Phytochemical, Pharmacognostic and Pharmacological aspect on the endemic plant species *Chionanthus mala-elengi* (Dennst.) P.S. Green (Oleaceae)

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By

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6. SUMMARY

Medicinal plants possess different types of phytochemicals that are used for various therapeutic properties. Medicinal products obtained from the natural sources are cost effective, eco friendly with no side effects. Analysis of pharmacological and pharmacognostic properties are helpful in identifying and characterising the herbal drug. Animal based studies confirms the authenticity, accuracy and importance of the drug molecule. It also ensures the useful health impacts in medicinal field.

Chionanthus mala-elengi (Dennst.) P.S. Green is an endemic tree species of peninsular India, Western Ghats belonging to Oleaceae family. It is occasionally near threatened species. These phenomena are taken into consideration to analyse the phytochemical, pharmacognostical and pharmacological properties of *Chionanthus mala-elengi* (Dennst.) P.S. Green and to bring out its medicinal uses. In our literature survey, we have collected the traditional uses of *C. mala-elengi*.

C. mala-elengi is a small ever green tree, leaves are simple, opposite, decussate, elliptic-obovate, exstipulate, glabrous grooved above. Trunk is straight with dark brown in colour, covered with raised corky lenticels. Cyme inflorescence. Flowers are yellowish white in colour, fragrant, bisexual and sessile. The fruit is drupe.

On microscopic evaluation, the leaf was dorsiventral, glabrous absence of hair on either side. The lamina of leaf is divided into upper epidermis, mesophyll and lower epidermis. Vascular bundle is collateral. Leaf constant such as stomatal number and stomatal index were analysed. The stomata is anomocytic type. The stomatal number was 30.33±1.20 and the stomatal index was 26.11±3.89.

The organoleptic properties such as colour, taste, touch and odour were observed in leaf and stem bark of *C. mala-elengi*. Leaves exhibit green colour, bitter taste, coarse touch with characteristic odour whereas in the stem bark, it was dark brown colour, astringent taste, coarse touch with no characteristic odour.

The powder study was carried out in *C. mala-elengi* leaf and stem bark to determine the quality of plant drug that led to safety and efficacy of herbal products. The leaf powder analysis showed the presence of pitted vessels, rosette crystals, stone cells, uniseriate

trichomes, calcium oxalate crystals and parenchyma cells whereas the stem bark showed the presence of calcium oxalate, primes, starch grains, macroscleride and periderm. The Fluorescence analysis of leaf and stem bark powder was conducted using various reagents under day light and UV 254 nm. It showed the characteristic results that could be used for identifying and ascertaining the quality of the crude drug.

Physicochemical analysis such as total ash, water soluble ash, acid insoluble ash, sulphated ash and moisture content (Loss on drying) were determined. From the analysis it was revealed that the leaf parameter values were higher than the stem bark values. The leaf and stem bark powder of *C. mala-elengi* were extracted with increasing polarity order of solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and water. The result suggested that ethanol leaf extract showed maximum extractive value than the other extract.

The presence of different phytocompounds in the leaf and stem bark were assessed by qualitative analysis. From the qualitative analysis result, leaf showed the presence of tannins, phenolic compounds, steroids, sterols, triterpenoids, flavonoids and balsams in chloroform, ethyl acetate, ethanol and water extracts. Qualitative analysis of stem bark extract showed the presence of triterpenoids, alkaloids, flavonoids were present in ethyl acetate, ethanol and water extracts. Saponins, tannins, phenolic compounds were present in petroleum ether, ethyl acetate, ethanol and water extracts.

The amount of phytocompounds such as phenolic compounds, flavonoids, tannins, alkaloids and saponins were tested by using quantitative method. From the result, it was revealed that the ethanolic leaf extract showed the maximum amount of tested phytochemicals than the other sample extracts.

Antioxidant activity of leaf and stem bark was investigated by using various assays such as DPPH free radical scavenging assay, Nitric oxide radical scavenging assay, Superoxide radical scavenging assay, Hydrogen peroxide scavenging assay and Total antioxidant capacity. Free radical scavenging mechanisms were estimated to be increasing with increase in extract concentration 10 µg/ml to 50 µg/ml. Antioxidant capacity of the extracts were expressed in terms of IC_{50} value and low IC_{50} value corresponds to a high

antioxidant activity. The results revealed that among the test samples, ethanolic leaf extract showed lower IC_{50} value in all the tested antioxidant assay. From the result, it was clearly observed that the ethanol leaf extract possess potential antioxidant activity than stem bark.

The result of antimicrobial activity of ethanol leaf extract showed the highest zone of inhibition against tested microorganism with *Bacillus cereus* (18.0±1.53 mm), *Staphylococcus aureus* (17.33±0.98 mm), *Citrobacter freundii* (17±1.15 mm), *Klebsiella pneumoniae* (16.33±1.86 mm), *Aspergillus niger* (30±1.15 mm), *Aspergillus flavus* (14.33±0.33 mm), *Candida albicans* (15±1.73 mm). Ethanol leaf extract inhibits maximum tested microbes than the other sample extracts.

The FT-IR analysis of *C. mala-elengi* showed broad spectrum of various position indicating the special evidence of the compounds present in ethanolic leaf and stem bark extract. Similar functional groups like alcohol, phenol, H bonded alcohol, alkane, alkene, alkyl halide, nitro compound, carboxylic acid, ether and ester are exhibited. The GC-MS analysis of ethanol leaf and stem bark extract exhibit similar compounds like 7,9-Di-tert butyl-1-oxaspiro (4,5) deca-6,9-diene-2-8-dione, Phytol and Ar-tumerone.

Antidiabetic potential of ethanolic leaf extract of *C. mala-elengi* showed significant inhibitory activity on α-amylase at the concentration of 25 µg/ml showed 14.5±0.69% and at the concentration of 200 µg/ml showed 67.22±1.40%. The concentration required for 50% inhibition (IC-50) was found to be 134±0.58 whereas the inhibitory activity on α-glucosidase at the concentration of 25 µg/ml showed 17.32±0.56% and at the concentration 200 µg/ml showed 69.28±0.98%. The concentration required for 50% inhibition (IC_{50}) was found to be 129.33±0.33 µg/ml. Our results strongly suggest that the ethanol leaf extract can be used as potential antidiabetic agent.

MTT calorimetric assay against HepG2 cells using the ethanol leaf extract of *C. mala-elengi* was conducted. The assay determines the cell cytotoxicity. Ethanolic leaf extract treated human hepato carcinoma (HepG2) cell lines showed significant inhibitory activity at the concentration of 10 µg/ml at 94±0.29% and at the concentration of 50 µg/ml it exhibited 28±0.46%. The IC_{50} concentration (The concentration of the extract have the capacity to kill 50% of viable cells) against HepG2 cells were at 22±1.0 µg/ml. The observation strongly suggested that the ethanolic leaf extract can be used as potential anticancer agent against Hepato carcinoma (HepG2) cancer cells.

The *in vivo* medicinal properties of ethanolic leaf extract was studied using Swiss albino mice and Wistar rats for different pharmacological activities. After oral administration of ethanol leaf extract, mortality was not observed up to 2000 mg/kg and was considered as safe as per OECD-423 guidelines.

Wound healing activity of ethanol leaf extract was tested using Excision repair model. The result showed the topical application of ethanolic leaf extract at the concentration 10% and 20% have showed good reduction in wound area. The 20% ointment test extract treated animals showed earlier epithelialization of wound than 10% extract ointment. The contraction of excision wound was promoted from 1st day of treatment till 12th day. The epithelialization period of 10% ointment was 19.1±0.4 while in 20% ointment was 18.2±0.2. From the study, it was observed that the test extract have potential wound healing activity.

Hepatoprotective activity of ethanolic leaf extract was evaluated. All the wistar rats were treated for a period of 14 days. On the 15th day, Paracetamol (negative control) at the concentration of 2 g/kg p.o was orally administrated to all the wistar rats. The results showed the elevated level of Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP), Bilirubin, Urea and Creatinine due to paracetamol intoxication reduced significantly ($P<0.01$) in rats after treatment with ethanolic leaf extract and silymarin at the dose of 50 mg/kg the level of the enzyme were found recovering towards to normal state. Among all the tested groups, the 250 mg/kg and 500 mg/kg of ethanolic leaf extract exhibit excellent significant effect which is equivalently potent to silymarin (standard drug). The study results, strongly suggested that the ethanolic leaf extract of *C. mala-elengi* can be used as potential hepatoprotective agent.

To conclude, the ethanolic leaf extract of *C. mala-elengi* (Dennst.) P.S. Green contain different bioactive compounds which is responsible for various medicinal properties. It has anticancerous and antidiabetic properties which was proven through *in vitro* studies. Our study strongly validates the traditional uses of *C. mala-elengi* in treatment of wounds and hepatoprotective activity.