla* found in Mussoorie hills of Uttarakhand. Phytosterols are obtained having 0.008% yield (β -sitosterol), 0.010% yield (γ -sitosterol), and a pentacyclitriterpene acid having 0.001% (Ursolic acid) from roots using petroleum ether extract given in Table-2. In this study the presence of γ -sitosterol and Ursolic acid in roots of *Girardinia heterophylla* has been reported for the first time. It has been reported to possess many biological activities like antiulcer, anti-diabetic, anti-inflammatory etc.

Given the attire of identified chemical compounds having potential of being utilized for commercial purposes, the plant can be integrated with region specific developmental activities. Indian Himalayas showcases vast varieties of such plants, many of which are still beyond the ambit of contemporary research. Thus, present study also paves the way for the researchers to carry out further phytochemical investigations in the Himalayan region to identify other plants that may have commercial potential to be used in various industries.

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