India is an emporium of medicinal and aromatic plants. It has been estimated that out of 15,000 higher plants found in India, 9000 are commonly used, of which 7,500 are used in various systems of medicine (Anonymous, 1994). Plant based drugs have been used worldwide in traditional medicines for treatment of various diseases. India is the largest producer of medicinal herbs and appropriately called the ‘Botanical garden of the world (Ahmedulla and Nayar, 1999). In most parts of the world, information on medicinal plants has generally been handed down from generation only by means of folklore. Now microscopic studies and chemical studies become an important part of pharmacognosy (Handa and Kapoor, 1989). The therapeutic value of a medicinal plant depends on the presence of one or more constituents possessing certain physiological and pharmacological activity. Plants based antimicrobials have enormous therapeutic potential. The plant based drugs are effective in the treatment of infectious diseases (Murray, 1995). The search for biologically active compounds from natural sources has always been of great interest to researchers looking for new sources of drugs useful in infectious diseases.

Two medicinal plants *Embelia ribes* and *Chonemorpha fragrans* were collected from Kakkayam forest, Kozhikode district, Kerala and the plant material was identified by experts at MS.Swaminathan Research Foundation, Wayanad, Kerala. All vouchers of the collections are deposited at the herbarium of St. Xavier’s College (Autonomous), Palayamkottai.

*Embelia ribes*

**Taxonomical Classification**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
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</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Angiosperms</td>
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<td>Order</td>
<td>Ericales</td>
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<tr>
<td>Family</td>
<td>Myrsinaceae</td>
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</table>
Genus : *Embelia*
Species : *ribes*

**Regional Names**

Sanskrit : Jantughna, Krmighna, Vella, Krmihara, Krmiripu  
Assamese : Vidang  
Bengali : Vidang  
Gujrati : Vavding, Vavading, Vayavadang  
Hindi : Vayavidanga, Bhabhiranga, Baberang  
Kannada : Vayuvilanga, Vayuvidanga  
Kashmiri : Babading  
Malayalam : Vizhalari, Vizalari  
Marathi : Vavading, Vavding  
Oriya : Bidanga, Vidanga  
Punjabi : Babrung, Vavaring  
Tamil : Vayuvilangam, Vayuvidangam  
Telugu : Vayuvidangalu  
Urdu : Baobarang, Babrang  

**Taxonomical Characters**

The plant is a climber with slender branches and long internodes. The leaves are elliptic, broad and covered with minute glands. The flowers are small, white racemes arranged in panicle inflorescence at the end of the branches. The fruits are berries, round, red to black colour and tipped with style. The seed resembles so much to pepper and often referred as false pepper and pepper is also used to adulterate *Embelia* (Figure 1).
Medicinal Uses

*Embelia* root and bark is acrid, astringent, anthelmintic, antifertility, antioestrogenic, carminative, digestive, laxative, soothing, stimulant, stomachic and thermogenic. It is used in treating intestinal parasites and works, abdominal disorders, skin fungal infections, flatulence, constipation, headache, hemorrhoids, lung diseases, obesity, piles, pneumonia, mouth ulcers, toothache and sore throat. Its decoction is useful in insanity and heart diseases.

Leaves possess astringent, thermogenic, demulcent and depurative properties. They are useful in skin diseases and leprosy.

*Embelia* fruits are acrid, astringent, anthelmintic, bitter, brain tonic, carminative, contraceptive, diuretic, febrifuge, laxative, stimulant and thermogenic. They are useful in leprosy, nervous debility, dyspepsia, flatulence, colic, tumors, asthma, fever, ascaris infestation, general debility and skin diseases. The fruit paste is used as a mouth wash to combat cavities, and also applied externally for skin related problems. The root decoction is taken for treating insanity and heart diseases.

*Chonemorpha fragrans* (Moon) Alston

Taxonomical Classification

- **Kingdom**: Plantae
- **Phylum**: Division
- **Class**: Angiospermae
- **Order**: Gentianales
- **Family**: Apocynaceae
- **Genus**: *Chonemorpha*
- **Species**: Fragrans
**Regional Names**

- Sanskrit : Murva, Morata
- Hindi : Garbhedaro
- Kannada : Manjinaru
- Telgu : Chaga
- Malayalam : Perunkurumpa

**Taxonomical Characters**

Woody climbers, branchlets tomentose. Leaves simple, 20-25 x 15-22 cm, broadly elliptic or ovate-orbicular, apex shortly acuminate, base cordate, pubescent above and tomentose beneath. Flowers 6-8 cm across, white, in terminal tomentose paniculate cymes. Calyx 1 cm long, glabrous, glandular within; lobes ovate, acute. Corolla fairly large, salver shaped, lobes longer than the tube, obovate, cuneate, rounded at apex. Stamens included anthers lanceolate, sagitate, disc copular, carpels distinct, ovules many, stigma bifid. Follicles to 30 x 2 cm, subtetragonal; seeds 2 cm long, coma 4.5 cm long, white (Figure 2).

**Medicinal Uses**

*Chonemorpha fragrans* is an endangered medicinal plant. It is used in different preparations, such as sudarsanasavam and Kumaryasavamin Ayurvedic System. Phytochemical investigations have revealed the presence of steroidal alkaloids, such as chonemorphine and funtumafrine in *C. fragrans* Camptothecin, a well-known anticancer alkaloid has been detected in ethanolic extracts of stem with bark and callus cultures derived from *C. fragrans*. The plant has a variety of pharmacological activities such as antiamoebic, antipyretic, antidiabetic, antiparasitic, anthelmentic, anticancer, HIV disorder, skeletal muscle relaxant and gynaecological disorder.
**Microscopic Characters of *Embelia ribes* leaves**

Thin transverse section of the leaf was taken for microscopic examination. Microscopic examination shows the presence of upper and lower epidermis. Stomata were viewed only on the lower epidermis. Mesophyll cells were well differentiated into palisade and spongy parenchyma, Pallisade parenchyma was seen under upper epidermis which is packed without any intercellular space.

Both upper and lower epidermis is made up of a single layer of cells that are closely packed. The cuticle on the upper epidermis was thicker than that of lower epidermis. Stomata are more in number on the lower epidermis than on the upper epidermis (Plate 3).

Below the epidermis, vertically elongated cylindrical cells in one or more layers without intercellular spaces form palisade parenchyma. Below palisade parenchyma towards lower epidermis spongy parenchyma cell was loosely arranged with numerous air spaces.

**Thin transverse cross sections of the midrib region of the *Embelia ribes***

Vascular bundles are seen very close without much intercellular space. Xylem is present towards the upper epidermis, while the phloem towards the lower epidermis. Phloem fibres are absent. Tracheids and xylem fibres are also not seen (Plate 3).

**Microscopic characters of *Chonemorpha fragrans* root**

Cortex consists of extensive vessel clusters including few wide and many narrow vessels, axial parenchyma diffuse-in-aggregates and scanty paratracheal, oval or rounded loosely arranged parenchymatous cells are observed. These cells may store food reserves. Vascular tissues are seen in radial arrangement. Xylem and phloem are separated by conjunctive tissue (Plate 3).
**Fluorescence analysis**

Fluorescence analysis of *Embelia ribes* leaf powder and root powder of *Chonemorpha fragrans* are carried out after treated with different chemical compounds. The fluorescence characteristics of *Embelia ribes* leaf powder and *Chonemorpha fragrans* root is displayed in Table 2.

*Embelia ribes* - Leaf

The leaf powder treated with acetone and petroleum ether showed light green and green colour in normal light, dark green colour in 265 nm and 365 nm of UV light respectively. Methanol and benzene showed light green colour observed under normal light and green colour observed in 265 nm and 365 nm of UV light respectively. Pale brown and pale yellow colours observed under normal light, light green colour observed under 265 and 365 nm of UV light when treated with chloroform and ammonia. Acetic acid and sulphuric acid treated powder showed light green and pale yellow colours under normal light and green and light green observed under 265 nm and 365 nm of UV light respectively. Ethanol, nitric acid and iodine treated powder showed light green, brown and red colours observed under normal light and green, black and dark green colours observed under 265 nm and 365 nm of UV light respectively. When leaf powder treated with 0.1 N HCL, 0.1 N NaOH and Dis. Water showed pale yellow, pale brown and Light yellow under normal light but 265 nm and 365 nm of UV light exhibited Light green colour. Brown and pale brown colour showed under normal light of Formaldehyde and Powder and Green colour showed under UV light of 265 nm and 365 nm respectively.

*Chonemorpha fragrans* (Root)

Acetone, petroleum ether treated powder showed Pale yellow and light yellow under normal light and light green observed under 265 nm and 365 nm of UV light. Yellow, pale yellow and pale brown colours reflected when the powder treated methanol, benzene and
chloroform under normal light also green and light green colours observed under 265 nm and 365 nm of UV light. Ammonia and acetic acid treated powder showed yellow and light yellow colours under normal light and light green colour under 265 nm and 365 nm of UV light respectively. Sulphuric acid, ethanol, nitric acid and iodine treated powder showed brown, light yellow, yellow and red colours under normal light and dark green colour showed under 265 nm and 365 nm of UV light. 0.1 N HCl and 0.1 N NaOH treated powder showed Light yellow and Pale yellow colour under normal light reflected light green colour showed under UV light of 265 nm and 365 nm respectively. Distilled water, formaldehyde treated powder showed yellow, pale yellow and yellow colours under normal light and green colour observed under 265 nm and 365 nm of UV light respectively.

The ash values such as total ash, water soluble ash, acid insoluble ash, sulphate ash, alcohol soluble extractive value and water soluble extractive value of *Embelia ribes* and *Chonemorpha fragrans* are determined and the results shown in Table 3 & Figure 3.

Total ash value of *Embelia ribes* is 84.9%, water soluble ash, acid insoluble ash and Sulphate Ash are 12.9, 72.4, 34.2%. Alcohol soluble Extractive and Water soluble Extractive Value are 45.6% and 67.9% respectively.

Total ash value of *Chonemorpha fragrans* is 92.7%, water soluble ash, acid insoluble ash and sulphate ash are 56.2, 12.8 and 12.7%. Alcohol soluble extractive and water soluble extractive value are 30.0% and 15.5% respectively.

The physical constant evaluation of the drug is an important parameter in detecting aduteration or proper handling of drugs. The moisture content of the drug is not too high. Thus it could avoid bacteria, fungi and yeast growth as the general requirement for moisture content in crude drug should not be more than 14% (African pharmacopoeia, 1986). Determination of ash value and acid insoluble ash value in crude drug is very important. The presence or absence of foreign organic matter such as metallic salts or silica particularly
important in the evaluation of purity of drugs. Total ash value of *Embelia ribes* is 84.9% and *Chonemorpha fragrans* 92.7%. The pharmacognostic constant of plants, the diagnostic microscopic features and standards reported could be useful for the compilation of a suitable monograph for their proper identification.

Total extractive values of *Embelia ribes* are represented in the Table 4 & Figure 4. The *Embelia ribes* consist of following extractive values in different solvents Petroleum ether, Benzene, Chloroform and Ethanol are analysed. The extractive value of Petroleum ether is 0.385 mg/g, the extractive value of benzene is 0.766 mg/g, the extractive value of chloroform is 0.205 mg/g and the extractive value of ethanol is 0.834 mg/g respectively.

Total extractive values of *Chonemorpha fragrans* are represented in the Table 4 & Figure 2. The *Chonemorpha fragrans* consist of following extractive values in different solvents Petroleum ether, Benzene, Chloroform and Ethanol were analysed. The extractive value of Petroleum ether is 0.922 mg/g, the extractive value of benzene is 0.342 mg/g, the extractive value of chloroform is 0.671 mg/g and the extractive value of ethanol is 1.023 mg/g respectively.

Extractive value plays an important role in the evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage. Ether soluble extractive value signifies the presence of amounts of fats, lipids and some steriods in the drug.

Comparing the petroleum ether soluble, benzene soluble, chloroform soluble and ethanol soluble extractive values of the drugs and formulations, it is concluded that the amount of ethanol soluble extractive values are higher than the other three (*Embelia ribes*–0.834 mg/gm and *Chonemorpha fragrans*–1.023 mg/gm). This indicates the presence of more amount of Ethanol soluble contents in the plants.
Preliminary Phytochemical screening

Screening results indicated the beneficial phytoconstituents like carbohydrates, anthocyanin, alkaloids, steroids, tannins, saponins, flavonoids, quinones, glycosides, cardiac glycoside, terpenoids, phenols, protein, anthraquinone, phlobatannins and coumarins.

*Embelia ribes* (leaf)

Preliminary phytochemical screening of leaf extracts of *Embelia ribes* is displayed in Table 5. Carbohydrates, saponins and cardiac glycosides are present in petroleum ether, benzene, chloroform and ethanol extracts, phenols, coumarins and flavonoids are present in petroleum ether, benzene and ethanol extracts. Steroids and phlobatannins are present in petroleum ether and ethanol extracts. Anthocyanin is present in benzene, ethanol and aqueous extracts. Alkaloids are present in petroleum ether, ethanol and chloroform extracts. Quinones are present in chloroform, ethanol and aqueous extracts. Terpenoids are present in chloroform and ethanol extracts. Tannins are present in all extracts and glycosides, protein and anthraquinone are not present in any extracts.

*Chonemorpha fragrans* (root)

The preliminary phytochemical screening result of root extract of *Chonemorpha fragrans* is shown in Table 6. Carbohydrates, cardiac glycosides and terpenoids are present in petroleum ether, benzene, chloroform and ethanol extracts. Glycosides, protein and anthraquinone are not present in any extracts. Anthocyanin is only present in ethanol extract. Alkaloids, steroids and flavonoids are present only in three extracts. Tannins are present in petroleum ether and ethanol extracts. Saponins are present in petroleum ether and chloroform extracts. Phenol is present in petroleum ether and ethanol extracts. Quinones and coumarins are present in petroleum ether and ethanol extracts only.

The presence of sugars, phenolic compounds flavanoids and saponins were established in anticarcinogenic, insecticidal, antifungal and antibacterial (Middleton, 1998;
Eastwood, 1999, Joshy et al., 2008; Pattanaik et al., 2002) pathogens (Okwu and Okwu, 2004). Polyphenols are the major plant secondary metabolites and represent the most studied phytochemicals. A number of polyphenols form medicinal plants have been shown to protect body against effects of oxidative stress. These phytochemicals act as free radical scavenging, reducing and metal chelating substances. Phenols have powerful antioxidant activities (Lau et al., 2005; Harafi and amrani, 2008 and Zbarsky et al., 2005, kris-Etherton et al., 2002).

The presence of phenols is considered to be potentiality toxic to the growth and development that lead to the development of malignant tumours, may play a role in inactivating carcinogens and inhibiting the expression of mutagens (Okwu, 2004).

The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulphydryl groups or through more nonspecific interactions with the proteins (Mason & Wasserman, 1987). Phenolic compounds possessing a C3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and often cited as antimicrobial as well. Eugenol is a well-characterized representative found in clove oil. Eugenol is considered bacteriostatic against both fungi (Duke, 1985) and bacteria (Thomson, 1978).

Flavonoids are a large group of polyphenolic compounds that have been used for a long time to exert diverse biological effects. Their wide range of biological and pharmacological activities include antioxidant, cytotoxic, anticancer, hepatoprotective, antibacterial and antimicrobial (Harborne and Williams, 2000). Nowakowska, 2007, Havsteen, 2002 and Forie, 2008).

Flavonoids may also have a beneficial effect through their impact on the bioactivation of carcinogens. Most chemical carcinogens require transformation by phase I metabolizing enzymes into a more reactive form able to bind to DNA. If the resulting mutation is not
repaired, it may initiate or promote the carcinogenesis process. The reactive chemical group introduced by phase I enzymes (or the original carcinogen) can be detoxified through conjugation by phase II metabolizing enzymes into a water-soluble compound which can then be eliminated from the body. A cancer protective effect from plant-derived foods has been found with uncommon consistency in epidemiologic studies. However, it has been difficult to identify specific evidence for the beneficial action of flavonoids on multiple cancer-related biological pathways (carcinogen bioactivation, cell-signaling, cell cycle regulation, angiogenesis, oxidative stress, inflammation). Although the epidemiologic data on flavonoids and cancer are still limited and conflicting, some protective associations have been suggested for flavonoid-rich foods (soy and premenopausal breast cancer; green tea and stomach cancer; onion and lung cancer).

Plants rich in saponins generally have immune boosting and antiinflammatory abilities (Kenner and Requena, 1996). Tannins are complex phenolic polymers, which can bind to proteins and carbohydrates resulting in reduction in digestability of these macromolecules, and thus inhibition of microbial growth. Tanins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sodipo et al., 1991). The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins (Chung et al., 1998).

In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins (Stern et al., 1996), often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinones antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesive, cell wall polypeptides, and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganisms.
Pure, isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodiac and bactericidal effects (Stray, 1998). Quinine with a molecular formula of C$_{20}$H$_{24}$N$_2$O$_2$ is an anti-malarial drug extracted from the bark of a cinchona tree (C. succirubra). Quinine is highly valued in the treatment of unusually resistant strains of malaria. The first medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy Papaver somniferum (Fessenden & Gessenden, 1982), the name morphine comes from the Greek Morpheus, god of dreams. Codeine and heroin are both derivatives of morphine.

Furthermore alkaloids are often toxic to man and many have dramatic physiological activities, hence their wide use in medicine for the development of drugs (Harbone, 1973; Okwu, 2005).

Cardiac glycosides are a class of organic compounds that increase the output force of the heart and decrease its rate of contractions of acting on the cellular sodium – potassium ATPase pump (Patel, 2016). Their beneficial medicinal uses are as treatments for congestive heart failure and cardiac arrhythmias; however their relative toxicity prevents them from being widely used (Ambrosy et al., 2014).

Most commonly found as secondary metabolites in several plants such as foxglove plants, these compounds nevertheless have a diverse range of biochemical effects regarding cardiac cell function and have also been suggested for use in cancer treatment (Riganti et al., 2011).

**Quantitative estimation of phytochemical compounds**

The quantitative estimation of different phytochemical compounds such as, carbohydrates, lipids, protein, phenol, flavonoid, amino acids, oxalate, alkaloids and phytates in Embelia ribes and Chonemorpha fragrans are investigated and their results are represented in the Table 7.
The leaf of the *Embelia ribes* exhibited different amount of phytochemicals. The content of Carbohydrates is 41.3 mg/g/FW, Percentage of lipids are 22.5%, the content of Protein is 4.6 mg/g/FW, The Phenol content is 17.5 mg/g/FW, Percentage of Flavonoid is 42.5%, Free Amino acids contents are 43.3 mg/g/FW, Percentage of Oxalate is 9.0%, the Percentage of Alkaloids are 30.3%, Percentage of Phytates are 11.3%

The root of the *Chonemorpha fragrans* exhibited different amount of phytochemicals. The content of Carbohydrates is 54.6 mg/g/FW, Percentage of lipids are 12.1%, the content of Protein is 12.0 mg/g/FW, The Phenol content is 10.4 mg/g/FW, Percentage of Flavonoid is 80%, Free Amino acids contents are 45.3 mg/g/FW, Percentage of Oxalate is 29.3%, the Percentage of Alkaloids are 50.2%, Percentage of Phytates are 55.4% respectively.

The present study has shown that the usefulness of the chosen plants for medicinal purposes. Leaves of *Embelia ribes* and root of *Chonemorpha fragrans* are the potential sources of useful drugs due to their rich contents of phytochemical such as flavanoids, saponins, tannins, quinones, alkaloids, polyphenols and cardiac glycosides.

**Estimation of Chlorophyll and Carotenoids**

The photosynthetic pigments of the leaf of *Embelia ribes* are also studied. Chlorophyll-a content is 0.479 mg/g, Chlorophyll – b content is 0.381 mg/g and the Total Chlorophyll content is 0.991 mg/g, the content of Carotenoides 58.757 mg/g (Table 8).

The photosynthetic pigments content of the bark of *Chonemorpha fragrans* are also studied. Chlorophyll-a content is 0.049 mg/g, Chlorophyll – b content is 0.038 mg/g and the Total Chlorophyll content is 0.1008 mg/g, the content of Carotenoides 15.975 mg/g.

**FT-IR**

FT-IR spectral values reveal the composition of solids, liquids and gases. The most common use is in the identification of unknown materials and confirmation of production materials. FT-IR stands for Fourier Transform Infrared, the preferred method of infrared
spectroscopy. The usefulness of infrared spectroscopy arises because different chemical structures (molecules) produce different spectral fingerprints.

FT-IR analysis peak values and functional groups of *Embelia ribes* are represented in Figure 5 & Table 9. The FT-IR Spectrum confirmed the presence of functional groups such as N-H Amines, O-H Alcohol, C-H Alkane, C=N Oxime (≡NOH), S=O Sulfate, S=O Sulfate, S=O Sulfone, P=O Phosphoramid, C=S Thiocarbonyl, Si-OR Silane and S-OR Esters which shows the following peaks at 3856.32, 3775.68, 3354.58, 2973.68, 2888.56, 1927.94, 1657.58, 1448.72, 1380.30, 1327.13, 1273.82, 1086.47, 1044.35 and 879.31 respectively.

FT-IR analysis peak values and functional groups of *Chonemorpha fragrans* are represented in Figure 6 & Table 10. The FT-IR Spectrum confirmed the presence of functional groups such as N-H Amines, O-H Alcohol, C-H Alkane, C=N Oxime, S=O Sulfate, S=O Sulfone, P=O Phosphoramid, C=S Thiocarbonyl, Si-OR Silane, S-OR Esters which shows the following peaks at 3856.46, 3775.67, 3355.69, 2973.63, 2887.55, 1927.37, 1657.73, 1449.46, 1380.30, 1327.00, 1273.66, 1086.49, 1044.32 and 879.34 respectively.

FT-IR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000 – 6000 cm\(^{-1}\).

The background emission spectrum of the IR source is first recorded, followed by the emission spectrum of the IR source with the sample in place. The ratio of the sample spectrum to the background is directly related to the samples absorption spectrum. The resultant absorption spectrum from the bond natural vibration frequencies indicates the presence of various chemical bonds and functional groups present in the sample.
FT-IR is particularly useful for identification of organic molecular groups and compounds due to the range of functional groups, side chains and cross links involved, all of which will have characteristic vibrational frequencies in the infra-red range. The result of the present study shows the presence of the functional groups such as amines, alcohol, alkane, oxime, sulfate, sulfone, phosphoramide, thiocarbonyl, silane and esters. It indicates the medicinal property of *Embelia ribes* and *Chonemorpha fragrans*.

**Thin Layer Chromatography TLC**

TLC analysis of all the fractions using different solvent system revealed the presence of promising spots. Thin Layer Chromatography can be used for purification, isolation and identification of natural products like volatile oil, waxes, terpenes, alkaloids, glycosides, steroids etc.

In the present investigation petroleum ether, benzene, chloroform, ethanol extracts of leaves and roots of *Embelia ribes* and *Chonemorpha fragrans* are studied. The plates are exposed to visible light (normal light) and then viewed through UV light and observed under iodine to observe the various colored bands.

**Solvent system I- Benzene: Chloroform (1:4)**

Thin layer chromatography studies of the petroleum ether extract of *Embelia ribes* leaf extract shows 7 spots under normal light with the Rf values of 0.04, 0.07, 0.07, 0.1, 0.11, 0.14 and 0.2. Rf values of spots detected in UV light are 0.05, 0.1, 0.15 and 0.45 and under iodine chamber were 0.05, 0.05, 0.1, 0.11 and 0.27 (Table 11 & Plate 4).

*Chonemorpha fragrans* extract shows 4 spots under normal light with the following range of Rf values 0.1, 0.12, 0.17 and 0.18. Rf values 0.07, 0.08, 0.11 and 0.31 are observed under UV light and 4 spots obtained from Iodine chamber with the range of the following Rf values 0.12, 0.12, 0.1 and 0.22 (Table 12 & Plate 4).
Solvent system II - Chloroform: Ethyl alcohol (4.75:0.25)

TLC studies of the benzene extracts of *Embelia ribes* extract shows the following results (Table 13). Under normal light 5 spots are observed with the following Rf values 0.05, 0.11, 0.12, 0.28 and 0.3. Under UV light 4 spots are detected and their Rf values are 0.11, 0.12, 0.27 and 0.32. Five spots are obtained under Iodine chamber and their Rf values are 0.04, 0.12, 0.17, 0.21 and 0.31.

*Chonemorpha fragrans* extract shows 5 spots under normal light and their Rf values are 0.07, 0.15, 0.17, 0.18 and 0.18. Four Rf values 0.08, 0.12, 0.15 and 0.32 are observed under UV light. Under Iodine 5 spots are obtained having Rf values of 0.07, 0.11, 0.14, 0.18 and 0.21 (Table 14 & Plate 5).

Solvent system III-Chloroform: Ethyl alcohol (4:1)

Chloroform extract of *Embelia ribes* leaves shows different results under different light. Under normal light 4 spots are observed with the Rf values of 0.04, 0.07, 0.15 and 0.18. Under UV light four spots are detected and their Rf values are 0.1, 0.18, 0.21 and 0.25. Three spots are obtained under Iodine chamber having the Rf values of 0.22, 0.3 and 0.42 (Table 15).

*Chonemorpha fragrans* extract showed five spots under normal light and their Rf values are 0.02, 0.14, 0.15, 0.21 and 0.21. Under UV light five spots were detected with the Rf values of 0.05, 0.11, 0.15, 0.17 and 0.21. Iodine chamber of TLC plates showed four spots with the Rf values of 0.04, 0.17, 0.24 and 0.27 (Table 16 & Plate 6).

Solvent system IV-Chloroform: Ethyl alcohol (2:3)

TLC studies of the ethanol extract of *Embelia ribes* extracts showed the following results (Table 17). Four spots under normal light with the Rf values of 0.11, 0.15, 0.18 and 0.27. Under UV light 4 spots are detected and their Rf values are 0.12, 0.17, 0.24 and 0.27. Under Iodine 4 spots are obtained having Rf values of 0.08, 0.2, 0.24 and 0.28.
In the *Chonemorpha fragrans* extract four spots were observed under normal light with the Rf values of 0.15, 0.15, 0.2 and 0.28. Under UV light, three spots are detected with the Rf values of 0.18, 0.3 and 0.38. Under Iodine chamber five spots are obtained and having Rf values of 0.07, 0.14, 0.14, 0.18 and 0.32 (Table 18 & Plate 7).

Petroleum ether, benzene, chloroform and ethanol extracts of leaves of *Embelia ribes* and root of *Chonemorpha fragrans* are used in the present investigation. Retention factor value shows presence of numerous compounds in different extracts. The related appearances of different colors in normal light, U.V. and Iodine by extracts reveals the compounds present in it (Rf values varies from 0.07 to 0.42). The TLC pattern can be used as adjunct to the physio chemical parameters to the pharmacopoeial standards.

**HPLC Analysis**

HPLC is also known as High-Pressure Liquid Chromatography. This separates compounds on the basis of their interaction with solid particles of a tightly packed column and the solvent of the mobile phase. High pressures of up to 400 bars are required to elute the analyte through the column before they pass through detector. HPLC is useful for compounds that cannot be vapourised or that decompose under high temperatures. HPLC provides both quantitative and qualitative analysis in a single operation.

The ethanol leaf extract prepared by hot extraction is also subjected to HPLC for the separation and identification of constituents present in the *Embelia ribes*. Two following compounds are separated at different retention times of 2.103 min and 2.750 min. The profile displayed one prominent peak at the retention time of 2.103 min followed by one moderate peak is also observed at the retention time of 2.750 min. (Figure 7 and Table 19). The samples shown characteristic peaks of embelin at the same that of standard embelin. In the earlier studies, embelin is isolated from the fruits of *Myrasine africana* L. using methods like high performance liquids chromatography (HPLC) and HPTLC, Paul *et al.*, (2007)
The ethanol root extract of *Chonemorpha fragrans* prepared by hot extraction is subjected to HPLC for the separation and identification of constituents present in the *Chonemorpha fragrans*. Four following compounds were separated at different retention times of 2.080 min, 2.757 min, 3.173 min and 3.427 min. The profile displayed only one prominent peak at the retention time of 2.080 min followed by three moderate peaks are also observed at the retention times of, 2.757 min, 3.173 min and 3.427 min. (Figure 8 and Table 20). HPLC analysis showed the presence of peak having same retention time as that of pure camptothecin in the ethenolic extract of root.

**GC-MS**

The results pertaining to GC-MS analysis of the ethanolic extract of *Embelia ribes* and *Chonemorpha fragrans* lead to the identification of a number of compounds. These compounds are identified through mass spectrometry attached with GC. The Retention time, Name of compound, Molecular Formula, Molecular Weight and Structure and Peak area (%) are also presented.

GC-MS spectrum of *Embelia ribes* showed 3 different major peaks which indicated the presence of 3 compounds. The compounds are Cyclopropane, 1,1-dimethyl-(9.06%), Toluene(82.56%) and 1,3,6-Octatriene, 3,7-dimethyl-, (Z)-(8.38%). The spectrum profile of GC-MS confirmed the presence of three major components with retention time 1.757min, 1.927min and 3.828min respectively (Table 21 & Figure 9).

Cyclopropane, 1,1-dimethyl compounds are used as anti asthamatics, Bronchodilators, Drugs for disorders of the urinary tract, Drug for gential or sexual disorders. 1,3,6 Octatriene, 3,7- dimethyl (z) –act as plant defence and have antifungal properties.
GC-MS spectrum of *Chonemorpha fragrans* shows 3 different major peaks which indicated the presence of 3 compounds. The compounds are 3,3-Dimethyl-1,2-epoxybutane(4.33%), Toluene(91.89%), 1,3,6-Octatriene, 3,7-dimethyl-, (Z)-(3.78%). The spectrum profile of GC-MS confirmed the presence of three major components with retention time 1.757min, 1.927min and 3.828min respectively (Table 22 & Figure 10).

3,3 – Dimethyl – 1,2 – epoxybutane is used as antiabortive agents, drugs for dermatological disorders, for treating wounds, ulcers and burns for joint disorders-arthritis and anti-asthmatics.

**Antimicrobial Activity Studies**

The antimicrobial activity of different solvent leaf extract of *Embelia ribes* and *Chonemorpha fragrans* are tested against *Staphylococcus aureus, Shigellasonnei, Vibrio cholera, Klebsiella pneumoniae* and *Streptococcus pyogenes* pathogenic bacteria shown in Plate 8, and fungi such as *Candida albicans, Candida tropicalis, Candida parapsilosis, Aspergillus niger* and *Aspergillus flaves* shown in plate 10.

**Anti-bacterial Activity**

Among the different solvent extracts petroleum ether and ethanol extracts of leaves of *Embelia ribes* shows maximum zone of inhibition compared to other solvent extracts. Maximum zone of inhibiton (7 mm) observed against *Staphylococcus aureus* in petroleum ether extract. Ethanol extracts showed remarmable zone of inhibition against *Staphylococcus aureus* and *Steptococus pyogens* (6.5 mm). Benzene and chloroform and aqueous extracts showed minimum activity against selected microorganisms. Overall all the extracts shows some activity against microorganisms (Table 23 & Figure 11).

The root extracts of *Chonemorpha fragrans* shows a specific activity in different solvent extracts against all the bacteria. Among all, maximum zone of inhibition is observed in Ethanol extract (7 mm) against *Streptococcus pyogenes*. Chloroform (6mm) Benzene
(4.5mm) and petroleum ether (4mm) extracts showed moderate activity against streptococcus pyogens. Benzene extract shows minimum zone of inhibition (1.5 mm) against Klebsiella pneumoniae (Table 24 and figure 12).

Antifungal activity

Antifungal activity of Embelia ribes is evaluated on eight different fungal species by employing various concentrations of seed extract (0.5-2.0 mg). All the concentrations of plant extract inhibited the fungal growth, whereas maximum activity was observed at 2.0 mg concentration of seed extract. Among different doses, the diameter of inhibition zones ranged from 9 to 18mm in various fungal species and increased with the increase in the concentration of test solution. Among all the fungi, high inhibition zones are observed in Aspergillus flavus (15 mm). This is followed by Aspergillus niger and Candida tropicalis (12 mm), Candida albicans (11 mm) of ethanol extract. Colletotrichum capsici (17 mm), Aspergillus niger (16.5 mm), Rhizopus oryzae (16.5 mm), respectively. Aspergillus terreus and candida albicans showed less inhibition zones (15.5 and 16.0 mm) compared to other organisms (Rani et al., 2011).

The ethanol extract of Embelia ribes (leaves) shows a high degree of inhibition against Aspergillus flavus followed by Aspergillus niger and Candida albicans. Chloroform extract also showed remarkable inhibition against Aspergillus flavus followed by Candida tropicalis. Aqueous, Benzene and Petroleum ether extracts did not show any antifungal activity (Figure 13, Table 25 & Plate 10).

The Ethanol extract of Chonemorpha fragrans (root) shows inhibition against Aspergillus flavus followed by Candida tropicalis, Aspergillus niger and Candida albicans. Chloroform and benzene extracts showed a good antifungal activity. Petroleum ether and aqueous extracts did not show any antifungal activity (Figure 14, Table 26 & Plate 11).
The broad spectrum of antimicrobial activity found in the present study may be due to the presence of secondary metabolites of various chemical types in the plants. Among the chosen, ethanolic and petroleum ether extracts showed a greater activity than other solvents. This may be due to the ethanolic and petroleum ether extracts more in phytochemicals than the Benzene, Chloroform and aqueous extracts.

This study has confirmed the antimicrobial potential of the plant and thus supporting its promising possibility of finding new clinically effective natural source of bioactive compounds. The presence of a broad spectrum of antibiotic compounds might be the reason for the antimicrobial activity of *Embelia ribes* and *Chonemorpha fragrans*.

In addition, the effectiveness of plant was not due to one main active constituent, but to the combined action of other chemical compounds involved in it Essawi and Srous (2000). Several types of tannins, flavonoids, saponins, and phenolic compounds have been reported to have antimicrobial properties (Vandeek *et al.*, 1985, Bader *et al.*, 1987).

The previous report on antimicrobial activity studies of this plant also supported present results. Chitra *et al.*, (2003) reported that embelin (100 µg) exhibited significant antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes, Shigella flexneri, Shigella sonnei* and *Pseudomonas aeruginosa*. Feresin *et al.*, (2003) reported that embelin inhibited both methicillin sensitive and methicillin-resistant strains of *Staphylococcus aureus* with MICs of 250 and 62 µg/ml, respectively.

In a study conducted by Radhakrishnan *et al.*, (2011) the antimicrobial activity of embelin extracted from *Embelia berries* was carried out. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of embelin against both Gram +ve and Gram-ve bacteria were studied using micro dilution method and agar plate method by sub-culturing 10 µl of the test dilutions from MIC tubes on to fresh Mueller-Hinton agar plates. About 1.9± 0.1 gram of pure embelin was obtained from 100 gram of powdered...
berries (*E. ribes*). The characteristics studied reveal that properties are on par with the standard embelin received from Sigma (USA). With regard to antibacterial activity, embelin showed bactericidal activity against Gram +ve organisms, and bacteriostatic against Gram –ve organisms. Thus, embelin finds application as potent antibacterial agent.

In the present study, extracts of *Embelia ribes* and *Chonemorpha fragrans* offers a remarkable antimicrobial activity against Gram positive and Gram negative organisms. These findings support the traditional knowledge of the local users and its preliminary scientific validation for the use of these plants for antimicrobial activity. Based on the results obtained from *Embelia ribes* and *Chonemorpha fragrans*, it is found to be an effective antimicrobial agent which can be used in various products including drugs, cosmetics and other products. This investigation has opened up the possibility of the use of *Embelia ribes* and *Chonemorpha fragrans* in drug development for human consumption and possibility for the treatment of microbial infections.

**Anticancer Activity studies:**

Medicinal plants have been traditionally used in folk medicine for centuries as natural healing remedies with significant proven therapeutic effects in many areas including prevention of cardiovascular diseases and anti-inflammatory, antimicrobial, and anticancer activity. In addition, the emergence of resistance to cancer chemotherapy has forced researchers to turn to natural products of plant and marine origin. Although many compounds isolated from plants are being rigorously tested for their anticancer properties, it is becoming increasingly recognized that the beneficial effects of plants are due to a complex interplay of the composite mixture of compounds present in the whole plant (additive/synergistic and/or antagonistic) rather than constituent single agents alone (Liu, 1998).

The present investigation mainly focused on the anticancer activity of methanolic extracts of leaves of *Embelia ribes* and roots of *Chonemorpha fragrans*.  

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In the present study, *in-vitro* cytotoxicity effects of leaf extracts of *Embelia ribes* is carried out with various concentrations for the following cancer cell lines U87 (brain cancer cell line), Hep G2 (human liver cancer cell line) and MCF7 (breast cancer cell line). The leaf of the plant was collected from Kerala and shade dried powdered and extracted with methanol solvent. Five different concentrations (0.4 µg/ml, 2 µg/ml, 10 µg/ml, 50 µg/ml and 250 µg/ml) of leaf extracts are used to study the cytotoxicity potential of the plant. The cytotoxicity potential of various concentrations of methanolic extracts with LC 50 and LC 90 values of *Embelia ribes* is displayed in Table 27, Figure 15, Plate 8. The results revealed that the cytotoxicity rate is increased when the concentrations of leaf extract increases. MTT assay measured the cell viability based on the reduction of yellow tetrazolium MTT to a purple formazan dye mitochondrial dehydrogenase enzyme. So, the amount of formazan produced reflected the number of metabolically active viable cells. Among the three cell lines U87 have the highly potent activity followed by MCF7 and Hep G2. But in Hep G2 cell line, the concentration of 250 µg/ml revealed more activity than MCF7 cell line. The LC50 and LC 90 values observed for Cell line U87 against methanolic extract of *Embelia ribes* is 13.36µg/ml and 127.98 µg/ml. The values observed in cell lines Hep G2 and MCF7 were 85.58 µg/ml, 49.98 µg/ml and 222.79 µg/ml, 235.79 µg/ml respectively for LC50 and LC 90 concentrations.

The plant has enormous medicinal properties and its various parts are used to cure many diseases. Embelin, a naturally occurring quinonoid compound, is found to be the major constituent of *Embelia ribes* (Rao *et al.*, 1961). Embelin is the anti-cancer compound, so the plant exhibited the potent cytotoxicity against cancer cell lines. Embelin is reported to decrease tumor size and inhibit the increase in activity of serum enzymes, viz. acid phosphatase, τ-glutamyl transferase, lactate dehydrogenase, aldose, etc in rats with experimental fibrosarcoma. Embelin interferes with carbohydrate and amino acid metabolism
in tumor bearing animals (Gupta et al., 1989). Joya and Lakshmi (2010) reported that the crude hexane extract of the fruits of *Embelia ribes* exhibited cytotoxicity against Human leukaemic cells (K562) and Dalton’s Lymphoma ascites cells (DLA). In-vitro studies on Embelin suggest the potential of the compound on those two cell lines. However the compound did not exhibit toxicity on normal lymphocytes isolated from human blood preferentially attacking the tumour cells. Many reports revealed the anticancer potential of Embelia species, which may be due to the presence of phyto constituent Embelin in this plant.

In a study conducted by Srinivasan et al., (2001) the anticancerous activity of *Embelia berries* was done on HBL-100 cell lines using MTT assay. This study has shown the cytotoxic effect of Embelin extracted from the embelia berries. Chitra et al., (1994) reported antitumor activity of embelin in methyl cholangthrene induced fibrosarcoma in albino rats and in addition enhancing their survival time. Coleska et al., (2004) reported embelin as a fairly potent, nonpeptidic, cell-permeable, small-molecule inhibitor of XIAP and represents a promising lead compound for entirely new class of anticancer agents that target the BIR3 domain of XIAP. Dai et al., (2011) reported embelin inhibits chemical carcinogen-induced colon carcinogenesis. Suvarna (2014) studied the in-vitro cytotoxic activity of methanolic extract of Embelia tsjeriam against human breast (MCF7) and colon cancer cell lines (COLO-205) using the Sulfarhodamine B assay. The methanolic extracts inhibiting at least 50% of tumor cell proliferation at dose of 6.25- 400µ g/ml. MCF7 cell lines also inhibited (15- 250 µg/ml) by methanolic extract of *E. ribes* in the present study.

In the present study, in-vitro cytotoxicity effects of root extract of *Chonemorpha fragrans* carried out with various concentrations for the following cancer cell lines U87 (brain cancer cell line). Hep G2 (human liver cancer cell line) and MCF7 (breast cancer cell line). The root of the plant was collected from Kerala and shade dried powdered and
extracted with methanol solvent. Five different concentrations (0.4 µg/ml) of root extracts were used to study the cytotoxicity potential of the plant. The cytotoxicity potential of various concentrations of methanolic extracts with LC 50 and LC 90 values concentrations of methanolic extracts with LC 50 and LC 90 values of *Chonemorpha fragrans* was displayed in Table 28, Figure16 and Plate 9. The results revealed that the cytotoxicity rate was increased when the concentrations of root extract increases. MTT assay measured the cell viability based on the reduction of yellow tetrazolum MTT to purple fromazan produced reflected the number of metabolically active viable cells. Among the three cell lines U87 have the highly potent activity followed by MCF7 and Hep G2. But in Hep G2 cell line, the concentration of 250µg/ml revealed more activity than MCF7 cell line. The LC 50 and LC 90 values observed for cell line U87 against methanolic extract of *Chonemorpha fragrans* was 76.56 µg/ml and 296.33µg/ml. The values observed in cell lines Hep G2 and MCF7 were 51.13 µg/ml 36.32 µg/ml and 175.68 µg/ml, 243.93 µg/ml respectively for LC 50 and LC 90 concentration.

MTT assay done on U87, Hep G2 and MCF 7 cell lines using the, methanol extract of *Chonemorpha fragrans* root have shown anticancerous activity. This may be due to the presence of camptothecin present in the plant extract which is a potent anti-tumour compound. Camptothecins are one of the most important anticancer alkaloids of the 21st century because of their clinical applications against cancer, HIV. *Chonemorpha fragrans* (*C. fragrans*), a liana belonging to family Apocynaceae, shows presence of widely used anticancer compound Camptothecin (CPT). CPT is a modified monoterpenoid indole alkaloid produced by very few species belonging to unrelated orders of angiosperms, especially plants belonging to the families Apocynaceae and Icaceneae. Localization study revealed that out of all the organs studied roots accumulated highest levels of CPT followed by stem and bark.