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Phytochemical Screening and Antimicrobial Activities of Ehretia Laevis Roxb Plant

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Abstract: *In India many Folklore plants are used traditionally for medicinal purposes. Ehretia laevis roxb also known as khandu chakka. It has many medicinal useful properties. Itscommonly known as ovate-leaved lvory wood, Gujrat; vadhavaradi, Sanskrit: charmavruksh Hindi:Bhairi; It is found in India. In ayurveda these plant mention as a charma vruksha and useful for prameha (diabetics) and vishaghana (anti venon). This plant commonly used in jointpain, wound healing, minor fractures by local people. It has very good pain relief activity. People can use plant leaves with oil for relief. It is very beneficial for old peoples suffering from joint pain. It will save side effect of painkiller medicine. Present study reports phytochemical screening and the antimicrobial activity of crude extracts of leaf of Ehretia laevis roxb. on some human pathogenic bacteria and fungi. There are many wound healing problems like infections, old age, stress, diabetics, chemotherapy drugs, obesity, alcohol consumption, smoking, mal nourishment. Lots of higher antibiotics are used to treat wound infection. This is not affordable by rural population. Day by day resistance of higher antibiotics is increase in human. Patients have to face untoward effect of higher antibiotics. The present study was hence under taken to evaluate thescience behind such wonderful herb.*

Keywords: Phytochemical screening, Antimicrobial activity Ehretia Laevis, Fracture, Joint Pain, Wound Healing

I. INTRODUCTION

Ehretia laevis roxb is a medium sized tree reaching up to the height of 12 m. Its dropping branches bear dark green colored leaves with varied size 2–7.8 cm in length and 1.2 cm to 3.8 cm in width. The shape of leaves is obtuse; with 5 to 7 lateral veins on each side of the mid rib with a slender 2–3 cm long petiole. The bark of the plant is irregular and light grey. The flowersare white, with round orange fruits when ripe or mature.

This plant commonly used in Wardha district for fractures, body ache by rural populations.local people from vidharbha are using these plant for wound healing since many years and these also proved on scientific ground.

Ehretia laevis is rapidly growing medium sized tree of boraginaceae. The genus of Ehretia contains more than 150 species. The plant is primarly distributed throughout tropical and subtropical regions of asia, Africa, and Australia. E.

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laevis is commomnly known by more than 120 names in diverse languages. Mostly it's found in Gujrat, Maharashtra, Rajasthan.

Another survey conducted on Gujjar tribes of Uttarakhand reported that E. laevis had been greatly used for the treatment of liver diseases, e.g., jaundice. A paste of soaked seeds of E. laevis was prepared, mixed with powder of Amomum subulatum and given thrice a day with milk by Nomadic people for the treatment of liver diseases . Furthermore, an ethnobotanicalsurvey has been conducted in the Tharu community of district Udham Singh Nagar, Uttarakhand, India. It was found that local villagers and health practitioners used the paste prepared from the seeds of E. laevis for the healing of skin diseases. Leaves, stems, barks and fruits of E. laevis are also used in the manufacturing of dyes, cosmetics and wines .

Ethnobotanical studies established that barks, leaves and fruits of E. laevis are potential sources of phytoconstituents. Phytochemical investigations had led to the extraction and isolation of secondary metabolites along with primary metabolites from petroleum ether, chloroform and methanolic extracts of its barks and leaves. These are pentacyclic triterpenoids,flavonoids, alkaloids, tannins, phenolic components, phenolic acids, hydrocarbons, aliphatic alcohols, fatty acids, ascorbic acid, amino acids, carbohydrates, benzoquinones, vitamins and minerals.

The present study "Efficacy of Folklore plant Khanduchakka (Ehretia laevis Roxb.) Patra Siddha Tail in Sandhivata" assumes significance due to absence of any planned study on the folklore drug, which is widely practiced in certain parts of Maharashtra state specially in Vidharbha region for the treatment of Osteoarthritis. This plant is commonly used in Wardha district for fractures, sandhivata and wound healing by rural population. Local name of this plant is Khanduchakka. People from Wardha District are using Kalka or Kalka mixed with oil of this plant since many years. It was routinely employed by rural people for Sandhivata management, with surprising output. This folk tribal herbal drug not mentioned in standard Ayurvedic text, was found to be very effective in Osteoarthritis. Majority of the patients (35%) were reported in the age group of 41-50 years followed by33.33% in the age group of 51- 60years.Demographic studies revealed that osteoarthritic changes commence between 4th-5th decades of life Sandhivata is the disease of vriddhavastha due to dhatukshya which has been found here also. Majority of the patients werefemale (55%) followed by male (45%). Women affect more than men. This shows the prevalence of the disease in females as mentioned in texts. The post menopausal hormonal variations play a role in bone mineralization.

II. MATERIALS AND METHOD

2.1 Plants Collection

The present work was carried out at Department of Chemistry, J.M.V. Chandrapur, Gondwana University. The plant named Phyllanthus amarus collected from Chandrapur forest region. Their botanical identity of plant was determined and authenticated from literature available in Department of Botany, J.M.V. Chandrapur. The leaves of Phyllanthus amarus plant was thoroughly washed with water and dried under shade for about ten days. The dried plant sample was grinding well into a fine powder in a mixture grinder. The powder was stored in air sealed polyethylene bag at room temperature before extraction

Preparation of Ethanol & Hexane Extract

100 g of the dried and powdered E.leavis roxb were extracted at room temperature with 500 mlabsolute hexane extract for 72 h. extraction was done using the soxhlet apparatus briefly 100gm of powder steam was stored in a air sealed polyethylene bag & placed in soxh let and extracted with absolute ethanol The extraction was done until the solvent in the soxhlet turnedcolourless. The extract was concentrated by recovering the solvent using the soxhlet apparatusuntil the extract became just pourable. It was poured into a beaker & this was then used for the analysis.

Phytochemical Analysis

There are two Extract

1) Ethanol

2) Hexane

The extracts were analyzed for the presence of Alkaloids, Terpenoids, Tannin, Saponin, Flavonoid, Phlobatannin, Anthraquionone, Reducing Sugar, Glycoside and Cardiac glycoside

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1. **Alkaloid:** About 0.2 g of the extracts was warmed with 2% H_2SO_4 for two minutes. It was filtered and few drop of Dragencloffs reagent were added. Orange red precipitated indicates the presence of alkaloids.
2. **Tannine:** Small quantity of extracts was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicatesthe presence of tannins.
3. **Anthraquinones:** About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allows cooling. Equal volume of $CHCl_3$ was added to the filtrated
   Few drop of 10% NH_3 were added to the mixture and heat. Formation of rose-pink colour indicates the presence of anthraquinones.
4. **Glycoside:** The extracts was hydrolyzed with HCl solution and neutralized with NaOH solution.A few drop of Fehling's solution A and B were added. Red precipitate indicates the presence of glycoside.
5. **Reducing Sugars**: The extracts was shaken with distilled water and filtered. The filtrate was boiled with drop of Fehling's solution for minutes. An orange red precipitate indicates presenceof reducing sugar.
6. **Saponin:** About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.
7. **Flavonoids:** Extracts of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colorless indicates the presence of flavonoids. Phlobatannins: The extracts (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2%HCl solution. Red precipitated show the presence of Phlobatannins.
8. **Terpenoids (Salkowski test):** 0.2 g of extracts was mixed with 2 ml Chloroform ($CHCl_3$) and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.
9. **Cardiac glycosides:** Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brow ring, while in acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Preparation of Aqueous Extract

100 g of the dried and powdered E.leavis roxb were extracted at room temperature with500 ml aqueous solution for 72 h. extraction was done using the soxhlet apparatus briefly 100gm of powder steam was stored in a air sealed polyethylene bag & placed in sox let& extracted with absolute ethanol The extraction was done until the solvent in the soxhlet turned colorless. The extract was concentrated by recovering the solvent using the soxhlet apparatus until the extract became just pourable. It was poured into a beaker & this was then used for theanalysis.

Preparation of Extracts

The microorganism used in the study: Gram-negative E-coli, Gram-Positive S-aurous and fungus Aspergillus were obtained from stock culture in the Department of Microbiology, J. M. V. Chandrapur.

Antimicrobial Screening of Extracts

Susceptibility test were carried out. The modified agar well diffusion method, to testthe antimicrobial activity of the extracts. The medium employed was diagnostic sensitivity agar.The culture were prepared in triplicate and incubated at 37°C for 24 to 72 h. 0.2 ml of the broth culture of the test organism was put in a sterile Petri-dish and 18 ml of sterile molten diagnostic sensitivity agar, was added. Well were bored into the medium using 0.1 ml of the extracts. Streptomycin and Chloramphenicol were used as the standard antimicrobial agents at a concentration of 10 mcg/disk, 30 mcg/disk respectively. The plates were kept in sterilized inoculation chamber for 2 h to facilitate diffusion of the antimicrobial agents into the medium. The plates were then incubated at 37o C for 24 h and the diameter of zone of inhibition of microbial growth were measured in the plates in millimeters.

III. RESULT

Phytochemical screening of ethanol, hexane and extracts of E.leavis roxb is shown in table 1. The susceptibility of test micro organism to the crude extract of E.leavis roxb shown in table 2

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- Phytochemical analysis of ethanol extract of plant E.leavis roxb[khanduchakka] leaves

Table 1

Chemical Composition	Ethanol Extract
Alkaloid	Present
Tannine	Present
Anthroquinone	present
Glycoside	Absent
Reducing sugar	present
Saponine	Absent
Flavonoid	Present
Terpenoid	Present
Cardiac glycoside	Absent

- Phytochemical tests of hexane extract E.leavis roxb [khanduchakka] leaves

Table 2

Chemical Composition	Ethanol Extract
Alkaloid	Absent
Tannine	Absent
Anthroquinone	Absent
Glycoside	Present
Reducing sugar	Absent
Saponine	Present
Flavonoid	Absent
Terpenoid	Absent
Cardiac glycoside	Present

Table 2.1: Antimicrobial activity of leaf extract in ethanol

1	Gram +ve s-aureus	Gram –ve E-coli
2	14 mm	13 mm

Table 2.2: Antimicrobial activity of leaf extract in hexane

1	Gram +ve s-aureus	Gram –ve E-coli
2	08 mm	10 mm

3.1 Discussion

The qualitative analysis of extracts from ethanol and leaves of E.leavis roxb. Showed the presence of photochemical constituents such as:

Ethanol extract present in alkaloid, tannin, anthroquinone, reducing sugar, flavonoid, tannin,terepenoid and absent in glycoside, cardiac glycoside, saponine. Hexane extract present in glycoside, saponine and cardiac glycoside.

The results are summarized in table 1. The above result indicates that, the leaves of plant investigated are rich in alkaloid, tannins, flavonoid, and cardiac glycoside. The Ethanol extracts have showed absence of, Glycoside, saponin and cardic glycoside. Extracts of leaf were tested against Gram positive S-aurous and gram negative E-coli. Extract also tested for antifungal activity against standard chloraphenol and showed the inhibition of growth. ethanol were found to be highly sensitive against Gram positive S-aurous and gram negative E-coli (with zone of inhibition above 14mm for gram +ve & 15mm for gram –ve means highly sensitive). Ethanol extract was showed more antimicrobial activity than standard antibiotics and chloramphenicol. The inhibitory activity of these extract confirmed the potential use of the plant in the treatments of microbial induced ailments.

The qualitative analysis of extracts from hexane and leaf of E.leavis roxb showed the presence of photochemical constituents such as alkaloid, tannins, flavonoid, and terpenoid. The results are summarized in table1.The above

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results indicate that, the leaf of plant investigated is rich in glycoside, saponine, cardic glycoside. The Hexane extracts have showed absence of alkaloid, flavonoid,terpenoid, tannis, anthraquinone, reducing suger. Extracts of leaf were tested against Gram positive S-aurous and gram negative E-coli. Methanol were found to be highly sensitive against Gram positive S-aurous and gram negative E-coli (with zone of inhibition above 21 mm for gram +ve& 25mm for gram –ve means highly sensitive). Ethanol extract was showed more antimicrobial activity than standard antibiotics streptomycin and chloramphenicol. The inhibitory activity of these extract confirmed the potential use of the plant in the treatments of microbial induced ailments.

The plant studied here can be seen as a potential source of useful drugs. Further studies are going on this plant in order to isolate, identify, characteristics and elucidate the structure ofbioactive compounds.

IV. CONCLUSION

The plant E.leavis roxb is rich in Alkaloid, flavonid, saponine, etc. The antimicrobial activity of the extract can be co-related to their specific contents loke alkaloids, Flavonoids and terepenoids. The ethanol extract was found to be antimicrobilly more effective than hexane extract. The antimicrobial activity of S-aurousis more effective than E-coli. E.leavis roxb and itscompound as well as, investigate if these natural products could moderate. The plant summarizes information concering the morphology, ecology and most importantly phytochemical constitutes and antimicrobial activity. This study has generated an evidence forantimicrobial activity, which will provide cost effective option for treating the wound infection. Also it can help to promote the cultivation of E.leavis roxb as a medicinal plant. This will ultimately aid in improving the economy of farmer to some extends.

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