



## Pharmacognostical Characterisation and Phytochemical Profiling of the Medicinal Plant, *Ehretia ovalifolia* Wight

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### ABSTRACT

Pharmacognostical characterisation and phytochemical profiling is more important for a plant before the plant was utilized in the drug industry. The plant, *Ehretia ovalifolia*, belongs to family Boraginaceae is an important medicinal plant. The present investigation was carried out to evaluate the pharmacognostical, phytochemical studies to explore the characters of this plant. The organoleptic characters of stem and leaf expressed with some characteristic bitter taste, odour, with brown and deep green in colour forms. Morphologically the plant with 23cm of Girth, 6.5 cm leaf area with 5-8 pairs of veins. The reaction of stem powders with various chemicals were made and some of the powders were well reacted and it was observed under various light sources. Some the powders appeared to be black under UV light of 256nm from Dark green. Similarly the green coloured leaf powder was also appeared to be black in colour. The qualitative phytochemical study showed presence of important phytochemical component like alkaloids, saponins, terpenoids, coumarins, flavonoids, tannins and phenols. The chloroform extract also expressed the presence of important phytochemicals like phenols and flavonoids with other phytochemicals. The leaf study showed the presence of all studied phytochemicals except glycosides and whereas the steroids and terpenoids were absent. The GCMS analysis of methanol extract was detected with some 6 important compounds i.e Ethyl. alpha. -d-glucopyranoside n-Hexadecanoic acid and 3,4-Secodammar-4(28)-en-3-oic-acid,20,24-epoxy-25-hydroxy-, (24s)-. The total ash value of stem is 8% and water soluble as of 6.67% and 0.7% of acid soluble ash. The ash value of leaf is 17.6% with 17% of water





soluble ash and only 8.7% of acid soluble ash. The study completely explain the characteristic features of the study plant *E.ovalifolia* which may useful for pharma companies during drug manufacture.

**Keywords:** organoleptic study, phytochemicals, GC –MS, alkaloids, flavonoids.

## INTRODUCTION

India is known as botanical garden of the world. The plants that are founded with medicinal properties are used in the indigenous treatment practices such as ayurveda, unani, siddha and also in modern medicines. According to WHO 80% people prefer tradition medicines for their health care. Pharmacognosy is the study of the physical, chemical, biochemical and biological properties of drugs, drug substances of natural origin as well as the search for new drugs from natural sources. Modern Pharmacognosy involves the broad study on natural products from various product including plants, Bacteria, Fungi, and marine organisms. The phytochemicals are naturally available bioactive compounds derived from plants with potential disease curing capabilities. The secondary constituents consisting of alkaloids, flavonoids, tannins, terpenoids, steroids, saponins, and other phenolic compounds etc. the various phytochemicals are present in medicinal plants are alkaloids, tannins, saponins, steroid, terpenoids, flavonoids, glycoside. Medicinal plants are involved in the discovering and screening of the new phytochemical constituents which are very helpful for the manufacturing of new drugs. The modern chromatographical techniques such as GC-MS were used widely for profiling of phytochemicals Kirshnananda (2017).

These constituents have beneficial aspect in therapeutic value of human. Plants are being used as medicine since ancient periods because drugs obtained from plants are easily available, safe, and when its intake it possesses less side effects. The bioactive materials obtained from extraction and their quantitative and qualitative analysis is considered as pharmaceutical activity. The genus *Ehretia* with a 150 species belongs to family Boraginaceae. Many species of this family distributed in tropical Asia, Europe and North America. The parts of leaves, barks, roots, branches, heartwood are used in traditional medicine in Japan, India and China (Rajasekar and Saravana Gantri (2019). These genus are may present the capability of curing capacities for anti- inflammatory, antibiotic and antibacterial activity. The present study was carried out on most widely distributed lesser known medicinal plant *Ehretia ovalifolia* for its pharmacognostical and phytochemical nature to understand and authenticate the species for drug making in Ayurveda, siddha and other indigenous way of treatment.

## MATERIALS AND METHODS

The study material *Ehretia ovalifolia* was collected and characterized as is a small perennial tree growing to 12m with branches, leaves oblong or elliptic apex are obtuse, nerves are 6-8 pairs, pubescent in the nerve axis petioles are present. Inflorescence corymbs terminal and axillary flowers are white slightly pubescent pedicles. Tubular calyx corolla rotate filaments four, ovary four celled style two fused.

### Collection of plant material

The leaves and stem of *Ehretia ovalifolia* were collected from the NGM campus, Pollachi Taluk, Coimbatore district, Tamil Nadu. The healthy parts were collected, shade dried and pulverized to powder, further the powder was stored in air tight container till further works to be carried out.

### Preparation of Crude

Solvents viz., chloroform and methanol were used to take the extract from leaves and stem powders according to Harborne (1998) method. The samples were sequentially extracted using a soxhlet apparatus and was subjected to





further studies as mentioned by (Kokate,1994). The extract was allowed to evaporate the excess solvent at room temperature and the crude consistent was obtained.

**Organoleptic Studies: (Aji *et al.*, 2014)**

Microscopic and macroscopic observations of the study plant were carried out like plant shape, size, surface and physical characters like texture, colour, odour, taste etc was noted.

**Preliminary analysis of phytochemicals**

The crude was dissolved in respective solvents for working consistency and screened for the presence of some important phytochemical such as alkaloids, tannins, flavonoids, saponins, glycosides, steroids, phenol, terpenoid, coumarins and glycosides.

**Test for Alkaloids: (Mayer's test) (Aji *et al.*, 2014)**

To a few drops of the plant extract, two drops of the Mayer's reagent is added along the sides of the test tube. The appearance of white creamy precipitate indicates the presence of the alkaloids.

**Test for Phenols: (Shreya *et al.*, 2013)**

Ferric chloride test: The 50 mg of the extract in 5 ml distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the phenolic compound.

**Test for Flavonoids: (Shreya *et al.*, 2013)**

An aqueous solution of the extract is treated with few drops of  $H_2SO_4$ . Formation of orange colour indicates that the presence of flavonoids.

**Test for Saponins: (Kokate, 1999).**

The extract is dissolved with distilled water and make up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of the saponins (Kokate, 1999).

**Test for Steroids: (Shreya *et al.*, 2013)**

The extract is treated with 2ml of chloroform and few drops of Con. $H_2SO_4$ . Formation of reddish-brown ring indicate the presence of steroids

**Test for Coumarin: (Yadav *et al.*, 2014)**

To 2ml of extract was taken 3ml of 10% sodium hydroxide was added. Appearance of yellow coloration indicates the presence of coumarins.

**Test for Glycosides: (Keller-Killiani test) (Shreya *et al.*, 2013)**

To total of 1 ml of glacial acetic acid, few drops of ferric chloride solution and concentric  $H_2SO_4$  (slowly through the sides of the test tube) were added to the extract. Appearance of reddish-brown ring at the junction of the liquids indicated the presence of deoxysugar.

**Test for Terpenoids: (Yadav *et al.*, 2014).**

To 2ml of extract 2ml of acetic acid was added. Then concentrated sulphuric acid was added. Deep red colour development showed the presence of terpenoids

**Test for Tannin: (Braymer's test) (Edeoga *et al.*, 2005 and Harborne *et al.*, 1973).**

To 1ml of extract was mixed with 2ml of water. To these, 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannin.





### Anatomical study

The study on anatomical characterisation with normal sectioning and the section treated with various reagents, solvents were used to study the ergastic substances and exudates of the plant.

The free hand sections were taken from the fixed specimen of the study plant are used for the present study.

### Fluorescence Powder analysis: (Kokashi et al., 1958).

Fluorescence powder analysis of leaf and stem were performed as per standard procedures (Kokashie *tal.*, 1958). A small quantity of the leaf powder was placed on the microscopic slide and 1-2 drops of freshly prepared reagents was added, mixed by gently tilting of the slide and waited for few minutes. Then the slide were placed in normal light, white light, and in the UV chamber (256 nm) were observed to determine the fluorescence characters and details of foreign particles /objects present in the powder.

### Ash test: (Madhubala and Shanthi 2019).

The 3g of powder material was accurately weighed and placed in crucible. The material was spread in even layer at the temperature of 550°C until it was white indicating the absence of carbon.

### Determination of water soluble ash:

The residual ash was allowed to cool, weighed ash material was washed with distilled water and filtered by Whatman filter paper and then it is dried.

### Determination of alcohol soluble ash:

The dried powder is then filtered using concentrated hydrochloric acid and the substance that can extract from the acids are removed for the further process for the indication of heavy metals.

### Gas chromatography (GC) and mass spectrometry (MS) analysis:

The chemical composition of the methanolic leaf extract of *E.ovalifolia* was analysed by GC-MS. The analytical work was carried using thermo GC-turbo mass version 5.4.2 coupled with thermo: MS DSQ11 instrument. . The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethyl polysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min<sup>-1</sup>; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

## RESULTS AND DISCUSSION

The pharmacognostical studies on medicinal plant considered to be more important to authenticate the species before preparing the herbal formulation. The present study was carried out on important tropical medicinal plant *Ehretia ovalifolia* of Boraginaceae. The morphological features of the study plant was observed as 23cm of GBH, Leaf surface area with 6.5 cm, Petiole length of 1.7 cm and internode length of about 0.4 cm and the veins of about 5-8 pairs in alternate pattern where tabulated in the Table: 2. The morphological features are very fundamental in all other activities regarding photosynthetic efficiency, Physiological perfection. The Table refers about powder characters and it is referred as organoleptic characters of the plant. This is first important characterisation made in pharmacognostical studies. The stem powders appeared generally as standard brown in colours since it is rich in tannins and have characteristic odour with bitter taste and have a coarse texture. Similarly, the leaf powder will be very fine in nature since there will be absence of fibres. The type of fruit is berry type and no. of seeds per fruit is 4 and weight per 10 seeds is observed as 1.6 g. The powder samples of stem were treated with some selected chemicals





like strong acids, strong bases, weak acids, and weak bases and with solvents. The stem powders when treated with KOH appeared to be yellowish green to brownish green in ordinary light sources and UV light source. The stem powder when added with  $\text{FeCl}_3$  the powder appeared to be in Dark green in normal light sources and it was appeared to blackish green in UV light sources. When the stem powder was treated with normal distilled water it appeared as dark brown from light brown in colour. The concentrated sulphuric acid also changes the powder completely to black in colour. The importance of pharmacognostical evaluation gains its importance to authenticate the species before using it for making formulations (Pooja Sharma *et al.*, 2021).

The leaf powder of the study plant *Ehretia ovalifolia* showed characteristic change in appearance in various light sources. The powders when treated with Conc.  $\text{HNO}_3$  it appears as deep brown which resemble the coffee brown in colour and in UV light sources it appeared as reddish brown in colour. The characteristic change was observed in by adding the KCl the colour appeared as reddish brown in colour in UV light sources. The powders treated with various solvents showed some changes and turns in reddish brown from green. The powder not reacted with water and remained as such. Hence drug manufacture and herbal formulation may be carried out using water in various forms. The results of qualitative phytochemical analysis were made on both stem and leaf powders with chloroform and methanol extract. Comparatively the methanol extract exhibited presence of major phytochemicals except steroids and glycosides. The phenolic and tannin compounds present comparatively more abundant than other phytochemicals. Similarly, the leaf extract also exhibited the presence of important phytochemicals except glycosides and flavonoids. The leaf also exhibited the presence of phenols and tannins. The other important phytochemicals also showed its presence in methanol extract. The above studies clearly suggests that the phytochemicals found to be more in the plant hence the study plant may use to prepare the medicines (Table: 7 and 8). The similar results were also proposed by many workers. The presence of polyphenols compounds may be responsible for antioxidants (Shahidi and Wanasundara, 1992). Similar to the above studies the presence of flavonoids was reported in methanol extract in the plant parts which is responsible for many important biological effects such as antioxidant, anti-inflammatory, hepatoprotective, antiulcer, antiallergic, antiviral and anticancer activities (Umamaheswari and Chatterjee, 2008).

The ash values of the *E.ovalifolia* nutrition qualities and to understand the presence of foreign particles. The ash values result clearly exhibited that leaf has more ash values with Total ash value was 17.6% but in stem only 8% of ash value has been observed. The lowest acid soluble ash values were observed with 0.7% and leaf 8.7% (Table: 9). The GCMS analysis of methanolic leaf extract showed the presence of important 6 compounds and its IUPAC names with molecular weight and Area % was found to be present. The medicinally important compound Ethyl alpha – D Glucopyranoside had 13.581 area percentage at 16.579 RT value. The compound used as anti-inflammatory was found to be present with area percentage of 6.951 at RT value of 2.984. The two medicinally important compounds having potential anti-cancer activity called pyridine was found to be at RT value 2.984 and high antioxidant compound N- Hexadecanoic acid at 20.02 of RT value with area percentage of 6.951 and 5.088 were observed respectively. Comparatively, the above compounds are found to be lesser but the compounds are medicinally more important were reported in the study plant species *Ehretia ovalifolia*. The above GCMS analysis clearly exhibited the presence of medicinally important phytochemicals. Many volatile compounds are reported previously by many workers similar to that of the results the aromatic phytoconstituents and its importance were clearly explained by the (Akintayo *et al.*, 2016)

## SUMMARY AND CONCLUSION

The present study clearly exhibits that pharmacognostical properties of the plant *Ehretia ovalifolia*. The genus *Ehretia* a well-adapted plant species of Boraginaceae where the plant can found only at typical dry deciduous forest as influenced by heavy monsoon rain. The poor regeneration capacity of the plant, fruit formation and seed setting habit of the plant leads to have very poor population in natural habitat. The present study clearly gives an idea on morphological nature of the plant, Phytochemical profile, pharmacognostical characters and GCMS analysis made to





understand the plant in various criteria. The present study helps to avoid unnecessary adulteration and misguiding or mismatching species to be added in the important medicinal formulation made in the indigenous system of medicine.

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**Table.1: Showing the organoleptic characters of the study plant *Ehretia ovalifolia***

S.No	Part	Colour	Odour	Taste	Texture
1.	Stem	Brown	characteristic	Bitter	Coarse
	Leaf	Green	characteristic	Bitter	Fine

**Table.2: Showing the results of Morphological parameters of the study plant *Ehretia ovalifolia***

S.No.	GBH (cm)	Leaf length (cm)	Petiole length (cm)	Internode Length (cm)	Veins	No. of seeds /Fruit(Berry)
1.	23	6.5	1.7	0.4	5-8 pairs	4

**Table.3: Showing the characters of stem powder of the study plant *Ehretia ovalifolia***

S.NO	Treatment	Light	White light	UV Light (256 nm)
1	Powder + Con.HCL	Brown	Muddy Brown	Dark Brown
2	Powder + Dil.HCL	Light Brown	Muddy Brown	Brown
3	Powder + Con.H <sub>2</sub> SO <sub>4</sub>	Black	Reddish Brown	Black
4	Powder + Dil.H <sub>2</sub> SO <sub>4</sub>	Muddy Brown	Muddy Brown	Muddy Brown
5	Powder + Con.HNO <sub>3</sub>	Reddish Brown	Reddish Brown	Dark Brown
6	Powder + Dil.HNO <sub>3</sub>	Light Brown	Light Brown	Muddy Brown
7	Powder + CH <sub>3</sub> COOH	Light Brown	Dark Brown	Deep Brown
8	Powder + NaOH	Dark Brown	Dark Brown	Black
9	Powder + KOH	Yellowish Brown	Yellow	Brownish Green





10	Powder + FeCl <sub>3</sub>	Dark Green	Dark Green	Black
11	Powder + KCl	Muddy Brown	Light Brown	Brown
12	Powder + Acetone	Muddy Brown	Light Brown	Dark Brown
13	Powder + Pet Ether	Light Brown	Muddy Brown	Brown
14	Powder + Chloroform	Brown	Light Brown	Muddy Brown
15	Powder + Ethanol	Light Brown	Light Brown	Muddy Brown
16	Powder + Methanol	Muddy Brown	Dark Brown	Light Brown
17	Powder + Water	Light Brown	Light Brown	Dark Brown

Table.4: Showing the characters of leaf powder of the study plant *Ehretia ovalifolia*

S.NO	Treatment	Light	White Light	UV Light (256 nm)
1	Powder + Con.HCl	Green	Green	Black
2	Powder + Dil.HCl	Dark Green	Dark Green	Dark Green
3	Powder + Con.H <sub>2</sub> SO <sub>4</sub>	Black	Dark Green	Black
4	Powder + Dil.H <sub>2</sub> SO <sub>4</sub>	Dark Green	Light Green	Dark Green
5	Powder + Con.HNO <sub>3</sub>	Coffee Brown	Brown	Reddish Brown
6	Powder + Dil.HNO <sub>4</sub>	Dark Green	Light Green	Dark Green
7	Powder + CH <sub>3</sub> COOH	Deep Green	Green	Black
8	Powder + NaOH	Green	Dark Green	Black
9	Powder + KOH	Green	Blackish Green	Black
10	Powder + FeCl <sub>3</sub>	Green	Blackish Green	Black
11	Powder + KCl	Dark Green	Dark Green	Reddish Brown
12	Powder + Acetone	Dark Green	Brown	Black
13	Powder + Pet Ether	Dark Green	Black	Green
14	Powder + Chloroform	Dark Green	Deep Green	Dark Black
15	Powder + Ethanol	Dark Green	Deep Green	Reddish Black
16	Powder + Methanol	Dark Green	Black	Black
17	Powder + Water	Dark Green	Light Green	Dark Green

Table.5: The Qualitative phytochemical analysis of the stem and leaves of study plant *Ehretia ovalifolia* using chloroform and methanol extract.

S.NO	Phytochemical Compounds	Stem		Leaf	
		Chloroform	Methanol	Chloroform	Methanol
1.	Alkaloids	+	+	+	+
2.	Steroids	-	-	-	+
3.	Saponins	+	+	+	+
4.	Terpenoids	+	+	-	+
5.	Coumarins	-	+	+	+
6.	Glycosides	-	-	+	-
7.	Flavonoids	+	+	+	+
8.	Phenols	+	+	+	+
9.	Tannins	+	+	-	-



**Table.6:** Components detected through GC-MS analysis on the methanolic leaf extract of the study plant *Ehretia ovalifolia*

S.No	RT	Compound name	Mol wt.	Molecular formula	Area %	CAS.No	Medicinal Properties
1	2.984	Pyridine	79	C <sub>5</sub> H <sub>5</sub> N	6.951	110-86-1	Anticancer, antimicrobial
2	15.759	Diethyl phthalate	222	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	10.001	84-66-2	-
3	16.579	Ethyl. alpha. -d-glucopyranoside	208	C <sub>8</sub> H <sub>16</sub> O <sub>6</sub>	13.581	900127-29-4	Anti-inflammatory
4	20.02	n-Hexadecanoic acid	256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	5.088	57-10-3	Anti-inflammatory, antioxidant
5	25.347	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	278	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	38.279	4376-20-9	-
6	27.083	3,4-Secodammar-4(28)-en-3-oic-acid,20,24-epoxy-25-hydroxy-, (24s)-	474	C <sub>30</sub> H <sub>50</sub> O <sub>4</sub>	26.101	56421-13-7	-

**Table.7:** The ash content of the study plant *Ehretia ovalifolia*

S.NO.	Ash type	% of Ash content	
		Stem	Leaf
1	Total ash value of powder	8	17.6
2	Water soluble ash	6.67	17
3	Acid soluble ash	0.7	8.7

