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GC-MS Analysis of Phytochemical Compounds present in the bark extracts of *Ehretia laevis* Roxb.

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ABSTRACT: *Ehretia laevis* is a fast-growing small deciduous tree generally found in Asia and Australian tropics. Medicinal value of bark of plant is yet unexplored, hence this study forms a basis for the active components present in it and further isolation of the compound. The aim of GC-MS study is to screen the phytochemicals present in the bark of plant *Ehretia laevis* and further analysis of the components present in it by GC-MS analysis. The bark was sequentially extracted based on the polarity viz., petroleum ether, chloroform, and methanol. The petroleum ether, chloroform, and methanol extract of plant *Ehretia laevis* showed the presence of all phytoconstituents studied. The GC-MS analysis of the petroleum ether, chloroform, and methanol extract revealed the presence of 13, 17 and 19 compounds. GC-MS analysis of extracts of plant forms a basis for the biological activity and importance of the compounds identified.

INTRODUCTION

India is generally called as the largest production of herbal medicine and is appropriately called as the botanical garden of the world [1]. Plants are having more ability of biosynthesis of variety of organic compounds called as secondary metabolites, which are generally unique and more complex structures. Many secondary metabolites have been found to possess interesting pharmacological and therapeutic values and have applications such as pharmaceuticals, insecticides, dyes, colors, sweeteners, in cosmetics as flavors and fragrances. Plants used for traditional medicine contain a wide range of active chemical constituents that can be used to treat acute, chronic and any infectious diseases [2]. Plant secondary metabolites which are biosynthesized in plant are called as phytochemicals that are naturally occurring and have potential disease inhibiting capabilities [3]. These phytochemicals are excellent sources of many bioactive compounds, like as essential or volatile oils, steroids, triterpenoids, alkaloids and natural antioxidant compounds like as flavonoids, tannins and other phenolic compounds, which have beneficial effects on

human health. Hence, the characterization and screening of these chemically active compounds and antioxidant activity determination from plants have led to the discovery and development of novel drugs from plant to be used against many diseases. Drugs obtained from plants are easily available, easily separated from plant, they are less expensive, safe and efficient and have less side effects [4]. There are many modern analytical methods which are helps in describing the identification, characterization and quantification of active chemical constituents in plant material may be useful for proper standardization of herbal drugs and their formulations. Recently, Mass spectrometry is coupled with many chromatographic separations, like as gas chromatography (GC/MS), liquid chromatography (LC-MS). It is normally used for the direct analysis of the chemically active constituents that present in traditional herbal medicines. Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical tool that combines the properties of separation of gas-liquid chromatography with the detector as special feature of mass spectrometry to carry out identification of different substances within a test sample.

Gas Chromatography is used to separate the volatile, essential and thermally stable substances in a sample whereas GC-MS fragments which are analyte to be identified on the basis of its mass to charge ratio. The again addition of mass spectrometer in GC-MS leads to GC-MS/MS [5-7].

In recent years, hyphenated tool GC-MS studies have been increasingly applied for the analysis of chemically active constituents present in medicinal plants as this technique has proved to be more valuable method for the analysis of many non-polar components and volatile-essential oils, fatty acids, lipids and alkaloids [8]. The use of chemically active constituents derived from herbal plants has been in practice for a very long time [9].

The herbal plants which are related to traditional medicinal system continues to provide primary health care. In all over the world, plant derived traditional medicines are play important role in the maintenance of health of peoples. Some major categories of plant derived products include personal care products, herbal medicines, natural health products, cosmetics and pharmaceuticals [10].

Now a days GC-MS analysis has been applied for the study of medicinal plants as this technique generally applied to be a most valuable method for the analysis of polar, semi-polar and non-polar components and volatile oil, fatty acids, lipids, alkaloids, flavonoids, tannins, proteins, terpenoids and steroids, and only few grams of plant material is required [11-13].

Ehretia laevis plant is fast-growing and small tree which is belonging to family Ehretiaceae. The plant is cultivated mainly in India, Pakistan, Myanmar, Vietnam, China, Bhutan. The plant *Ehretia laevis* is located mainly at hilly forests, in ravine and on hill slopes. The plant is known as Dant-Rang, Vadhvarni, Chamror [14].

The inner bark of *E. laevis* is used as food. Leaves are used in treatment of ulcers and applied to skin diseases and in headache. Fruit are used in urinary passage, lung and spleen diseases, astringent, anthelmintic, diuretic, demulcent, expectorant. Powdered kernel is used in treatment of ringworm with oil. Seeds are anthelmintic. Barks are used in throat infection. Root for venereal diseases. The plant contains chemical constituents like as fatty acids, phenolic acids, flavonoids, cyanogenetic glycosides and benzoquinones [15].

MATERIALS AND METHODS

Plant Collection

The fresh barks of plant *Ehretia laevis* were collected from region of Taluka Yawal, District Jalgaon, India. The plant was identified and authenticated by Dr. D. A. Dhale, Asst. Professor, PG & Research Dept. of Botany SSVPS's, L. K. Dr. P. R. Ghogrey Science College, Dhule, Maharashtra. Barks of plant were dried under sunlight and milled with the aid of grinding machine to make powder.

Preparation of Plant extract

The coarse powder of bark of plant was extracted with increasing polarity of solvents like as Petroleum ether (60-80°C), Chloroform and Methanol by Continuous Soxhlet extraction method. Finally, the extracts were evaporated by vacuum evaporator and dried in tray dryer to obtain thick sticky dried extract [16].

Gas chromatography-mass spectrometry (GC-MS) profiling of Petroleum ether, Chloroform and Methanolic extracts of bark of plant *Ehretia laevis*.

The Petroleum ether, chloroform and methanolic extracts of plant *Ehretia laevis* were analyzed by the Gas chromatography-mass spectrometry (GC-MS) technique and were performed at SAIF Panjab University Chandigarh, India. The chemical composition of the Petroleum ether, chloroform and methanolic extracts was determined using an instrument Thermo Scientific TSQ 8000 gas chromatograph-mass spectrometer which consist of direct capillary interface fused with silica capillary column Trace GOLD 5MS (30 m X 0.25 mm, 0.25 μ m). Samples (Extracts) were injected under the following conditions: Helium was used as carrier gas at constant rate 1 mL/min, pulsed splitless mode. The solvent delay was 2-3 min and the injection size was 1.0 μ L. The run time for Gas Chromatography was 21.76 min.

The mass-spectrophotometric detector was operated in form of electron impact ionization mode with an ionizing energy of 75 eV and scanning from m/z 50-700. The GC temperature program started at 60°C, then elevated to 300°C at a rate of 10°C/min, with a 10 min hold at 300°C. The injector, ion source and detector temperatures were set at 250, 230 and 300°C, respectively [17, 18]. The peaks which are separated in GC-MS were identified by NIST (National Institute of Standards and Technology) mass spectral databases. The components present in plant extracts were identified based on comparison of their relative retention time and mass spectra. The Name of component, Molecular weight and structure of the components of the test material was ascertained.

RESULTS AND DISCUSSION

The phytochemical components present in the petroleum ether, chloroform and methanolic extract of bark of plant *Ehretia laevis* were identified by a hyphenated tool GC-MS analysis (Figure 1, 2, and 3). The active chemical compounds with their retention time (RT), Molecular formula and Molecular weight (MW) in the petroleum ether, chloroform and methanolic extract of bark of *Ehretia laevis* are presented in Table 1, 2 and 3. Thirteen, seventeen and nineteen phytochemical components were identified in petroleum ether, chloroform and methanolic extract of bark of plant *Ehretia laevis*. This type of hyphenated tool GC-MS analysis of extracts is very first step towards understanding the chemical nature of active constituents in this plant. Thus, the plant *Ehretia laevis* studied can be used as a potential source of chemically active components as new useful drugs. The phytochemical characterization of the extracts, the isolation of responsible bioactive chemical compounds and their pharmacological activity are necessary for future studies.

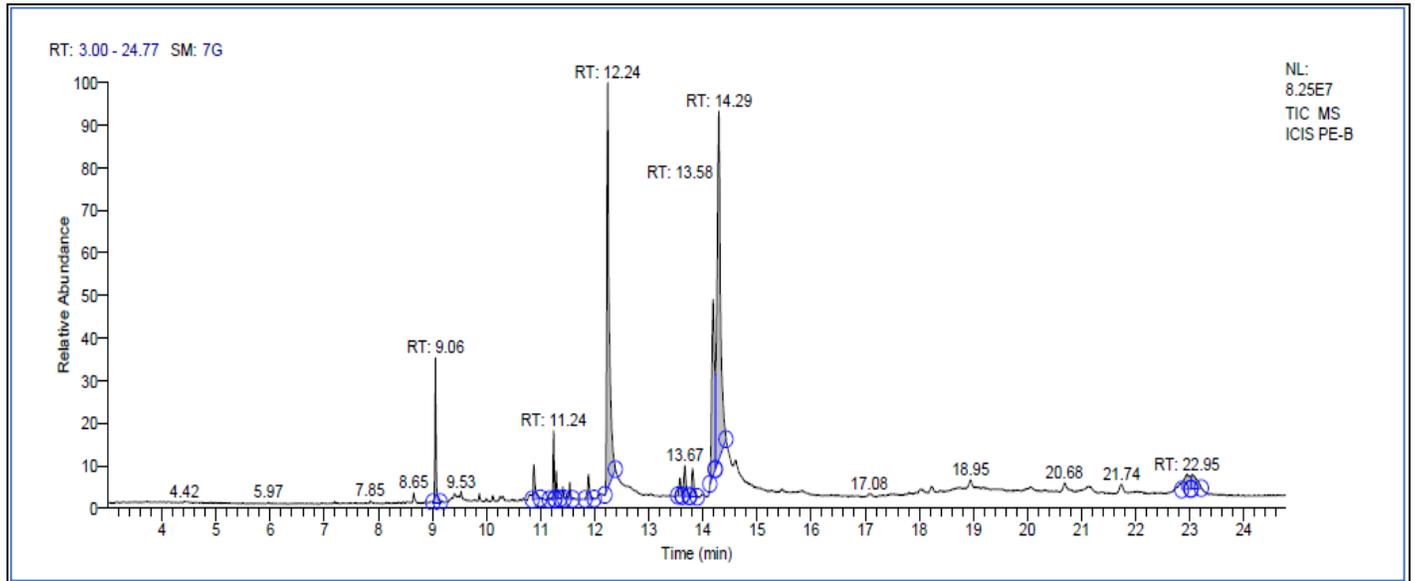


Figure 1: GC-MS chromatogram of Petroleum ether extract of *Ehretia laevis*

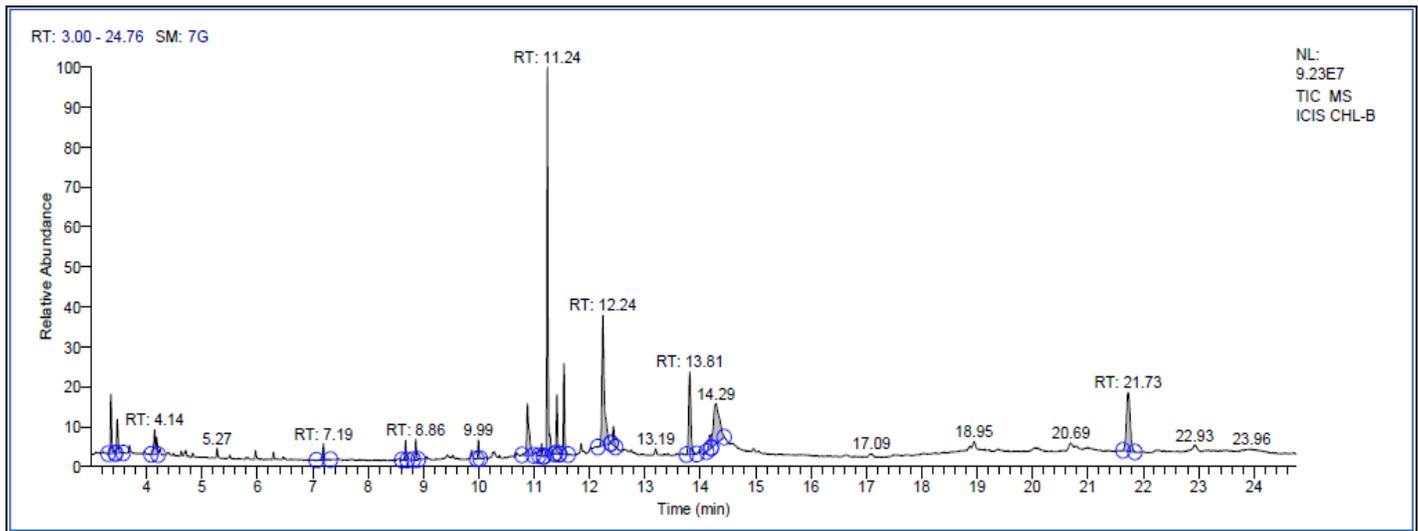


Figure 2: GC-MS chromatogram of Chloroform extract of *Ehretia laevis*

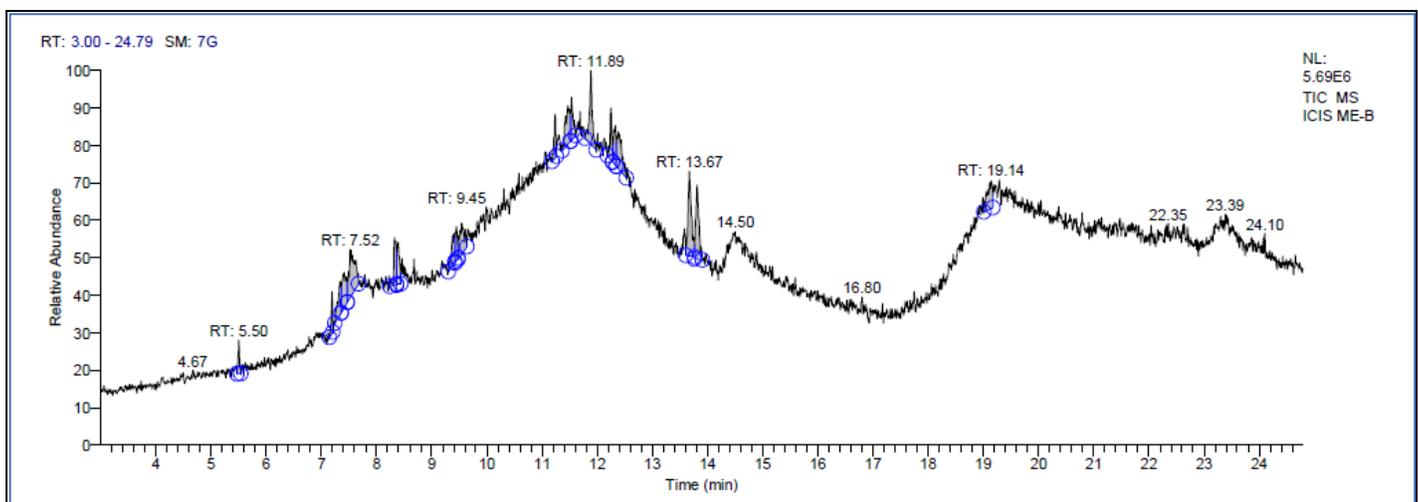


Figure 3: GC-MS chromatogram of Methanolic extract of *Ehretia laevis*

Table 1: Phyto-compounds present in Petroleum ether extract of *Ehretia laevis* using GC-MS Profiling

Sr. No.	Rf (min)	Name of compound	Molecular formula	Molecular weight	Area %
1	9.06	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	C ₁₁ H ₁₆ O ₂	180	5.01
2	10.88	Ppropionic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)	C ₁₃ H ₂₀ O ₃	224	1.94
3	11.24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	2.28
4	11.29	2-Pentadecanone, 6,10,14-trimethyl	C ₁₈ H ₃₆ O	268	1.04
5	11.89	Hexadecanoic acid, 15-methyl-, methyl ester	C ₁₈ H ₃₆ O ₂	284	1.39
6	12.24	Tridecanoic acid	C ₁₃ H ₂₆ O ₂	214	28.49
7	13.58	12,15-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294	0.95
8	13.67	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	C ₁₉ H ₃₂ O ₃	308	1.95
9	13.81	1,2-15,16-Diepoxyhexadecane	C ₁₆ H ₃₀ O ₂	254	1.72
10	14.19	8,11-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294	13.78
11	14.29	Methyl 8,11,14-heptadecatrienoate	C ₁₈ H ₃₀ O ₂	278	35.65
12	22.95	5-Chloro-3beta-hydroxy-6beta-nitro-5alpha-androstan-17-one	C ₁₉ H ₂₈ C ₁ NO ₄	369	2.70
13	23.04	Propenenitrile, 2-(4-chlorophenylsulfonyl)-3-cyclohexylamino-	C ₁₅ H ₁₇ C ₁ N ₂ O ₂ S	324	2.31

Table 2: Phyto-compounds present in Chloroform extract of *Ehretia laevis* using GC-MS Profiling

Sr. No.	Rf (min)	Name of compound	Molecular formula	Molecular weight	Area %
1	3.35	Propane, 1,2-dichloro-2-methyl	C ₄ H ₈ Cl ₂	126	3.52
2	3.47	1-Chloro-2-ethoxy-2-methoxy-propane	C ₆ H ₁₃ ClO ₂	152	2.39
3	4.14	4-Chloro-2,4-dimethylhexane	C ₈ H ₁₇ Cl	148	2.86
4	7.19	Cyclohexasiloxane, dodecamethyl	C ₁₂ H ₃₆ O ₆ Si ₆	420	1.27
5	8.68	Cycloheptasiloxane, tetradecamethyl	C ₁₄ H ₄₂ O ₇ Si ₇	518	1.11
6	8.86	Phenol, 2,4-bis(1,1-dimethylethyl)	C ₁₄ H ₂₂ O	206	1.25
7	9.99	Cyclooctasiloxane, hexadecamethyl	C ₁₆ H ₄₈ O ₈ Si ₈	592	0.96
8	10.88	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-di ol	C ₁₂ H ₂₀ O ₂	192	5.97
9	11.13	Octasiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	C ₁₆ H ₅₀ O ₇ Si ₈	578	0.70
10	11.24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	22.50
11	11.54	9-Eicosyne	C ₂₀ H ₃₈	278	5.41
12	12.24	1,2-Benzenedicarboxylic acid, butyl octyl ester	C ₂₀ H ₃₀ O ₄	334	15.17
13	12.43	Phthalic acid, isobutyl octadecyl ester	C ₃₀ H ₅₀ O ₄	474	1.35
14	13.81	Oxirane, hexadecyl-	C ₁₈ H ₃₆ O	268	8.67
15	14.18	Methyl 6,10-octadecadienoate	C ₁₉ H ₃₄ O ₂	294	1.83
16	14.29	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	278	11.45
17	21.73	Phthalic acid, octyl 2-propylpentyl ester	C ₂₄ H ₃₈ O ₄	390	10.36

Table 3: Phyto-compounds present in Methanolic extract of *Ehretia laevis* using GC-MS Profiling

Sr. No.	Rf (min)	Name of compound	Molecular formula	Molecular weight	Area %
1	5.5	Benzoic acid, 2,6-bis [(trimethylsilyloxy)-trimethylsilyl ester	C ₁₆ H ₃₀ O ₄ Si ₃	340	1.8
2	7.19	Silane(pregn-5-ene-3á,11á,17,20á-tetrayltetraoxy)tetrakis[trimethyl	C ₃₃ H ₆₆ O ₄ Si ₄	638	1.77
3	7.33	(5á)Pregnane-3,20á-diol,	C ₂₈ H ₄₃ NO ₆	489	3.69
4	7.41	2-Trimethylsiloxy-6-hexadecenoic acid, methyl ester	C ₂₀ H ₄₀ O ₃ Si	356	5.76
5	7.52	4-(Dimethylaminomethyl-5-hydroxybenzofuran-3-yl)(4-methoxyphenyl)methanone	C ₁₉ H ₁₉ NO ₄	325	9.76
6	8.32	Chromone, 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-	C ₁₄ H ₁₆ O ₆	280	4.16
7	8.39	6,7-Epoxyregn-4-ene-9,11,18-triol-3,20-dione	C ₂₅ H ₃₂ O ₈	460	3.79
8	9.41	3,9-Epoxyregn-16-ene-14-18-diol-20-one, 7,11-diacetoxy-3-methoxy-	C ₂₆ H ₃₆ O ₉	492	3.92
9	9.45	5,8,11,14-Eicosatetraynoic acid, trimethylsilyl ester	C ₂₃ H ₃₂ O ₂ Si	368	2.31
10	9.54	2,7-Diphenyl-1,6-dioxypyridazino[4,5:2',3']pyrrol o[4',5'-d]pyridazine	C ₂₀ H ₁₃ N ₅ O ₂	355	5.36

11	11.24	2,15-Heptadecadiene, 9-(ethoxymethyl)	C ₂₀ H ₃₈ O	294	2.68
12	11.47	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	6.36
13	11.53	4-Methoxyphenoxyformamide N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]	C ₁₇ H ₂₂ N ₂ O ₃	302	3.37
14	11.89	Hexadecanoic acid, 14-methyl-, methyl ester	C ₁₈ H ₃₆ O ₂	284	7.55
15	12.25	Phthalic acid, butyl oct-3-yl ester	C ₂₀ H ₃₀ O ₄	334	3.51
16	12.38	Methyl 9,12-epithio-9,11-octadecanoate	C ₁₉ H ₃₂ O ₂ S	324	6.80
17	13.67	Methyl 4,7,10,13,16,19-docosaenoate	C ₂₃ H ₃₄ O ₂	342	10.36
18	13.81	Z,Z-4,16-Octadecadien-1-ol acetate	C ₂₀ H ₃₆ O ₂	308	8.48
19	19.14	2,7-Diphenyl-1,6-dioxypyridazino[4,5:2',3'] pyrrolo [4',5'-d] pyridazine	C ₂₀ H ₁₃ N ₅ O ₂	355	5.16

CONCLUSION

The correlation between the phytochemical compounds and their biological activities is now being the matter of innovative thought. *Ehretia laevis* is a plant, traditionally used for the ulcers, skin diseases, in headache, urinary passage, lung and spleen diseases, astringent, diuretic, demulcent, expectorant, throat infection. Root for venereal diseases etc. But till date, there are few reports on chromatographic analysis of petroleum ether, chloroform and methanolic extract of the plant. Here we report the presence of some important compounds in the plant *Ehretia laevis* isolated by GC-MS analysis. GC-MS study of plant extracts may give information on nature of active principles present in the medicinal plants. These phytoconstituents presumed to be responsible for eliciting the traditional activity of this plant *Ehretia laevis*.

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