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In Vitro studies in important medicinal plant *Ehretia laevis* Roxb

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Abstract
The plants of the genus *Ehretia* composed of about 150 species mainly distributed in tropical Asia, Africa, Australia, and North America. They have been used as traditional and folk medicines to treat various ailments in Japan, India, and China for a long time. Previous phytochemical screenings demonstrated that the *Ehretia* plants mainly contain fatty acids, phenolic acids, flavonoids, cyanogenetic glycosides, and benzoquinones and other constituents from different chemical classes. The pharmacological studies confirmed that the crude extracts or individual compounds from the genus showed antioxidant, anti-inflammatory, antibacterial, antiarthritic, antitubercular, and antiallergic activities, as well as anti-snake venom property. Taking into consideration we have undertaken *in vitro* studies on this important medicinal plant.

Keywords: *ehretia*, traditional and medicinal importance, essential oil, antiparasitic activity, future prospects

Introduction

Ehretia genus has around 150 species belongs to the family Boraginaceae [1-3]. Many species are mainly distributed in tropical Asia, Africa, Australia, Europe, and Northern America [4-14]. All species of *Ehretia* are trees (*Ehretia* acuminata) and shrubs [15] (*Ehretia rigida*). The leaves, barks, roots, branches, fruits, and heartwoods are used as the traditional medicines in China, Japan, and India. Some species produce small fruits are visited by a broad variety of opportunistic avian frugivores, and some species could be a valuable supplementary feedstuff for ruminant livestock and wild animal due to its *in vitro* fermentation characteristics as well as low fiber [15-19].

In India, genus *Ehretia* is reported for many species such as *Ehretia laevis* Roxb., *E. acuminata* R. Br [20], and *Ehretia microphylla* [21, 22]. These species are used in many herbal and traditional medicines in India and China because of their good response in many biological activities. *Ehretia* genus has reported the presence of phenolic acids, lignans, flavonoids, nitrile glycosides, quinonoids, steroids, triterpenoids, and pyrrolizidine alkaloid [23, 24]. Many species of *Ehretia* genus are reported for anti-inflammatory, antidiabetic, and antibacterial activity. Some important species of this genus are *Ehretia*longiflora, E. laevis, E. acuminata, E.microphylla, and *Ehretia*obtusifolia. In an effort to provide the up-to-date information of the genus *Ehretia*, in previous, some chemical constituents and activities have published up to 2010 [25], and this article represents the results of an extensive investigation of the chemotaxonomy, secondary metabolites, biological activities, and pharmacological applications of this genus up to 2017, which would assist further researches and potential applications of the plants.

Traditional and medicinal importance of *Ehretia*

Different plants of genus *Ehretia* are widely used traditionally in many herbal and Chinese medicines from last few decades in China, India, and Japan [26, 27].

Chemical constituent present in genus *Ehretia*

Species present under the genus *Ehretia* contains many phytoconstituents such as-phenolic acids, flavonoids, benzoquinones, cyanogenetic glycosides, fatty acids, and some other important compounds.

Phenolic acids

Plants are potential sources of natural bioactive compounds such as secondary metabolites and antioxidants. Phenolic acids are the secondary metabolites and therapeutic agent present in medicinal plant. Phenolic compounds confer unique taste and health-promoting properties found in vegetables and fruits. The effect of dietary phenolics is currently of great interest due to their antioxidative and possible anticarcinogenic effect.

Flavonoids

Flavonoids are the polyphenolic compounds among secondary metabolites in different parts of plants that possess a wide range of biological activities. They are present in fruits, vegetables, nuts, spices, and herbs and derived products such as wine, tea, and chocolate. The flavonoid class includes more than 6000 compounds as found in nature and comprises several subclasses including flavonols (e.g.,quercetin, kaempferol, myricetin, and rhamnazin), flavones (e.g.,apigenin, luteolin, and tangeretin), flavanones (e.g.,hesperetin, naringenin, and eriodictyol), flavanols (e.g.,catechins and epicatechins), anthocyanidins (e.g.,cyanidin, delphinidin, and malvidin), and isoflavones (e.g.,genistein, daidzein, and glycitein).

Alkaloids

Alkaloids are quite important secondary metabolites of plants. They are internal constituents of plants so-called biomolecules. Uses of alkaloids primarily mean their use in health care. They act as lifesaving drugs in various serious disorders such as heart failure, blood pressure, and cancer. Several alkaloids isolated from natural herbs exhibit antimetastasis and antiproliferation effects on various types of cancers such as vinblastine and camptothec in and have already been successfully developed into anticancer drugs. Lycorine, indicine n-oxide, alstonine, cocaine, quinine, and quinidine are some alkaloids present in plants.

Fatty acids
Plants synthesize a huge variety of fatty acids although only a few are major and common phytoconstituents. Fatty acids are important dietetic sources of fuel for animals because, when metabolized, they yield large quantities of ATP (Adenosine triphosphate).
Many types of cell can use either fatty acids or glucose for this purpose. Long-chain fatty acids are not capable to cross the blood–brain barrier and so cannot be used as fuel by the cells of the central nervous system.

Benzoquinones
Quinones, a type of plant-derived secondary metabolites and benzoquinones, are widely distributed in the plant kingdom and mainly exist in higher plants, such as those from the Polygonaceaee, Rubiaceae, Leguminosae, Rhamnaceae, Labiatae, and Boraginaceae families, among others. Moreover, a number of benzoquinones show significant biological activities such as anticancer and antibacterial activities.

Glycosides
In the chemical constituents occur in a glycoside are important, secondary metabolites. However, biological activities of glycosides are, in many cases, susceptible to the nature of sugar moieties. They are an essential resource of natural medicine, health food, cosmetics, and food supplements.

Essential oil
Essential oil extracted from leaves of *Ehretia cymosa* by hydro distillation. Some chemical constituents separated by comparison of their mass spectra with NIST from essential oil which mainly comprised sesquiterpene hydrocarbon compounds. The other classes of compounds identified in these essential oils were monoterpenes, alcohols, phenylpropanoids, esters, and fatty acids. In addition, some of the studied volatile oils have exhibited biological activities such as antimicrobial, phytotoxicity, insecticidal, and cytotoxicity.

Material and method
Preparation of Explants
Seeds of *Ehretia laevis Roxb* were collected from Goga hills and grows in the green house, Botanical garden, department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Apical shoot, Axillary bud, node and Meristimatic tissue of *Ehretia* were collected from two month old plants grown in the Botanical garden, department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. All these explants were used from donor plants during present study. The explants were washed carefully in running tap water for 10 minute and followed by distilled water for 5 minutes. For surface sterilization, chemical such as 70% ethanol, Hgcl2 (0.3 %) was used. Explants were surface sterilized for 5 minute by 0.3% mercuric chloride followed by three subsequent rinses with sterilized double distilled water in a laminar flow. All these explants were dissected into small pieces and treaded so that maximum part can be exposed to media.

Culture media
Murashige and Skoog, 1962 medium was used for multiple shooting for apical shoot, Axillary bud, node explants of *Ehretia laevis Roxb*. Axillary bud, apical shoot tip multiplication of shoots was examined using MS medium variously supplemented with BA, KIN, for rooting, half strength MS medium Supplemented with various concentrations of auxins IAA, IBA, and NAA were examined.

Culture conditions
Murashige and Skoog medium contains with 3% sucrose and gelled with 3 gm/L solidified agent Clerigel, and the pH was adjusted to 5.8 after adding the growth regulators. The media were steam sterilized in an autoclave under 15 psi and 121° C. after the inoculation culture tubes and culture vessels were transfers to culture room under a 16 h photoperiod supplied by cool white fluorescent cool tubes light and 25 ± 0C temperature. At least ten cultures were raised for each treatment.

Table 1: Effect of growth hormones on regeneration of different explants

Explants	Conc. of Growth Regulator (mg/L)		Shoot Length (Mean)	% of shoot Formation
	BAP	IAA		
Aapical shoot tip	1.0	0.2	1.88	30
	1.2	0.2	2.64	32
	1.4	0.2	2.52	37
	1.6	0.2	2.90	51
	1.8	0.2	2.16	49
	2.0	02	2.24	47
Axillary Bud	1.0	0.2	1.70	35
	1.2	0.2	2.04	37
	1.4	0.2	2.14	44
	1.6	0.2	2.62	52
	1.8	0.2	1.76	50
	2.0	0.2	1.82	49

Results and discussion
Explants such as apical shoot, axillary bud and nodal explants of *Ehretia* were grown on hormone free MS medium no effect on multiple shoots formation. MS media with different concentrations of BAP 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mg/l and combination of IBA, NAA gives maximum average percentage of multiplication.

During the present investigation of an apical shoot tip, Axillary bud and nodal explant was essential for the development and formation of multiple shoots in *Ehretia*. The two Cytokinins was tested, BAP and KIN respectively. BAP was more effective than KIN for multiplication. MS media containing 3% sucrose, 3 mg/L Clerigel and different concentration of BAP 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mg/l alone with IBA 0.2, 0.4, 0.6, 0.8, 1.0 mg/L, concentration of BAP with combination NAA 0.2, 0.4, 0.6, 0.8, 1.0 mg/L gives average percentage of multiple shooting of *Ehretia*. Maximum average percentage of multiple shoot was recorded BAP 1.6 mg/L, with combination IBA 0.2 mg/L.

Apical shoot tip and Axillary bud explants were inoculated on MS medium supplement with 3% sucrose, 2.5% Clerigel and various combinations of growth hormones as shown in the table No. 1 Maximum average shoot length and multiple shoot formation percentage of *Ehretia* was recorded in 1.6 mg/L BAP combination with 0.2 mg/L IBA. Various combinations of IAA were added into the MS medium to achieve rooting. *In vitro* rhizogenesis was achieved by adding 0.5 mg/lit IAA. Plants were hardened and introduced in soil for *in vivo* trails. *In vitro* regenerated plants had shown 65 % viability *in vivo*.

Conclusion

Low seed germination rate and habitat destruction threatens its population in natural habitat. The vegetative propagation by root tubers and stem cuttings is very arduous. The tuberous roots of many *Ehretia* species are having medicinal importance. The leaves are contains valuable constituents in many traditional Indian Ayurvedic drug preparations against many diseases, such as Fever, diarrhea and dysentery. The root tubers contain starch, sugar, gum, albuminoids, fats and crude fiber and are valuable constituents in many traditional medicinal systems in India. Hence present *in vitro* studies are important for propagation of this important medicinal Plant.

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