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Isolation and Identification of γ- Sitosterol by GC-MS from the Leaves of *Girardinia heterophylla* (Decne)

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Abstract: *Girardinia heterophylla*, known as Himalayan Nettle commonly grows in temperate and subtropical Himalayas. Plant leaves were collected in winter season and analyzed using appropriate chemical methods and the biochemical constituents were ascertained. An unknown compound was isolated from leaves of the plant. After being collected, analyzed by GC-MS and compared with NIST standard chart library, it was declared to be γ-sitosterol, an epimer of β-sitosterol. γ-sitosterol has been reported for the first time in *Girardinia heterophylla* and has potential to be used as an antidiabetic owing to its remarkable medicinal properties.

Keywords: *Girardinia heterophylla,* GC-MS, γ-sitosterol, β-sitosterol.

INTRODUCTION

Girardinia heterophylla (Decne) is known as Himalayan Nettle *(Bicchu Butti)*, commonly found in temperate and subtropical Himalayas [1]. It can be seen growing extensively as an underutilized biomass in the forest areas situated in the outskirts of the villages. The plant belongs to the family *Urticaceae* and has remarkable medicinal properties [2] apart from its indispensable role in the local livelihood.

The leaves of this plant are boiled and cooked for vegetables and for preparing traditional recipes by the local forest dwellers [3]. The whole plant (leaf and stem) is used as cattle fodder to improve milk production. Root and leaf extracts are taken orally to cure abdominal pain [4]. Its root and swollen base are used as soap/shampoo for washing. Morphological characteristics of the plant viz. simple, opposite and sharply toothed leaves (5-10 cm long) with persistent stipules and stinging trichomes, 8-12 feet height, etc. [5] help to identify plant with ease.

Though a number of applications and uses of *Girardinia heterophylla* have been reported, so far, holistic scientific evaluation of the plant finds a rare mention in the contemporary literature. Similarly, studies on availability, uses and phytochemical examination of the plant have remained a far cry in Uttarakhand (India). Therefore, scientific study about abundance, relevant uses and phytochemical examination is expected to provide an insight into its unexplored potential.

Girardinia heterophylla has a remarkable association with the local communities owing to its unique properties and variety of uses. A review of traditional and contemporary knowledge reveals chemical constituents of the plant comprised of biologically active compounds that render its versatile properties, which were traditionally harnessed for local livelihood and domestic uses, valuable from economic view point. In the context of the unique and underexplored properties of the plant, the present study was therefore undertaken with the objectives to isolate and characterize biochemical compounds present in leaves. Findings of the study revealed the presence of γ-sitosterol, an epimer of β-sitosterol.

γ-sitosterol has been reported for the first time in *Girardinia heterophylla* and has potential to be used as an antidiabetic owing to its remarkable medicinal properties while β-sitosterol is the common sterol found in almost all plants which has Δ^5 double bond and α- ethyl at C-24 [6].

EXPERIMENTAL

Plant samples collection: For the phytochemical examination, leaves (Fig. **1**) of the plant were collected in winter season from the Middle Himalayas of Mussoorie Hills, Uttarakhand, India at an altitude of 2200m to 2500m.

Isolation and identification of unknown compound: The leaves (800g) of *Girardinia heterophylla* were milled after air drying and were soxhlet extracted with the solvent petroleum ether (60-80^{o}C) under vacuum, yielding petroleum ether extract (3.35%) which was separated by column chromatography over silica gel and elution of the column with varying amount of ethyl acetate in petroleum ether afforded one unknown compound. The details of the values during packing the column with petroleum extract, silica gel, retention volume and fraction volumes are given in (Table **1**).

The unknown compound was eluted with petroleum ether: ethyl acetate (97:3), fraction number 199-282, volume 83x50ml on concentrating yielded white crystals (20mg, 0.003% and m.p.147-148^{o}C). Solvent system for TLC was

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petroleum ether: ethyl acetate (93:7) having Rf value = 0.60. It gave a positive reaction in the Liebermann-Burchard test and was soluble in chloroform. The unknown compound was analyzed by GC-MS and it shows the retention time at 21.642.

Fig. (1). *Girardinia heterophylla* leaves from Mussoorie hills of Uttarakhand (India).

Table 1. Details of Column Chromatography of Leaves Extract of Petroleum Ether

Parameters Used	Quantity
Weight of petroleum ether extract	26.813gm
Weight of silica gel used for adsorption of extract	30gm
Weight of silica gel used for building of column	60gm
Solvent used for packing column	Petroleum ether
Retention Volume	125 ml
Volume of each fraction	50 ml

GC-MS analysis: GC-MS conditions for analyzing the unknown compound were TRAC-MS (Finnigan company), OV-1071 column (30m x 0.25mm x 0.25μm) from vertical chromatography Co. Ltd. Mobile phase: He gas (99.99% purity), with flow speed was 0.81/min and split ratio was 10:1. Sample temperature: 280^0C. Column temperature: from 240^oC and rose up to 265^0C at 10^0C/min. Remained at 265^oC for 10 minutes. Ionization mode: EI^+, Electron energy: 70eV. Interface temperature: 250^0C. Ion source temperature: 200^0C. Detection voltage: 350V.

RESULTS AND DISCUSSION

The mass spectrum of the compound was matched with that of γ-sitosterol provided in the standard reference database 69 of NIST Chemistry Web Book http://webbook.nist.gov/chemistry/[7], which led to the identification of γ-sitosterol. Details of information related to the mass spectrometry (MS) of the unknown compound are given in (Fig. **2**) below. As revealed by the corresponding information consulted on NIST Standard Chart Library, the unknown compound was identified as an isomer of β-sitosterol.

The sitosterol is most widely distributed in the plant sterols from which β-sitosterol and γ-sitosterol are found. β-sitosterol and γ-sitosterol are stereoisomers but they differ in the spatial configuration of the C-17 side chain. β-sitosterol, Δ^5 4-desmethyl sterol, has α-chirality at its 24-ethyl substituent [8] while unknown compound revealed 24-ethyl substituent has β-chirality, indicated as 24s in the NIST Standard Chart Library. Hence, given the GC-MS information of the compound, the unknown compound has been identified as an epimer of β-sitosterol, known as γ-sitosterol (Fig. **3**).

β-sitosterol is mainly found in cotton seed oil and wheat germ oil and γ-sitosterol in soyabeen oil [9]. β-sitosterol and γ-sitosterol are closely related but differ in that they are not appreciably adsorbed from the digestive tract [10]. A lowering of serum cholesterol in human subjects fed a usual diet plus crude soy sterol presumably largely γ-sitosterol has been reported [11].

γ-sitosterol has been reported for the first time in the leaves of *Girardinia heterophylla*. γ-sitosterol reduces hyperglycemia in STZ- induced diabetic rats due to increased insulin secretion and inhibition of glucogenesis. It can be used in the treatment of *Diabetes mellitus* [12]. Docking studies of the ligand γ-sitosterol with four different target proteins showed it as a good molecule which docks well with various targets related to *Diabetes mellitus*, thus γ-sitosterol can be considered for developing into a potent antidiabetic drug [13]. It has been also reported that γ-sitosterol may af-

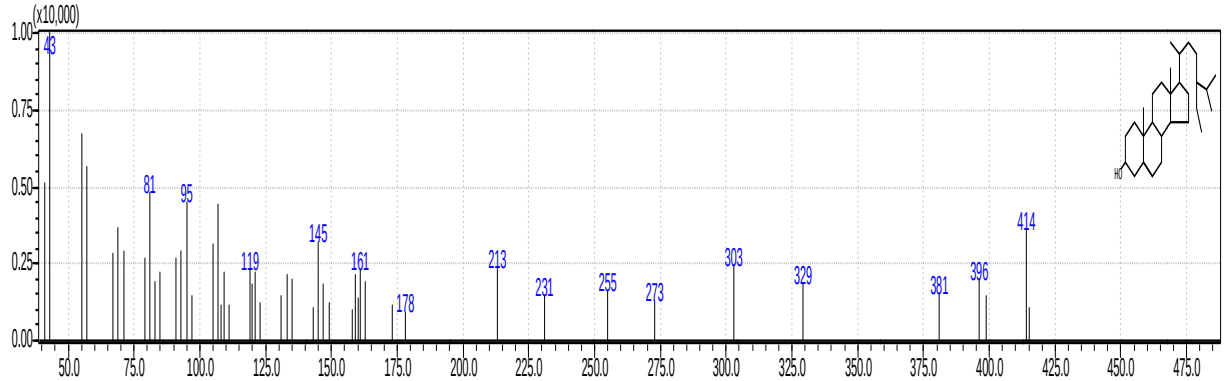

Fig. (2). Mass spectra information of unknown compound isolated from the leaves of *Girardinia heterophylla*.

fect the amount and activity of components of the extrinsic apoptic pathway in human lung and breast adenocarcinoma cells [14].

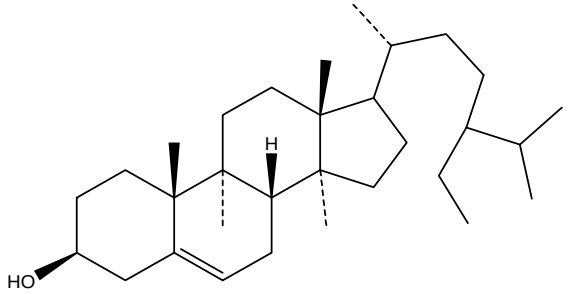

Fig. (3). Chemical structure of γ-sitosterol.

Though the presence of γ-sitosterol has already been reported from soya [10], *Abelmoschus manihot* [15] and *Polygonum bistorta* [16], yet its production for industrial purposes in developing countries is subjected to several limitations like utilization of these crops as cash food crops, limited agricultural land, etc. while abundant herbage of *Girardinia heterophylla* containing γ-sitosterol, which otherwise remain unutilized, can be used for commercial production. Therefore, in depth investigation of *Girardinia* species is required to assess its potential to be used in medical sciences specially pharmacology. Compounds identified in the present study may help in validating the various medicinal applications of *Girardinia heterophylla* found in the areas of Mussoorie hills of Uttarakhand (India). Thus, this study elucidated the medical importance of this plant and many other properties like antidiabetic, antihyperglycemic activity and it shows antihyperlipidemic activity which was evidenced by a significant decrease in serum total cholesterol, triglycerides and very low density in lipoprotein-cholesterol levels coupled with the elevation of high density lipoprotein-cholesterol levels in treated rats [12].

Given the suite of identified biochemical compounds having potential of being utilized for commercial purposes, the plant can also be integrated with region specific developmental activities. Indian Himalayas showcases a variety of such plants, many of which are still beyond the ambit of contemporary research. At present phytochemical investigation, provides an avenue for taking up future research and development activities for strengthening the existing pharmacological sciences besides, refining and utilizing traditional knowledge for the welfare of the society. Findings of the present study may be utilized to develop resource based local livelihood practices by screening of the economically viable and commercially important chemical compounds from such plants predominantly inhabiting hilly areas.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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