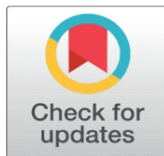
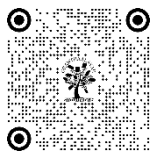


PHYTOCHEMICAL AND PHARMACOLOGICAL STUDY OF EHRETIA MICROPHYLLA (BORAGINACEAE FAMILY) BY USING GC-MS

Shweta Sain¹, Dr. Manjul Mishra²

¹ Research Scholar, Chemistry Department, Apex University, Jaipur, Rajasthan, India

² Professor, Chemistry Department, Apex University, Jaipur, Rajasthan, India



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ABSTRACT

The present examination is about the primary investigation and the compound constituent of Boraginaceae species, Ehretia microphylla, by the utilization of GC-MS. Qualitative assessment of Ehretia microphylla by the utilization of GCMS affirmed the presence of eleven selective phytochemical compounds. The leaf phase of the plant was once analysed by way of GC SHIMADZU, model no. GCMS-QP2010 and software program GCMS solution version 4.45 SP1. The GC SHIMADZU utilized in the examination utilized a section loaded with elite 5MS and the factors have been separated by the usage of helium (1mL/min) as the service gas. The compounds were identified by evaluating their maintenance duration and fragmentation patterns with the records saved in the NIST library. The stated main constituent is gamma-sitosterol, 13-Docosenamide, and Benzoic acid, 4-ethoxy-, ethyl ester.

Keywords: Ehretia-Microphylla, Gamma-Sitosterol, GC-MS, Phytochemicals, Secondary-Metabolites

1. INTRODUCTION

Natural herbs are the backbone of corresponding and elective medication that acquired more attention globally and is continuously being coordinated into standard medical services frameworks. Its minimal expense, wide acknowledgment because of its worth as a characteristic item with low poisonousness, viability in a few serious illnesses, and adaptability in its accessibility, planning, and use are significant elements in the development of customer relations and usage¹.

Today Natural herbs are a significant piece of essential medical services in numerous country networks in Africa and Asian nations. It is additionally a basic piece of the way of life of numerous social orders all over the planet. Numerous spices and herbal medicines have a long history of conventional people use and medical advantages. Scientific investigations have shown that herbs contain complex synthetic mixtures answerable for pharmacological impacts compared to medical advantages as well as toxicity².

The pharmacological activity of natural products is liable for their advantages and a large portion of their poisonousness. These bioactivities are basically because of the presence of specific complex substance elements: secondary metabolites³ where some are answerable for the extreme activity, others go about as supports that regulate and change the pharmacological impacts of the dynamic parts, making them less harmful or more dynamic.

With the remarkable improvement of the worldwide utilization of conventional medication, the security and viability and quality control of natural drugs and customary treatments have arisen as a main pressing issue for both well-being specialists and people in general.

There is as yet a critical absence of exploration Material around here. Since there are no pharmacopeial insights for a few plant extricates, it is unimaginable to expect to disconnect or rank the dynamic element of plants with the ideal impact. The assessment of plant- inferred dynamic parts is sharp for the improvement of new drugs with an efficient reinforcing and healing job against different sicknesses. Screening of dynamic parts from plants has paid all due respect to the advancement of newly restorative medications that have an effective stronghold and cured different diseases⁴.

Ehertia microphylla is a medicinal shrub. The arrangement of leaves is in bunches, cutting edge obovate. Blossoms are undivided or expanded. Fruit is globose, maturing natural products are caramel orange in color⁵. A decoction of E. Microphylla leaves is utilized to treat stomach aches, an accurate remedy. Ehretia microphylla leaves are being utilized to cure cold and abdominal problems⁶, escalate fertility⁷ and for the treatment of looseness of the bowels and diarrhea. Leaves act as inflammatory⁸. Part of restorative plants and their refined constituents have shown favorable and helpful possibilities.

Today, scrounging for medicinal herbs which are wealthy in cancer prevention agents or other pharmacological properties arising because of therapeutic activities to cure various persistent diseases⁹. From the roots, Ehretianone a quinoid xanthan was separated from the methanol concentrate which has the counter snake toxin action¹⁰. By means of this, the current review intended to detect the phytochemicals present in Ehretia microphylla by method for GCMS examination.

Materials and Methods Material Used

Pestle mortar, Vortex mixer, Blender, stirrer, Evaporator, Centrifuge, syringe, filter
Solvents: Hexane, Chloroform, Ethyl acetate, Methanol.

Instrument Used

GC SHIMADZU, model number GCMS-QP2010

Instrument conditions

- Injection volume -1 µl
- Mode -split: 30:1
- Infusion temperature: 250°C
- Identifier temperature -230°C
- Flow 1.5 ml per minute
- Oven program (Total Run time; 26 Minutes)

Rate/Minute	Final temperature	Hold time
-	40°C	0.0
10°C	300°C	0.0

Protocols

A leaf sample of E. microphylla was shaded for 5 days and blended to get a fine powder with 100 mg of leaf powder of E. microphylla. A solvent mixture [Chloroform, Hexane, Ethyl acetate, Methanol] in equal ratio (1:1:1:1) is added to it. It is stirred for 6 hours in a magnetic stirrer. After six hours, the solvent is collected and evaporated under a nitrogen evaporator. The contents are reconstituted in methanol. Then place in Vortex mixer for 1 minute and transfer to the centrifuge tube. The clear supernatant fluid is gathered and separated through a 0.22 µm needle channel. The filtrate is transferred into a 2 ml GC vial and injected into GC.

GC-MS analysis Components of GCMS:

The following are the major components present in the GC.

- A. Injector – AOC 5000 Auto injector
 - a. The automatic liquid sampler system can include One injector tower and 2 trays.
 - b. Injector tower - houses a 10-µl syringe for Liquid samples and 2500 for headspace gas injection.

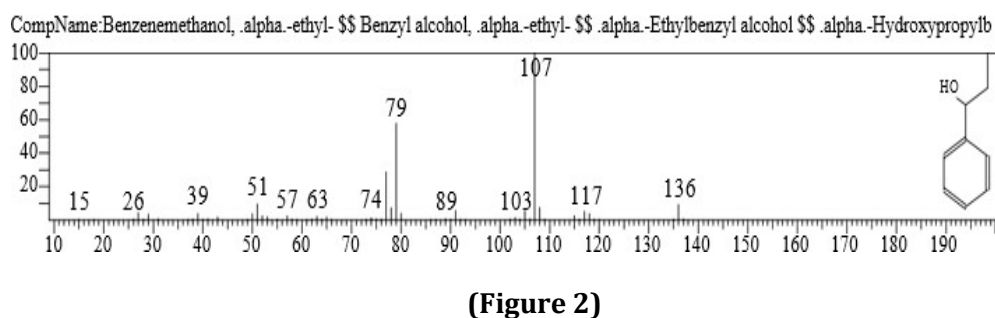
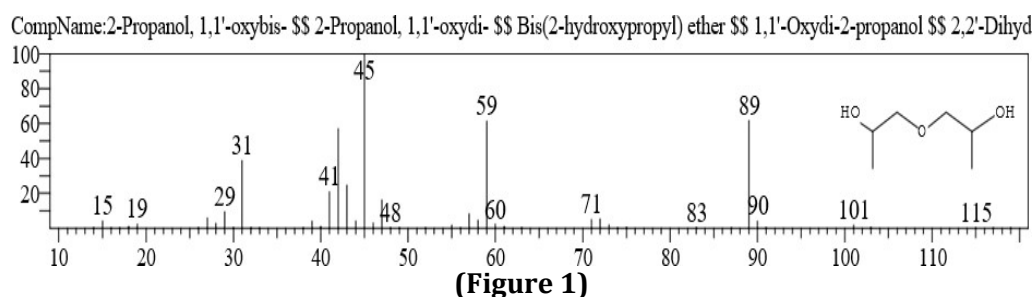
- c. Sample tray - holds a maximum of 98 vials for liquid & 32 headspace sample vials. d. Wash vial trays hold washing solvent waste which holds 4 vials.
- B. Column Oven
- Programmable column oven with maximum temperature of 380°C.
 - Ramp rate 0 to 100°C
 - The oven holds one inlet and detector
- C. Detector - Single Quad MS
- D. Software acquisition system -Real-time Analysis The GC-[Make SHIMADZU (GCMS-QP2010) was used in this examination. It utilized a melded silica segment loaded with DB - 5MS and the parts were isolated involving Helium gas as a transporter at a consistent progression at 1.5 mL/min. The 1 µL test separate was infused into the instrument The infusion temperature was set on at 250°C. MS recognition was finished in 26 min. The NIST 14/NIST 17 library was utilized for detection and theChecking range is 40m/z to 500m/z.

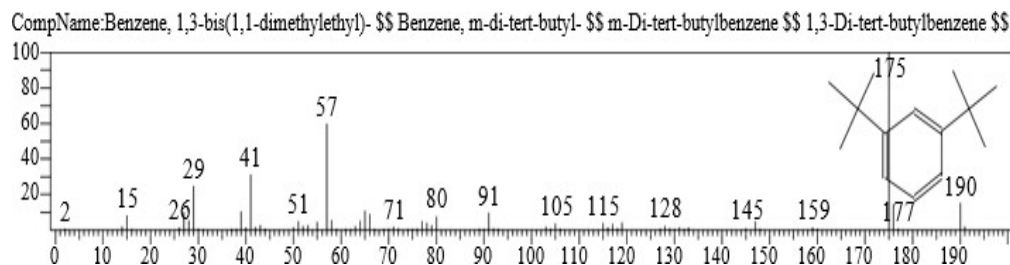
RESULTS AND DISCUSSION

The outcome of the GCMS examination promptedacknowledgmentof various compounds through methanolic concentrate of *E. microphylla*. These compounds were recognized through mass range connected with GC. The result shows the presence of the following compounds as below. (Table 1)

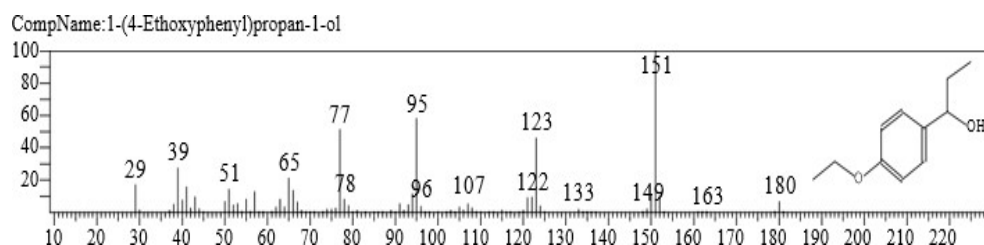
- 2-Propanol,1,1'-oxybis-(0.26%)
- Benzenemethanol, alpha,-ethyl (0.30%),
- Benzene,1,3-bis(1,1-dimethylethyl) (0.45%)
- 1-(4-Ethoxyphenyl)propan-1-ol (1.53%),
- Benzoicacid,4-ethoxy-,ethyl ester(5.02),
- Tetradecanoic acid(5.58), vii)Neophytadiene (5.54%),
- Phytol (3.39%),
- Hexadecanoicacid,2-hydroxy-1-(hydroxymethyl) ethyl ester (3.47),
- gamma-Sitosterol (48.95%),
- 13 -Docosenamide(25.51%)

The range value of GC-MS well established the appearance of eleven parts with the maintenance period 6.75, 8.865, 10.303, 13.387, 14.013, 16.698, 17.570, 20.291, 23.763, 25.035, 25.811min.shown in below figures(1-11)

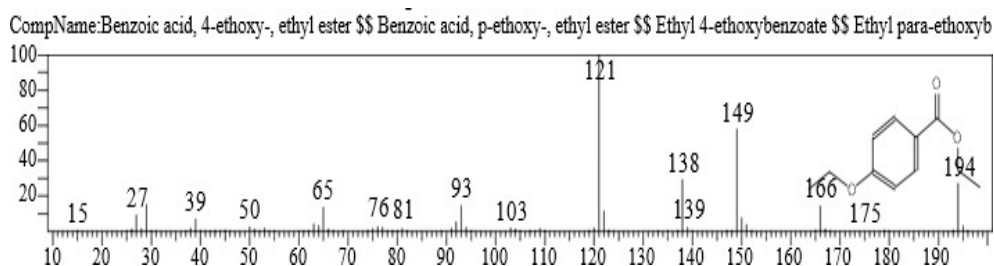




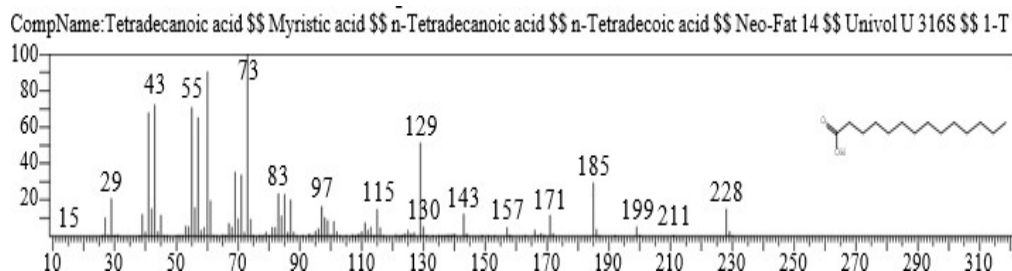
(Figure 3)



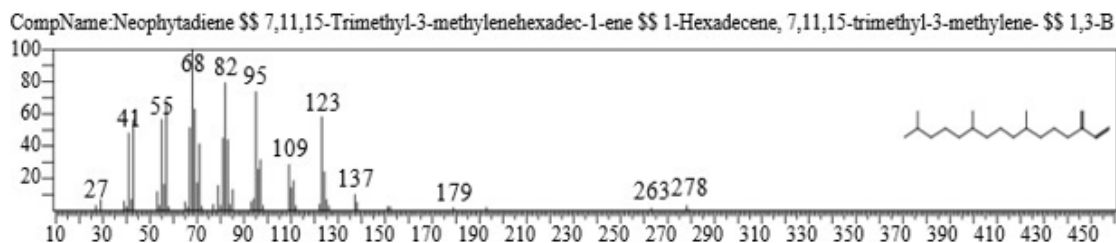
(Figure 4)



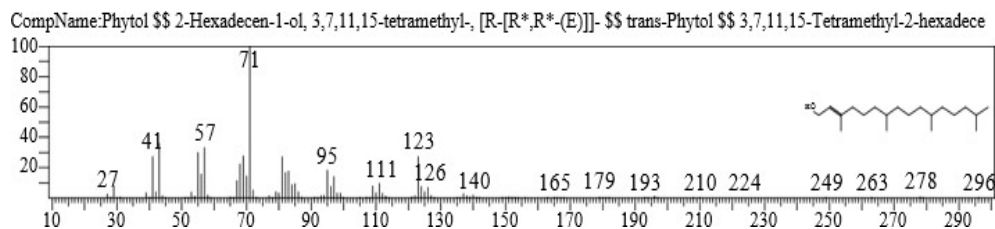
(Figure 5)



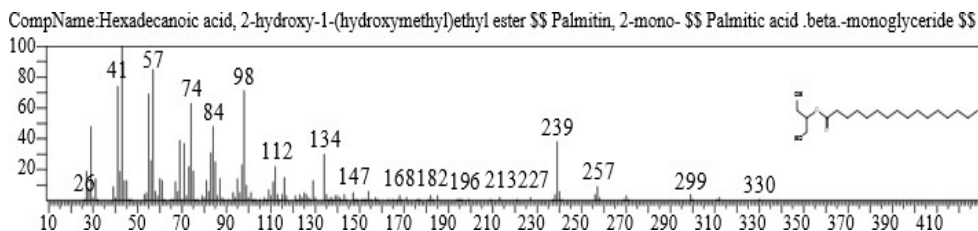
(Figure 6)



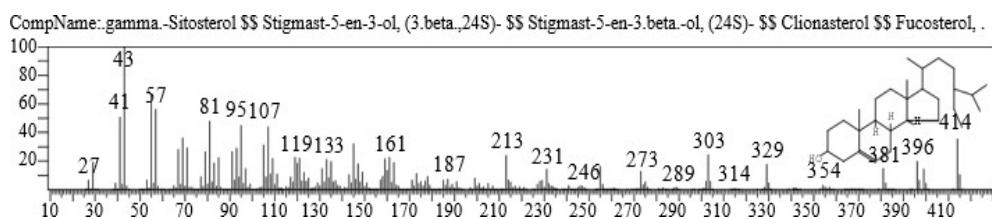
(Figure 7)



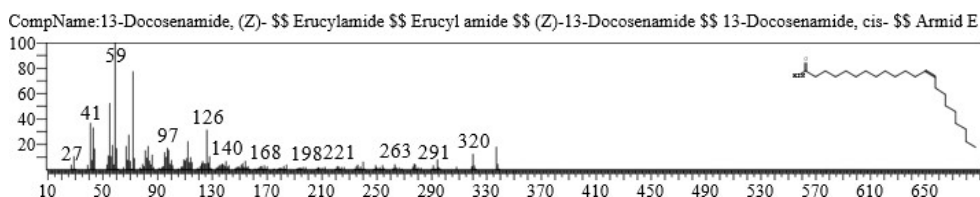
(Figure 8)



(Figure 9)



(Figure 10)



(Figure 11)

Fig (1-11): The important fragment patterns of compounds identified in *E. microphylla***Table1- Components identified in leaves of *E.microphylla***

S.N o.	Compound Name	Molecular Formula	Mol. Weight	Retention Time	Area%
1	2-Propanol,1,1'-oxybis-	C ₆ H ₁₄ O ₃	134	6.751	0.26
2	Benzene methanol, alpha. -ethyl-	C ₉ H ₁₂ O	136	8.865	0.30
3	Benzene,1,3-bis (1,1-dimethylethyl)	C ₁₄ H ₂₂	190	10.303	0.45
4	1-(4-Ethoxyphenyl) propan-1-ol	C ₁₁ H ₁₆ O ₂	180	13.387	1.53
5	Benzoic acid,4-ethoxy-,ethylester	C ₁₁ H ₁₄ O ₃	194	14.013	5.02
6	Tetra decanoic acid	C ₁₄ H ₂₈ O ₂	228	16.698	5.58
7	Neophytadiene	C ₂₀ H ₃₈	278	17.570	5.54
8	Phytol	C ₂₀ H ₄₀ O	296	20.291	3.39
9	Hexa decanoic acid,2-hydroxy-1-(hydroxyl methyl) ethyl ester	C ₁₉ H ₃₈ O ₄	330	23.763	3.47
10	gamma. -Sitosterol	C ₂₉ H ₅₀ O	414	25.035	48.95

11	13-Docosenamide,(Z)-	C22H43NO	337	25.811	25.51
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Table 2- Pharmacological Properties of Phyto Components identified in leaves of E.microphylla

S. No	Compound Name	Properties
1	2-Propanol,1,1'-oxybis-	No activity reported
2	Benzenemethanol, alpha. - ethyl-	No activity reported
3	Benzene,1,3-bis (1,1-dimethylethyl)	No activity reported
4	1-(4-Ethoxyphenyl) propane-1-ol	No activity reported
5	Benzoic acid,4-ethoxy-,ethyl ester	antimicrobial and preservative properties; antioxidant and anti-inflammatory properties
6	Tetradecanoic acid	larvicidal and repellent action against Aedes aegypti and Culex quinquefasciatus
7	Neophytadiene	anti-inflammatory agent, an antimicrobial agent, a plant metabolite, and an algal metabolite
8	Phytol	antimicrobial, anticancer, anti-inflammatory and diuretic
9	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	anti-inflammatory activity ; anticancer cytotoxic potential ; potential antioxidant and anticancer activity
10	gamma. -Sitosterol	thyroid-inhibitory and insulin-stimulatory nature; antidiabetic and antiperoxidative properties

The current review described the substance profile of E. microphylla utilizing GCMS. The chromatogram of GC describes different compounds through the maintenance period. The compound is distinguished from the NIST information library. The properties of different phytocomponents distinguished in leaves of E.microphylla are shown in Table 2:

- i) Phytol acts as an antimicrobial, anticancer, and diuretic.
- ii) Benzene methanol, alpha, - ethyl is prescribed to be a liquor compound in nature.
- iii) Hexadecanoic acid is anti-inflammatory activity¹¹; anticancer cytotoxic potential¹²; potential antioxidant and anticancer activity¹³.
- iii) Gamma-Sitosterol is insulin-stimulatory in nature and thyroid inhibitory; antidiabetic and antiperoxidative properties¹⁴; anticatabolic properties¹⁵
- iv) Tetra decanoic acid shows larvicidal and repellent movement against Aedes aegypti and Culex quinquefasciatus¹⁶.
- v) Benzoic acid, 4-ethoxy-,ethyl ester is antimicrobial and additive properties¹⁷; cell reinforcement and mitigating properties¹⁸.
- vi) Neophytadiene shows an anti-inflammatory agent, an antimicrobial specialist, a plant metabolite, and an algal metabolite.

CONCLUSIONS

Gas Chromatography-Mass Spectrometry (GCMS) is a valuable instrument for authentic finding of biologically potent compounds. The experiment describes that E. microphylla has different helpful applications. Plentiful phytoconstituent experiments have been done by using GC-MS worldwide. The present review described the substance profile of E. microphylla by utilizing GC-MS. The Gas Chromatographic pattern explains the centralization of different mixtures that are separated with the end goal of a maintenance period. The Mass analyser investigates mixtures that are separated at various intervals to perceive the behavior and construction of mixtures. The mass figure prints of the compound could be perceived from the information library. The event of different biologically potent mixtures affirms the use of E. microphyll for various illnesses. A number of mixtures have recently been accounted for from various other natural herbs. Subsequently, the discovery of various compounds from E. microphyll could have organic meaning.

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CONFLICTS OF INTEREST

None.

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None.

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