



Phytochemicals Isolation from Leaves of *Girardinia Heterophylla*

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ABSTRACT

A chemical study was done on the leaves of *Girardinia heterophylla* commonly called as Dans Kandali in Uttarakhand. The separation of chemical compounds was carried out by different chromatographic techniques and their structures were elucidated by spectroscopic methods. The three compounds, β -sitosterol, (24R)-Ergost-5-en-3- β -ol)/Campesterol and chlorogenic acid isolated from the leaves of *Girardinia heterophylla*.

KEY WORDS: *Girardinia heterophylla*, β -sitosterol, (24R)-Ergost-5-en-3- β -ol, chlorogenic acid.

INTRODUCTION:

Girardinia heterophylla belongs to the family *Urticaceae* is commonly known as 'Dans Kandali' in Uttarakhand. It is dioecious herb, up to two meter high with grooved stems abundantly armed with stinging hair and is found in the Himalayas from Kashmir to Kumaun hills of Uttarakhand at altitude ranging from 2,100m to 3,200m [1]. It grows naturally around human habitats. We report here on the isolation and characterization of three compounds namely β -sitosterol, [(24R)-Ergost-5-en-3- β -ol)]/Campesterol and chlorogenic acid.

MATERIAL AND METHOD:

The leaves (800g) were chopped, powdered after air drying and were sequentially extracted with the solvents of increasing polarity of [petroleum ether (60-80°C), chloroform and methanol]. Removal of the solvents under vacuum yielded three respective extracts (given in Table-1). The yield of chloroform extract (0.06%) was poor and not considered for chemical examination only petroleum ether extract (3.35%) and methanol extract (4.45%) were, therefore, examined (Figure-1). The methanol extract was suspended in water in the ratio of 1:10 and sequentially fractionated with dichloromethane and ethyl acetate. The yield of dichloromethane extract (0.05%) was poor and not considered for chemical examination, only ethyl acetate extract (0.49%) was therefore, examined.

Table-1: Percentage Yield of Different Leaf Extracts of *Girardinia heterophylla*

Solvent	Yield of extract (%)
Petroleum ether	3.35
Chloroform	0.06
Methanol	4.54

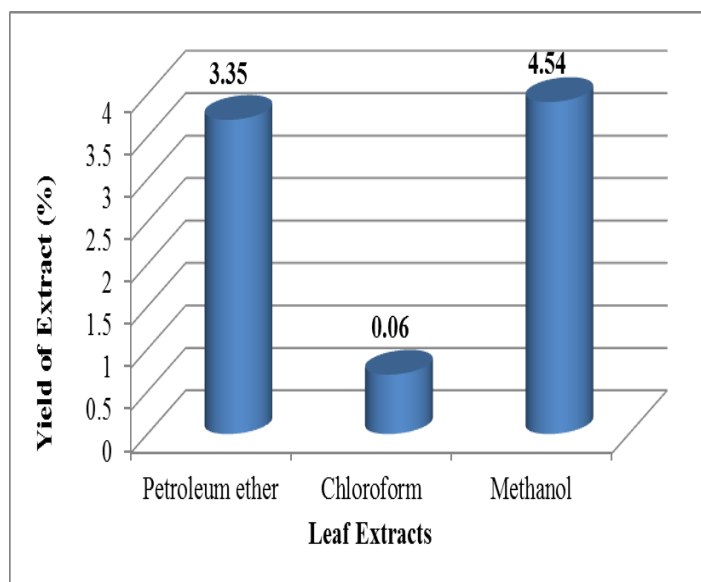


Figure-1: Percentage yield of leaf extracts in different solvents

RESULTS AND DISCUSSION:

(A) CHEMICAL EXAMINATION OF PETROLEUM ETHER EXTRACT:

The petroleum ether extract was column chromatographed over silica gel and elution of the column with varying amount of ethyl acetate in petroleum ether afforded two compounds, as described in the following subsection:

(I) COMPOUND GHLPA (B- SITOSTEROL):

The fractions were eluted with petroleum ether: ethyl acetate (98:2) on concentrating obtained as white silky needles (50mg), m. p. 136-137°C, $[\alpha]_D^{25} 100^\circ\text{C}$ (0.05 in CHCl_3). The yield of β -sitosterol was 0.007%. It gave Liebermann Burchard test for terpenoids and steroids

having molecular formula $C_{29}H_{50}O$. (M^+ , m/z 414). The GC-MS spectrum (Figure-4A) with that provided in the NIST standard chart library.

[Figure-1(A) and (B)]. 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 activity [2]. It reduces the symptoms of Benign Prostatic Hyperplasia (BPH) and also found as an anti-inflammatory agent [3-4]

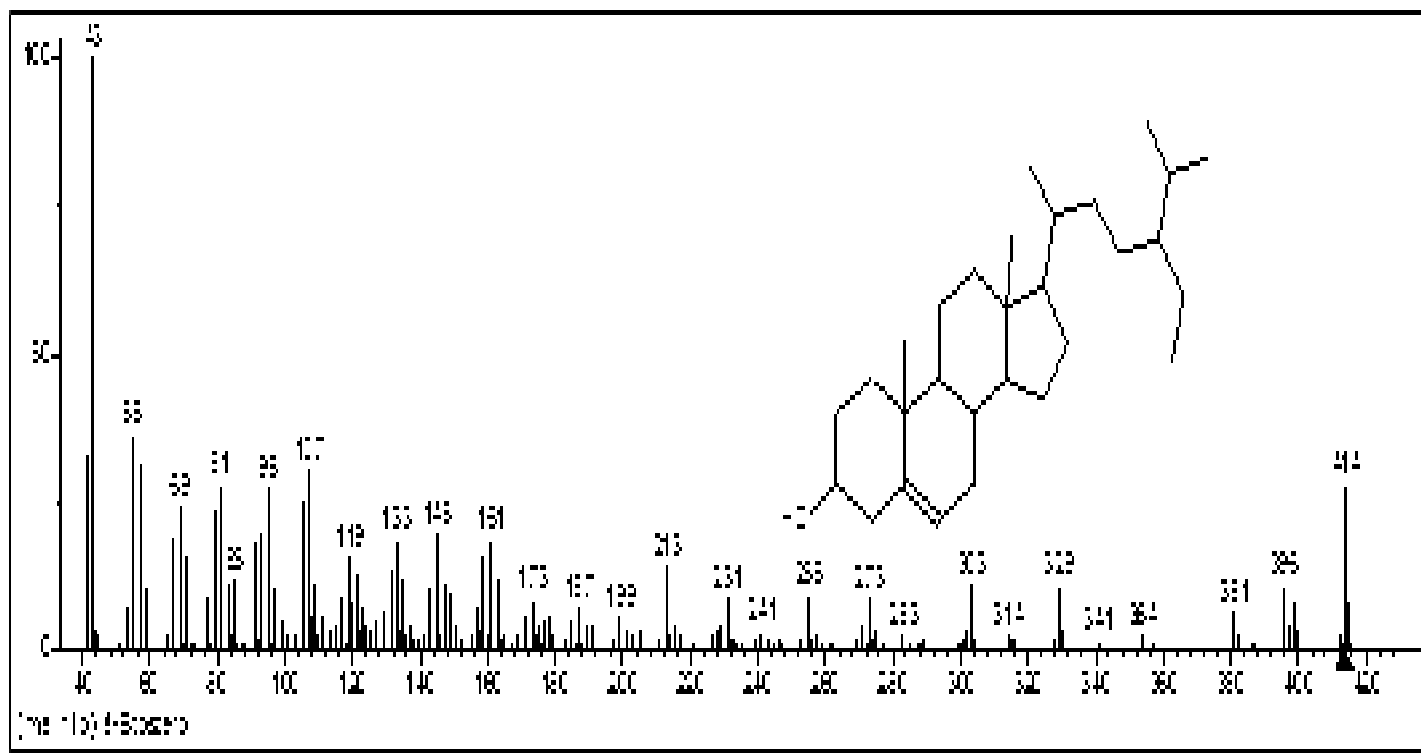


Figure 2 (A): Mass Spectra of β -Sitosterol

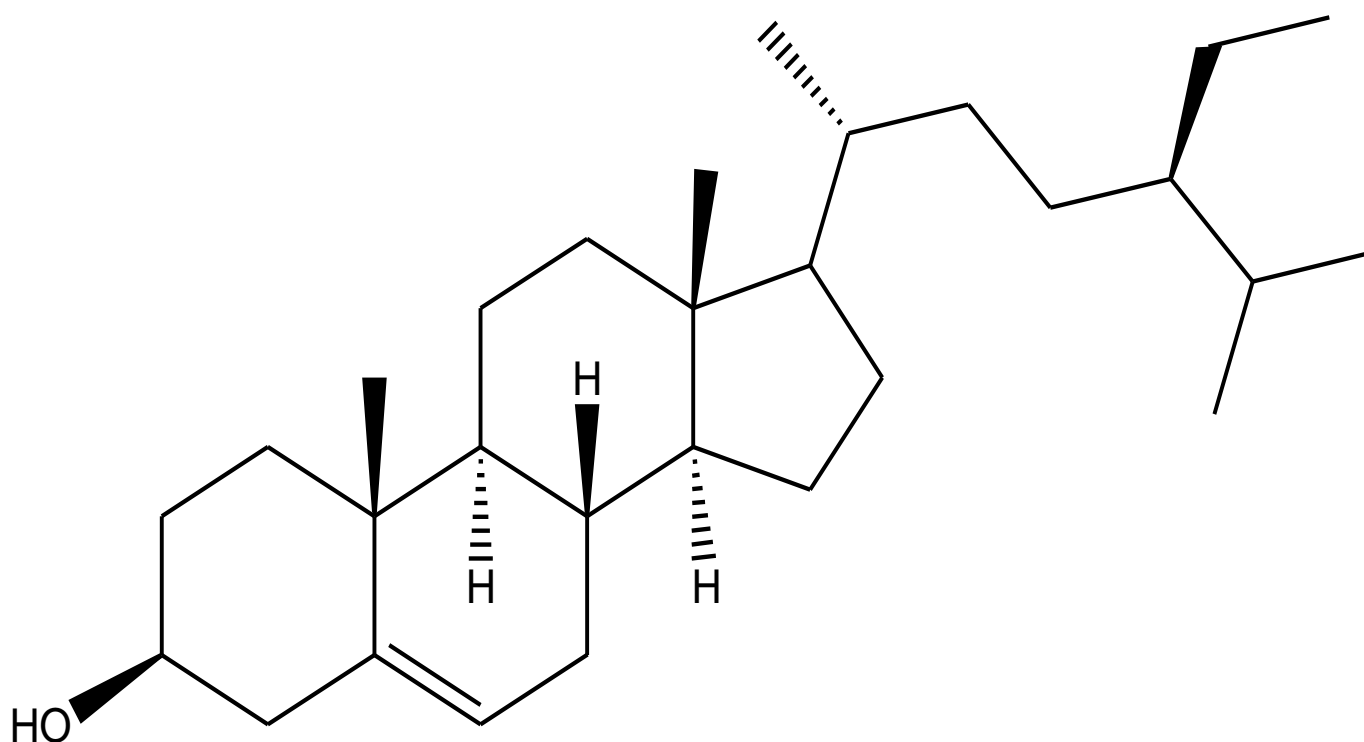


Figure 2 (B): Structure of GHLPA (β -Sitosterol)

(II) COMPOUND GHLPB [(24R)-Ergost-5-en-3-β-ol] / CAMPESTEROL:

The fractions were eluted with petroleum ether: ethyl acetate (95:5) on concentrating yielded as white crystalline solid (15mg), m.p. 156-160°C with 0.002 % yield. It gave Liebermann Burchard test for terpenoids and steroids. It was identified as (24R)-Ergost-5-en-3-β-ol given in Figure [3(A), 3(B) and 3(C)] and by direct comparison with an authentic sample (m. m. p., Co-TLC and

Superimposable IR) purchased from Sigma Aldrich. This is the first report of isolation of (24R)-Ergost-5-en-3-β-ol commonly called as campesterol in the leaves of *Girardinia heterophylla*.

It reduces the absorption of cholesterol in the human intestine [5]. It is growth hormone of animals and plants which has antiulcer, antiphlogistic and antipyretic function [6]. It has antiinflammatory property [7].

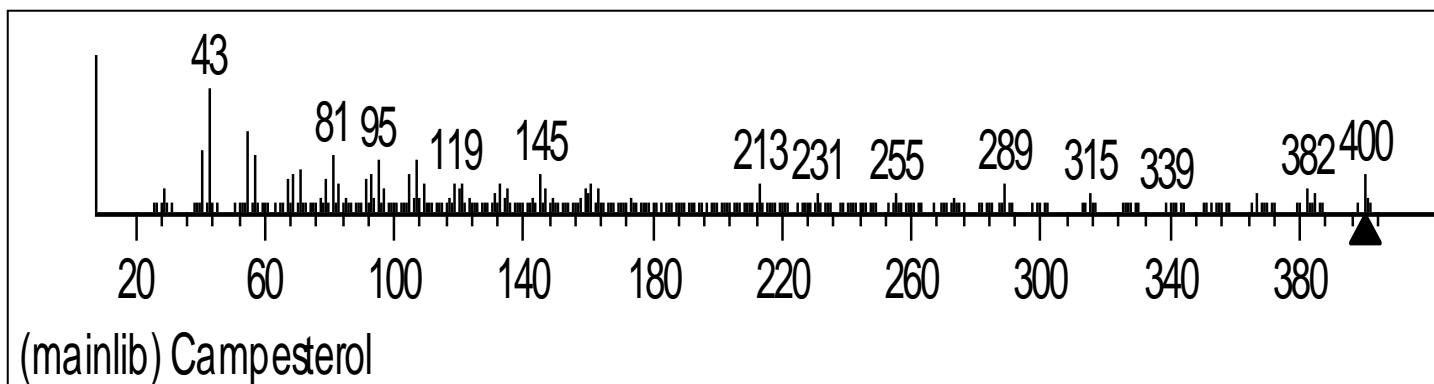


Figure-3(A): Mass Spectra of (24R)-Ergost-5-en-3-β-ol (Campesterol)

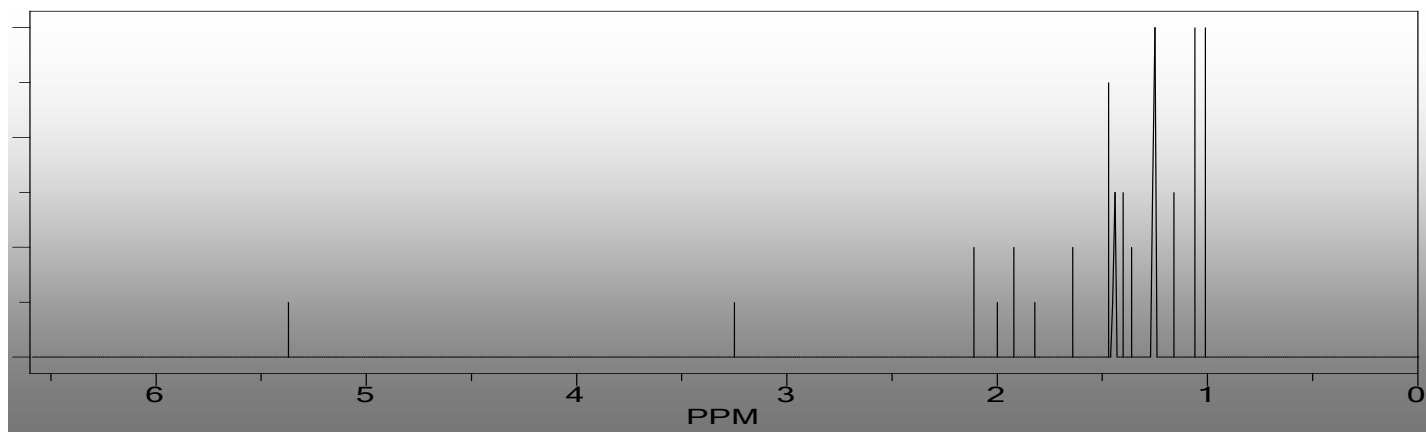


Figure-3(B): ¹H NMR of (24R)-Ergost-5-en-3-β-ol (Campesterol)

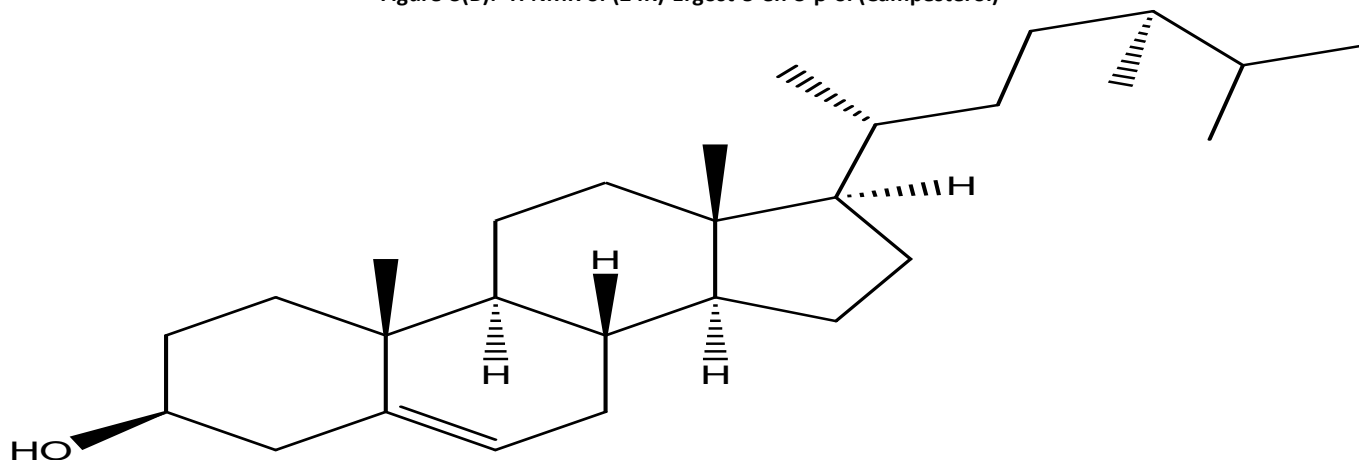


Figure-3(C): Structure of Compound GHLPC (24R)-Ergost-5-en-3-β-ol

(B) CHEMICAL EXAMINATION OF THE ETHYL ACETATE EXTRACT:

The ethyl acetate extract was column chromatographed over silica gel and elution of the column with varying amount of methanol in chloroform afforded one compound.

(I) COMPOUND OBTAINED (CHLOROGENIC ACID):

The fractions were eluted with CHCl₃: MeOH (90:10), on concentrating afforded as white amorphous powder (12 mg), m.p. 209-210°C with a yield of 0.002%. It gave ferric

chloride test for phenols/phenolic acids. It was identified as chlorogenic acid [Figure-4(A), 4(B), 4(C) and 4(D)] and by direct comparison with an authentic sample (m.m.p., Co-TLC and superimposable IR) purchased from Sigma Aldrich. This is the first report of isolation of chlorogenic acid in the leaves of *Girardinia heterophylla* from methanolic extract.

It shows cytoprotective effect [8,9]. It possesses various biological and pharmacological activity including antiviral [10], antibacterial [11], antiinflammatory [12] and antifungal [13].

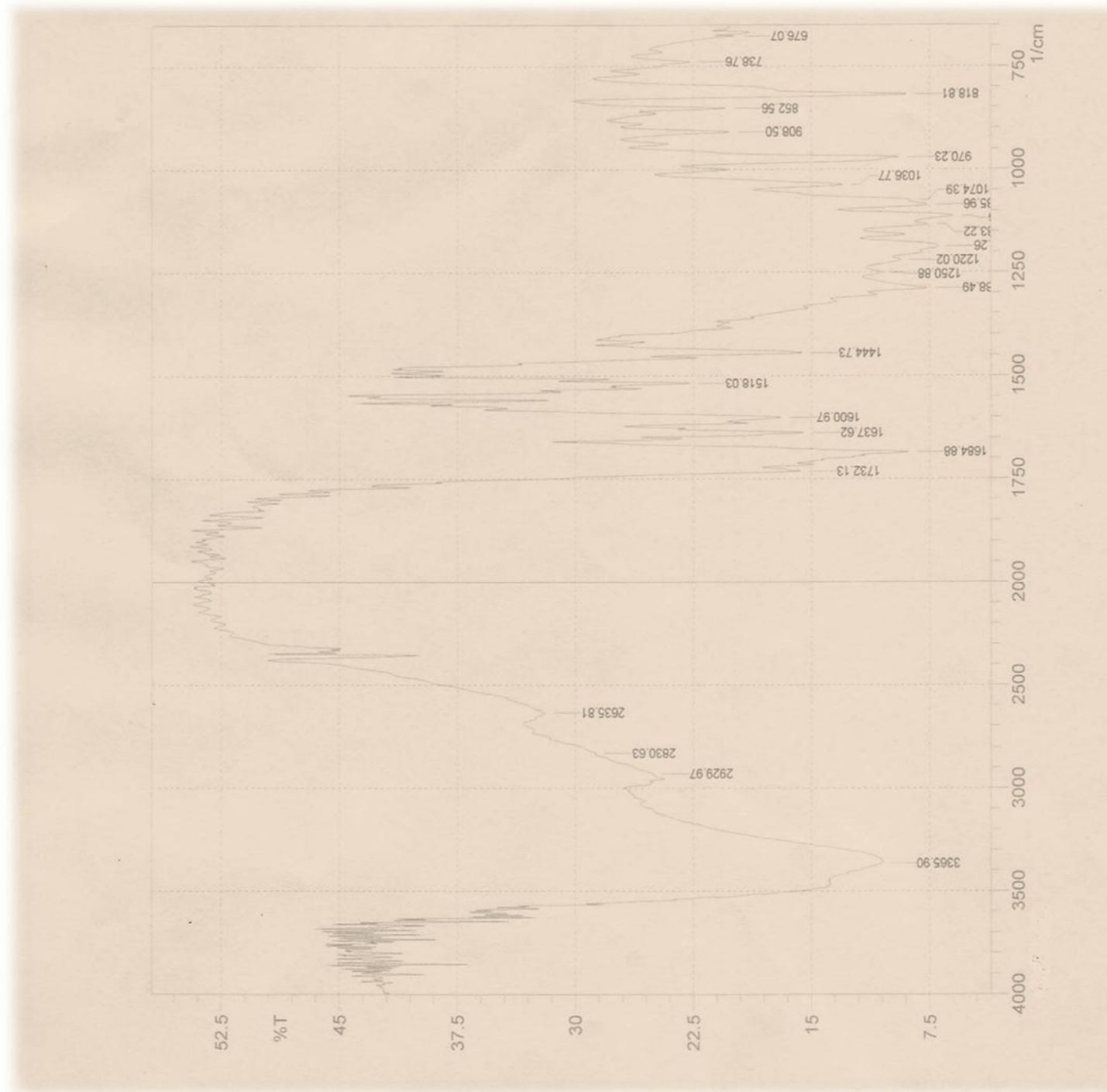


Figure 4(A): FT-IR spectra of chlorogenic acid

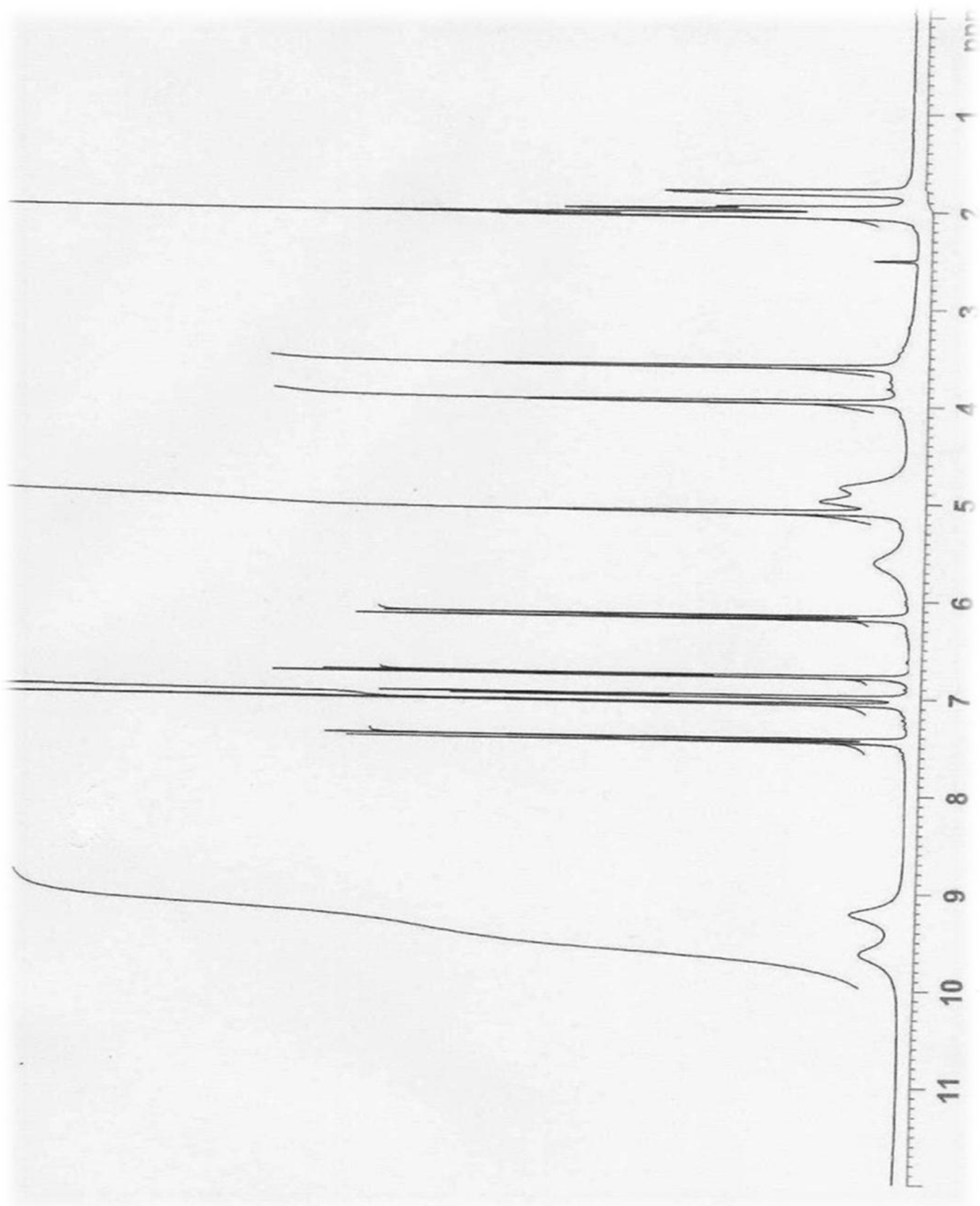


Figure 4(B): $^1\text{H-NMR}$ Spectra of Chlorogenic Acid

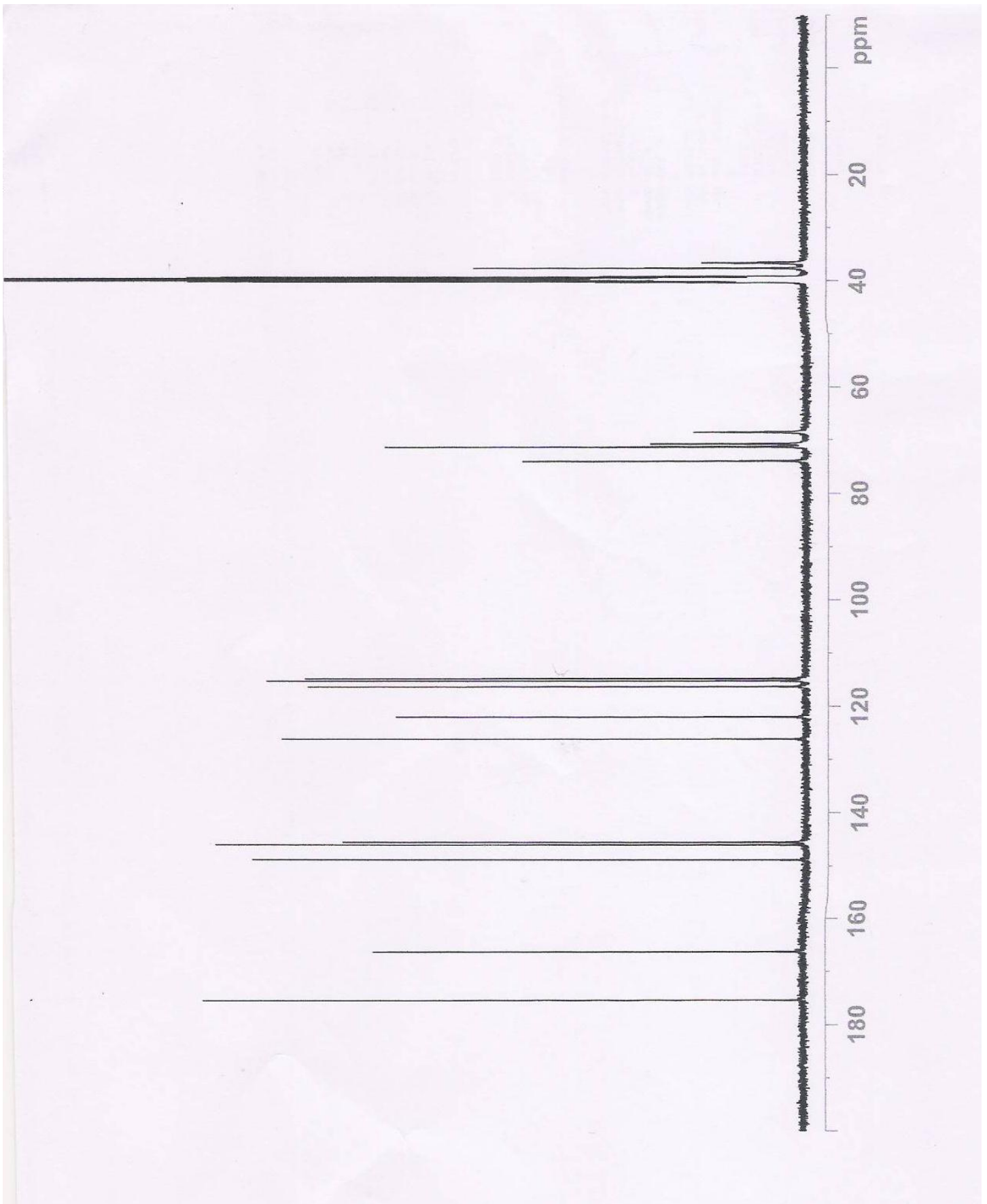


Figure 4 (C): ^{13}C -NMR of Chlorogenic Acid

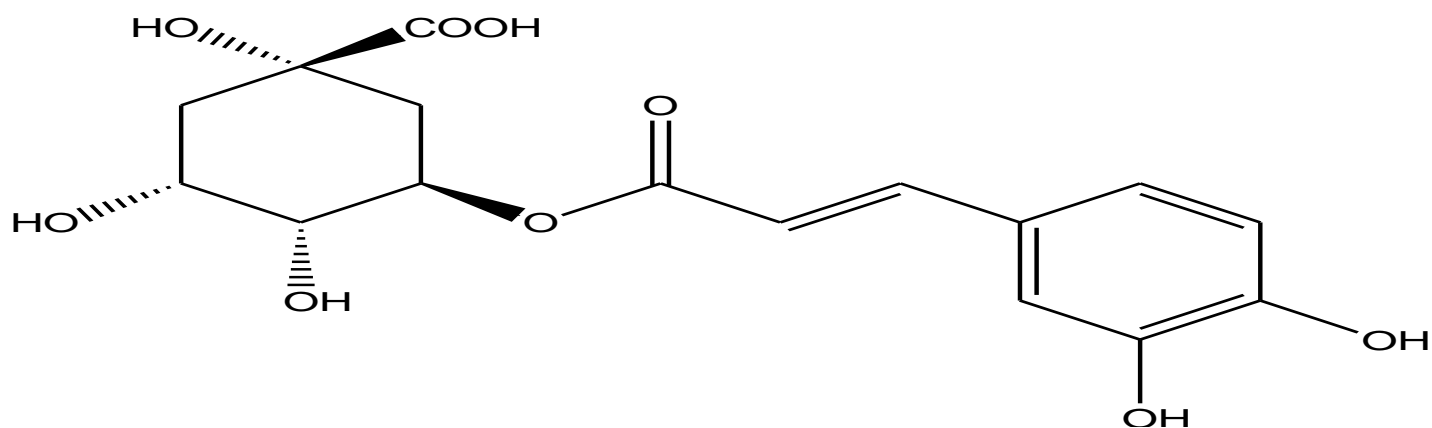


Figure 4 (D): Structure of Compound GHLED (Chlorogenic Acid)

CONCLUSION:

Chemical examination of the leaves of *Girardinia heterophylla* was conducted. Results of leaf extract with petroleum ether revealed presence of β -sitosterol, (24R)-Ergost-5-en-3- β -ol (commonly called as campesterol) while leaf extract with ethyl acetate showed presence of chlorogenic acid. All the compounds (β -sitosterol, campesterol, chlorogenic acid, were isolated from the leaves of *Girardinia heterophylla* have been reported to show many biological activities. Thus, this study elucidate the medical importance of this plant and puts light on isolated compounds and their biological and pharmacological properties

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