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Antidermatophytic Potential of Selected Ethno Medicinal Plants from Paderu, Visakhapatnam, India.

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ABSTRACT

In the present work ethylacetate and methanolic bark extract of *Aegle marmelos(L) Correa*, *Alstonia scholaris (L)R.Br*, *Alstonia venenata (L)R.Br*, *Cyclea peltata (Lam) Hook .F.Thoms*, *Dilenia pentagyna Roxb*, *Macaranga peltata Roxb Mueller*, *Oroxylum indicum (L)Vent*, *Pterocarpus marsupium Roxb*, *Zanthoxylum armatum DC* and *Zizipus oenoplia (L) Mill*, were tested against *Trichophyton rubrum* a clinical isolate from skin scrapings of *Taenia Corporis* using Agarwell diffusion method and its minimal inhibition concentration (MIC) was also determined by Broth dilution method. Out of all the Methanolic bark extracts *Oroxylum indicum* Vent showed highest zone of inhibition followed by *Alstonia venenata* Linn, at 50 µgrams concentration. Clotrimazole was used as standard antifungal drug. MIC was found to be between 10µg - 1000µg per ml. *Oroxylum indicum* and *Alstonia venenata* showed MIC proportional to their zone of inhibition. Both bark extracts could be potential natural healing agents against Ringworm or *Taenia* disease . Further investigation is needed to identify the compound which was present in both the plant barks to develop a novel drug.

Keywords : Antidermatophytic , *Oroxylum indicum*, *Alstonia venenata*, *Trichophyton rubrum*.

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INTRODUCTION

Superficial mycoses of humans caused by dermatophytes remains a public health problem especially in tropical countries like India^{1,2}. High humidity, over population are conducive for the growth of dermatophytes which causes a disease known as Taenia or Ringworm. In recent years there has been an increasing interest in the use of natural substances for therapy and it is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds³. Coincidentally herbal medicines have been found to possess one or more medicinal properties^{4,5}, several plants have been screened and found to have antimycotic activity^{6,7}.

Aegle marmelos (L) Correa, belongs to family Rutaceae, known as wood apple (thorny tree). It is one of the important plants, with several medicinal and nutraceutical properties⁸. Different organic extracts of the leaves of *A. marmelos* have been reported to contain alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavonoids and steroids⁹.

Alstonia scholaris (L)R.Br., is ever green tropical tree known as Indian devil tree, belongs to Apocynaceae. The milky juice of the tree is applied to ulcers. The bark contains the alkaloid ditamine, the decoction of the bark is used to treat diarrhoea and malaria¹⁰.

Alstonia venenata (L)R.Br., is an important ethno botanical plant of the Apocynaceae family. It grows as shrub or small tree, the fruits are stated to possess tonic and antihelmintic properties which is a remedy for syphilis, insanity and epilepsy¹¹.

Cyclea peltata (Lam) Hook .F.Thoms, belongs to family Menispermaceae, known as Rajapatha is an excellent wound healer and also used as diuretic drug¹².

Dilenia pentagyna Roxb, belongs to Dilleniaceae known as dog teak is a tall deciduous tree, Barks and Leaves are used for the treatment of diarrhoea and dysentery described in medicinal plants of Bangladesh, stem bark of this plant used for gastric cancer and also it helps to increase erythrocyte count¹³.

Macaranga peltata Roxb Mueller, is a plant found in northern Thailand and India, is one of the most widely occurring early successional woody species belongs to Euphorbiaceae, leaves and stem barks are tested for its invitro antioxidant, antimicrobial and cytotoxic properties¹⁴.

Oroxylum indicum (L)Vent, is a flowering tree belongs to the family Bignoneaceae, commonly known as Indian trumpet flower or midnight horror, the root bark is used as astringent and also for anticancer treatment¹⁵.

Pterocarpus marsupium Roxb, is commonly known as Indian kino tree, is a deciduous tree used in reducing type II diabetes belongs to family Leguminaceae¹⁶.

Zanthoxylum armatum DC, known as winged prickly ash belongs to Rutaceae. It is known for its GIT, respiratory, and cardiovascular modulatory activity¹⁷.

Zizipus oenoplia (L) Mill, known as wild jejobe belongs to the family Rhamnaceae is used as anti bacterial agent¹⁸.

The present study validates the above ethnomedicinal plants towards their antidermatophytic activity.

MATERIAL AND METHODS

Plant material

Plants were collected from the forests of Paderu, a tribal area near Visakhapatnam, plants were identified and authenticated by plant taxonomists in department of Botany, Andhra University, Visakhapatnam.(A.P.), India.

Test organism

Trichophyton rubrum is a clinical isolate from the skin scrapings of the patients attending outpatient department of Dermatology, King George Hospital of Andhra Medical College, Visakhapatnam, India, with suspected dermatophytosis. Isolation and identification of dermatophytes has been already published¹⁹, it shows that *Taenia corporis* was predominant clinical presentation and *T.rubrum* is the causative agent.

Collection and Isolation of *Trichophyton rubrum* from Dermatophytosis

Skin scales, nail and hair Specimens were collected from patients with suspected dermatophytosis. Sample was obtained from patients attending the Dermatology outpatients department, King George Hospital of Andhra Medical Collage, Visakhapatnam (India). The affected area was thoroughly cleaned with 70% alcohol to remove the surface contaminants. Whatman no. 1 filter paper was used for collecting specimens²⁰. After disinfection with alcohol, skin lesions were scraped with a scalpel to collect epidermal scales.

Samples were also cultured on duplicate plates of Sabouraud Dextrose Agar (Himedia) and Dermatophyte Test Medium (DTM) prepared according to the manufacturers instructions. The plates were inoculated with finely divided pieces of each sample and incubated at 28⁰C in BOD incubator (Remi) for recovery of dermatophytes. The dermatophyte test medium (DTM) is an alternative culture medium that suggests the presence of dermatophyte pathogens, even though it does not identify specific species macroscopically²¹, the ability to hydrolyze urea provides additional data that can be used to aid in the differentiation of *Trichophyton rubrum* (urease negative) from other *Trichophyton* sps²². In – vitro hair perforation test demonstrates the

inability of *T.rubrum* to penetrate the hair shaft. Similarly the cultures were identified on the basis of their macro and microscopic features²³.

Preparation of plant extracts

Collected plant material was air dried for one month, then ground into powder and dissolved in organic solvents ethyl acetate and methanol so as to make 40% respective solvent extracts. The extract was kept in orbital shaking incubator for 3 days and centrifuged to remove the debris, clear solvent extracts were collected and the solvent was evaporated by using rotavapour (Buchi India) to get the concentrated residue of the solvent. This was dissolved in DMSO and tested for antidermatophytic activity.

Preparation of inoculum

21 days old grown culture of *T. rubrum* was scraped with sterile needle and dissolved in 0.9% sterile solution so as to make different dilutions, the diluted suspension which has the absorbance of 0.600 at 450nm determined spectroscopically (EI, India) was used as inoculum²⁴.

Agar well diffusion method

Twenty ml of Sabourauds dextrose agar medium was poured into a 15 cm petridish. Twenty μ l of inoculum suspension of *T. rubrum* was distributed evenly over the surface, A 6mm well was cut in the plate using a sterilized cork borer .50 μ l of plant extracts were placed into the wells. Clotrimazole disk (Hi media) was used as standard , the plates were incubated for 10-15 days at 28° C in BOD(Remi), pure organic solvent used as a control. Results were determined based on size of inhibitory zone surrounding the wells containing the test solution. The diameter of zones of inhibition was measured in mm using Hi media zone reader^{25,26}.

Broth dilution assay

The medium containing different concentrations of plant extracts viz. 100mg.-1 μ g per ml, was prepared by serial dilution (10⁻¹ dilution) and inoculated with 20 μ l of culture, the tubes were incubated for 72 hrs at 28°C in BOD (Remi). The MIC of each sample was noted by measuring the O.D in the spectro photo meter (EI, India) at 520 nm and, compared the result with those of the non- inoculated broth used as a blank. Control was prepared using media and inoculum without plant extract²⁷. The experiment was conducted according to CLSI Standards^{28,29}.

Data Analysis

Three different experiments results average taken as data

RESULT AND DISCUSSIONS

Table 1 shows antidermatophytic activity of different solvent extracts of various plant barks in

which *Aegle marmelos*, *Alstonia scholaris*, *Alstonia venenata*, *Oroxylum indicum*, *Zanthoxylum armatum*, showed inhibitory action against *T. rubrum* while *Cyclea peltata*, *Dilenia pentagyna*, *Macaranga peltata*, *Pterocarpus marsupium*, *Zizipus oenoplia* extracts did not show any inhibitory activity. *Oroxylum indicum* methanol extracts showed highest zone of inhibition (32mm) followed by *Alstonia venenata* in methanol (24mm) and then *Aegle marmelos*, *Zanthoxylum armatum* (16mm). *A. scholaris* showed highest zone of inhibition in ethylacetate extract (19.3mm) followed by *Aegle marmelos* (16mm) MIC found to be between 10 μ g to 1000 μ g. Up to now more than 200 different biologically active substances have been isolated from plant extracts, among them organosulphur compounds such as allicin, azoones and diallyltrisulfide, eugenol, a phenolic compound (the most biologically active compound) were found in many plant extracts³⁰.

Only few substances are known to inhibit human pathogenic fungi, which are often completely resistant to anti-fungals, and most of them are relatively toxic. The increased incidence of therapeutic failure in the treatment of fungal infections and the prevalence of opportunistic infections has renewed interest in the search for new antifungal agents, including those obtained from higher plants. Present results show that these natural compounds could be useful agents in the topical treatment of fungal infections. Medicinal plants have been used in developing countries as alternative treatments to health problems. Many plant extracts and essential oils isolated from plants have been shown to exert biological activity in vitro and in vivo, which justified research on traditional medicine focused on the characterization of antimicrobial activity of these plants³¹. Brazil, Cuba, India, Jordan and Mexico are examples of countries that have a diverse flora and rich tradition in the use of medicinal plants for both antibacterial and antifungal applications^{32, 33, 34}. *Oroxylum indicum* is one among the group of ten drugs named Dasamoola and rasayana widely used in Ayurvedic system of medicine, root bark is used as astringent, bitter tonic. It is included in famous tonic formulation such as Chavanprasha. TLC studies of Radika³⁵, revealed a glycosides, Terpenoids, Phynols and Alkaloids from root of *Oroxylum indicum*. Previous chemical studies of *Oroxylum indicum* led to isolation of ellagic acid³⁶, 5-7-dihydroxyflavone (*Chrysin*)³⁷, 5-hydroxy-8-methoxy-7-O- β -D gluco pyranuronosyl flavone³⁸, stigmast-5-en-3-ol³⁹, 5,6,7-trihydroxy flavone (*biacalein*)⁴⁰, 4',5-dihydroxy-7-methoxy isoflavone (*Pratenson*)⁴¹.

Alstonia venenata devil tree is used as snake antivenom by tribals. An indol alkaloid venenatine showed antifungal activity⁴², From the leaves *alstonia venenata* compounds were isolated from ethanolic extract⁴³, of which anti oxidant activity exhibited by n-hexadecanoic acid squalene.

Oroxylum indicum , Alstonia venenata, in methanol extracts show comparable inhibitory activity (32 mm , 24mm) with clotrimazole (33mm). All tested plant extracts showed similar results with standard T.rubrum culture(MTCC 3272).

Table 1: Inhibitory effect of Methanolic solvent extract of various plant barks on a clinical isolate of Trichophyton rubrum .

S.no	Name of the Plant	Type of Extract	Zone of Inhibition (mm)	MIC(μ g)
1	Aegle marmelos	M	16	1000
		E	13	1000
2	Alstonia scholaris	M	-	-
		E	19.3	1000
3	Alstonia venenata	M	24	100
		E	-	-
4	Cyclea peltata	M	-	-
		E	-	-
5	Dilenia pentagyna	M	-	-
		E	-	-
6	Macaranga peltata	M	-	-
		E	-	-
7	Oroxylum indicum	M	32	10
		E	-	-
8	Pterocarpus marsupium	M	-	-
		E	-	-
9	Zanthoxylum armatum	M	16	1000
		E	-	-
10	Zizipus oenoplia	M	-	-
		E	-	-
11	Clotrimazole	M	33	1
		E	32	1

(M=methanol), (E=ethylacetate)

50 μ l of plant extract (1 μ g / μ l concentration) per well. Clotrimazole 30 PPM (30 μ g). Results are the average of three readings

CONCLUSION

T.rubrum was the most common etiologic agent isolated from T.corporis in and around Visakhapatnam region, barks of Oroxylum indicum, Alstonia venenata methnolic extracts showed potential inhibitory activity against T.rubrum than other tested plant extracts on par with Clotrimazole, further investigation is needed to identify the responsible compound present in the bark of above plants to develop a novel drug.

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