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ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF STEM BARK OF *OUGEINIA OOJEINENSIS* (ROXB.)HOCHR

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ABSTRACT

Ougeinia oojeinensis (Roxb.) Hochr, is an ethnomedicinal plant of Western Ghats seen in deciduous forests. Since ancient times, plant bark has been used to treat several ailments based on traditional knowledge such as inflammation, constipation, leprosy, skin allergy, leucoderma, diarrhoea, dysentery, gonorrhoea, and diabetes. It is also useful in vitiated conditions like urrorrhagia, verminosis, haemorrhages, fever, ulcers, and general debility. This investigation was carried out to assess the antimicrobial activity of ethanolic extract of its stem bark. This is a first report of antimicrobial study done on this plant. *Methodology:* The stem bark was collected, washed and dried completely. The dried bark was then coarsely powdered .Approximately 100gm of the powdered drug was extracted with 0.5 litre of ethanol (95%) by using the cold maceration technique for three days with occasional shaking. After completion of three days, the ethanol layer was decanted off. The process of maceration was repeated thrice. The solvent from total extract was distilled off and the concentrate was evaporated to syrupy consistency and then evaporated to dryness. The antimicrobial activity was screened by using agar diffusion method against 10 bacterial strains and 1 fungal strain. *Result:* The ethanolic extract of *Ougeinia oojeinensis* was found to exert significant antimicrobial activity against all the tested bacteria particularly against *Salmonella typhimurium* and the yeast *Candida albicans*. ***Conclusion****:* The claim on use of this plant bark in folk medicine is justified and extract has phytoconstituents which have potential anti-*candidiasis* and anti-*salmonella* properities.

KEYWORDS: *Ougeinia oojeinensis* (Roxb.) Hochr, *Salmonella typhimurium*, *Candida albicans.*

INTRODUCTION

Human beings have been dependent on plants since ancient times for curing ailments, providing long lasting good health, relieving bodily distress and for imparting flavour and aroma to food. Plants have been the centre of healthcare system in India and China for more than 5000 years. Plants were being used as mainstream medicine in Europe till about 50 years ago. Countries like India, China, Greece and Arabia independently developed their own traditional system of medicine. [1] Presently on our planet, approximately 250,000 known species of higher plants are present and out of them about 35,000 species has been used by people for indigenous purpose. [2] As per the survey done by the WHO it has been reported that 80 % of more than 4000 million inhabitants of the world dependent on the traditional medicines for health care need. [3] and presumed that the use of plant extract or their active principle is a major part of traditional therapy .[4]

Since the Vedic period and as early as the dawn of human civilization, the Ayurvedic system of medicine is prevalent in India. Even though Ayurvedic system has undergone phenomenal row during course of time but still it is used as a prime source of medical relief to a large population of the nation. [5] Advances in research of medicinal plants have undergone many changes in the last decade. Presently, research on the medicinal plants has become more popular due to their lesser side effects, lesser price, broad spectrum of activity and non – narcotic in nature. As medicinal plants add value to mankind, hence there is a necessity to increase the safety, effectiveness, and efficacy of novel as well as current medicinal plants. There is a tremendous research work going on to unleash the secret treasure present in medicinal plants viz. pharmacologically and therapeutically active photochemical. [6]

DESCRIPTION

Fig 1. Stem bark of *ougeinia oojeinensis* (roxb.) hochr.

Ougeinia oojeinensis(Roxb.)Hochr is a large deciduous tree, under good condition grows up to 20-40 m height, with a short crooked trunk; bark dark brown, deeply cracked; branches slender, terete. Leaves pinnately 3 foliolate, often reaching 12 inches long (including the petiole); common petioles 1 ½-2 in. long; stipules ¼ in. long, lanceolate, acute, caduceus. Leaflets rigidly coriaceous, the terminal broadly elliptic or roundish, sometimes trapezoidal,3-6 by 2-4 in. ,the lateral leaflets opposite ,obliquely ovate ,cordate ,3-4 by 1 ½ - 3 in., on petiolules 1/8 in. long ,all glabrous above ,glabrous or more or less downy beneath , distinctly and shallowly crenate ,bluntly pointed ;main nerves 4-8 pairs, prominent; stipules subulate. Flower numerous, in short fascicled racemes from the nodes of old branches; pedicels coloured, 1/6-1/4 in. long, filiform; bracts 1/20in .long, ovate, acuminate, broader than long, ovate, acuminate, broader than long, villous outside; bracteole 1 beneath the calyx, minute, villous. Calyx 1/6-1/4 in. long, pubescent; teeth short, triangular. Corolla 3/8 -1/2 in. long, white or rose –coloured, somewhat fragrant. Pods 2-3 in. long; joints reticulatately veined, 2-3 times as long as broad. [7]

GEOGRAPHICAL DISTRIBUTION

It is small or medium sized tree, found in sub-Himalayan tracts and outer Himalayan valley and slopes up to an altitude of 5000 ft. from Punjab to Bhutan ,also in Oath, Bundelkhand , Chota Nagpur, Central India ,Orissa Madras state ,Madhya Pradesh, Bombay state and Marwar i.e. Rajasthan. [8] It is sparely distributed in mixed forest of hilly slopes. [9]

MEDICINAL USE

The bark is astringent, acrid, cooling, anti-inflammatory, constipating, urinary, anthelmentic, sudorrific, depurative, stypic, and rejuvenating. It is useful in vitiated condition urrorrhagia, verminosis, haemorrhages, fever, ulcers, and general debility. [10] In Ayurvedic text, both tinishaa and tinisha have mentioned .Tinishaa is used for alleviating burning syndrome during illness, while tinisha for skin diseases, urinary disorder and anaemia and for treating obesity. It was also known as Atimuktaka, the drug for reducing obesity which has now equated with Hiptage benghalensis. Charaka prescribed fresh juice of the bark and stalks to be taken internally for fever, debility and as a tonic for recuperation. Sushruta administered the drug internally in obesity, jaundice, urethral discharge, chronic skin diseases, and oil of seed as a digestive in bilious affection. A decoction of tinisha in combination with other intestinal antiseptic and astringent was administered in haemorrhagic, diarrhoea and dysentery. [11] Bark also has stimulant and astringent properties and used to treat diarrhoea and dysentery. [12]

Bark when incised, furnished gum (a Kino like exudation) which is useful in diarrhoea, dysentery [13] and in digestive trouble [14] .A decoction of bark is given when urine is high coloured. [15] It alleviates three doshas, purifies the blood in constipation and is also used in urinary disorders, bleeding from rectus and diseases of medas. [16] Bark indicates it's use in leprosy, leucoderma, gonorrhoea, and diabetes [17] and have cooling effect.[18] Decoction prepared from mixture of bark of *Ougeinia oojeinensis* and *Terocarpus marsupium* is taken orally with milk by the tribal ladies for 6 days after completion of menstrual period to increase fertility and chances of pregnancy. [19] Bark paste is used externally to treat skin allergy [20] .Bark powder is applied on wound to aid quick healing of wounds. [21]

AIM OF RESEARCH

To study the antimicrobial activity of the ethanolic extract of the stem bark of *Ougeinia oojeinensis* (Roxb.)Hochr

MATERIALS

Sources of microorganisms

The microorganisms used were both bacteria and fungi which were procured from Microbiology department of Goa Medical College, Bambolim-Goa, India and NCL, Pune and were used for testing antimicrobial activity The organisms include Bacteria(*Escherichia coli ATCC 25922, Pseudomonas aeruginosa ,Klebsiella pneumonia ,Salmonella paratyphi A, Staphylococcus aureus ATCC 25923, Shigella soneii, Shigella boydii, Shigella dysenterica, Salmonella typhimurium*) and *Candida albicans* was the fungi used.

Equipments

Autoclave, Incubator, Cyclomixer, Nichrome loop, Conical flask, pipette, petridishes, micropipette, glass spreader, glass rod, Weighing balance, glass cylinder.

Collection and Authentification

Ougeinia oojeinensis (Roxb.)Hochr was procured from Chitradurga, Karnataka, India and was identified and authenticated Prof. Gopal Krishna Bhatt, Department of Botany, Poornaprajna College, Udipi, Karnataka, India

Extraction Process

The stem bark were collected, washed, shade dried completely. The dried bark was then coarsely powdered .Approximately 100gm of the powdered drug was extracted with 0.5 litre

of ethanol (95%) by using the cold maceration technique for three day with occasional shaking. After three day, the ethanol layer was decanted off. The process repeated thrice. The solvent from total extract was distilled off and the concentrate was evaporated to syrupy consistency and then evaporated to dryness.

Preparation of test solution

DMSO was used as a solvent. It is neutral and is used as a universal solvent in most of the anti-bacterial sensitivity procedure to dissolve compounds. 55mg of the dried extract was weighed and 1ml of DMSO was added and the mixture was cyclomixed on a cyclomixer gently untill a uniform brown solution was obtained .

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Microorganism

The antimicrobial activity of ethanolic extract of *Ougeinia oojeinensis* was screened by using agar diffusion method.10 bacterial strain and 1 fungal strain were used *Escherichia coli ATCC 25922, Pseudomonas aeruginosa ,Klebsiella pneumonia ,Salmonella paratyphi A, Staphylococcus aureus ATCC 25923, Shigella soneii, Shigella boydii, Shigella dysenterica, Salmonella typhimurium)* and *Candida albicans.*All bacterial and fungal strain were maintained on nutrient agar and were freshly subculture by using nichrome loop for 24-48 hr at 37°C.

Preparation of media

The antimicrobial activity of ethanolic extract of *Ougeinia oojeinensis* was screened by using Mueller – Hinton agar. The required quantity of Mueller –Hinton agar was prepared in a conical flask and sterilized at 121 °c at 15lbs pressure for 15-20 mins .The required number of previously sterilized petridishes were taken .The saline suspension of each strain was prepared separately .The Mueller –Hinton plate were prepared by pouring 20ml of molten media and 1ml of above suspension after cyclomixing into sterile petridishes. The plates were allowed to solidify for 5 mins. For each bacterial and fungal strain pure solvent (DMSO) is used as control.

Antimicrobial activity determination

The Muller Hinton Agar plates were prepared as mentioned above. Using a sterile cork borer, wells were bored at the centre of each plate. While boring the next plate the borer was dipped in alcohol and flamed to avoid cross contamination. A specific quantity and concentration of

test solution were introduced into the well by using sterile micropipettes. The inoculated plates were kept in the refrigerator at 2-8° C for 10-15 mins for the diffusion of the standard and test solution. The plates were then incubated for 24 hrs for bacterial strains and 48 hrs for fungal strains in an incubator at 37° C. At the end of incubation, inhibition zones formed around the well were measured with transparent ruler in millimetre. [22](For each strain, pure solvent (DMSO) is used as control. Ciprofloxacin (10 µg/ml) for antibacterial studies and Metronidazole (10 µg/ml) for antifungal studies were used as positive control. These studies were performed in triplicate and mean values were presented. The results are tabulated in tables 1.

Table 1. Antimicrobial activity of ethanolic extract of the stem bark of *Ougeinia oojeinensis* by well diffusion method

	Diameter of zone of inhibition in mm										
	Bacterial strain										Fungal strain
Culture	KP	SPT	SD	SB	SS	ST	EC	PA	SA	ST*	CA
Extract	22	25	24	24	24	25	24	22	24	27	28

Concentration of stock solution was 55mg/ml

KP (Klebsiella pneumonia), SPT (Salmonella paratyphi A) ,SD (Shigella dysenterica) , SB (Shigella boydii), SS (Shigella soneii), ST (Salmonella typhimurium) EC (Escherichia coli ATCC 25922),PA (Pseudomonas aeruginosa),SA (Staphylococcus aureus ATCC 25923), ST Salmonella typhimurium ATCC 23564) ,CA (Candida albicans) .*

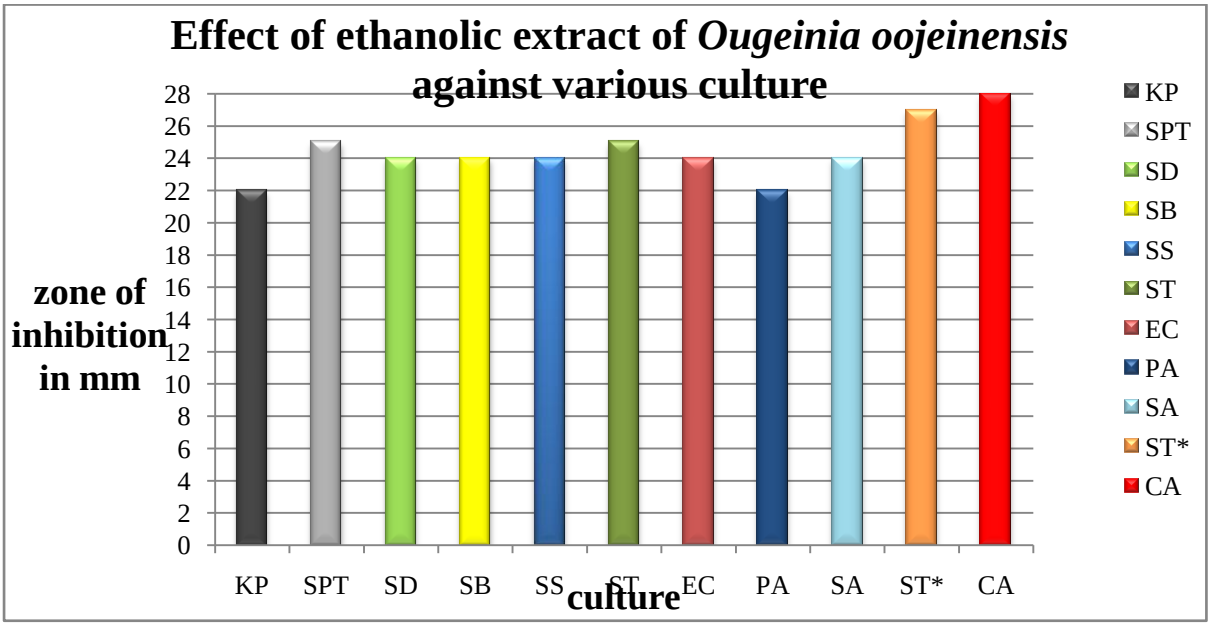

Fig 2. Graph of Effect of ethanolic extract of *Ougeinia oojeinensis* against various culture.

RESULTS

Since no antimicrobial work has been carried out on the Stem bark of *Ougeinia oojeinensis*, thus attempt have been made to study the antimicrobial activity of the Stem bark of *Oojeinensis* on different strains of micro-organisms.

The results indicated that the ethanolic extract of *Ougeinia oojeinensis* was found to exert significant antimicrobial activity against all the tested micro-organism which is cited by the zone of inhibition (refer table 1 and fig. 2)but from all the tested micro organism , ethanolic extract showed a potential activity against *Salmonella typhimurium* ATCC 23564 and the yeast *Candida albicans*.

DISCUSSION

Since ancient times, *Ougeinia oojeinensis* bark has been used to treat several ailments based on traditional knowledge such as inflammation, constipation, leprosy, skin allergy, leucoderma, diarrhoea, dysentery, gonorrhoea, and diabetes. It is also useful in vitiated conditions like urrorrhagia, verminosis, haemorrhages, fever, ulcers, and general debility, thus it was decided to analyse the extract against various microbial strains.

Candida albicans which causes nosocomial infections , white mucosal plaque term ‘thrush’ which occur on the tongue, palate, and other mucosal surfaces as single or multiple, ragged white patches, and Vaginitis which may be recurrent in female [23]and *Salmonella typhimurium* which causes Typhoid fever, enterocolitis, diarrhea,vomiting, and abdominal pain after an incubation period of 12 to 72 h. [24] appear to be strongly inhibited by ethanolic extract by agar diffusion method which is indicated by significant larger zone of inhibition (show in table 1 and fig.2)thus indicating a potential anti-*candidiasis* and anti-*salmonella* properities.Turbidometric method was not done as the extract was coloured . In the GC-MS study of the bark, the ethanolic extracts showed the presence of 8 constituents ,which were alcoholic compounds 1-octanol, 2-butyl, sugar moiety 3-o-methyl-d-glucose, palmitic acid, linoleic acid, oleic acid, 1, 2 benzene dicarboxilic acid, fatty acid ester,and triterpine squalene .It is well documented that 4 constituent from above 8 constituent posses antimicrobial activity[25] {refer table 2} and hence this reinforces our investigation on antimicrobial activity of ethanolic extract of the stem bark of *O.oojeinensis* and it confirmed .

Table .2. GC-MS study-activity of phytocomponents identified in the bark extract of *ougeinia oojeinensis* (roxb.) hochr . [25]

Name of the compound	Compound Nature	Activity
1-Octanol, 2-butyl	Alcoholic compound	Antimicrobial
1, 2-Benzenedicarboxylic acid, diisooctyl ester	Plasticizer compound	Antimicrobial, Anti-inflammatory
Dodecanoic acid, 1, 2, 3-propanetriyl ester	Fatty acid ester	Antioxidant, Antibacterial, COX-1 & COX-2 inhibitor, Antiviral Hypocholesterolemic
Squalene	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant

CONCULSION

The present study established that the ethanolic extracts of the stem bark of *O.oojeinensis* show a significant antimicrobial activity against all the tested micro-organism and showed a potential activity against *Salmonella typhimurium* ATCC 23564 and the yeast *Candida albicans* indicating its anti- *Salmonella* and anti- *candidiasis* properities. The above activity has been reported for the first time for the ethanolic extract of the stem bark of *O.oojeinensis.*

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