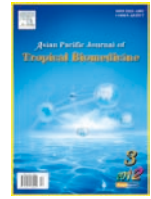




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A study on anti dermatophytic potential of selected ethno medicinal plants against *Trichophyton rubrum*, a common etiologic agent in and around Visakhapatnam region (India).

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ABSTRACT

Objective: The purpose of this study was to evaluate the clinical patterns and etiology of dermatophytosis and the anti dermatophytic potentiality of some selected medicinal plants used by tribal people in and around Visakhapatnam region, India. **Methods:** 62 patients with dermatophytosis who attended the dermatology clinic of King George hospital, Visakhapatnam were studied. Isolation and identification was done by direct microscopic observation, cultural characteristics and by using biochemical tests in the microbiology laboratory. Some ethno medicinal plant parts like *Albizia lebbek* bark, *Annona reticulata* leaf and bark, *Cassia fistula* leaf, *Wrightia tinktoria* bark and *Couroupita guianensis* leaf were tested for anti dermatophytic activity by agar well diffusion method and minimum inhibitory concentration (MIC) studies were carried out by broth dilution assay. **Results:** 51 patients out of 62 were positive (82.2%) by direct smear and culture. Tinea corporis was the most common dermatophytosis which was predominantly caused by *Trichophyton rubrum* in and around Visakhapatnam. Two dermatophyte species were isolated and identified. *Trichophyton rubrum* was the most frequent isolate (58.8%) followed by *Trichophyton mentagrophytes* (19.6%). *Albizia lebbek* bark, *Annona reticulata* bark and leaf extracts showed inhibitory against *T. rubrum* while *Cassia fistula* leaf extract did not show significant inhibitory activity. *Wrightia tinktoria* bark and *Couroupita guianensis* leaf extracts did not show inhibitory activity. **Conclusions:** The results showed that tinea corporis was the most common dermatophytosis in and around Visakhapatnam region. *Trichophyton rubrum* was the most common etiologic agent. *Albizia lebbek* bark, *Annona reticulata* leaf and bark extracts showed potential inhibitory activity against *Trichophyton rubrum* than other tested ethno medicinal plants.

1. Introduction

Skin, hair, nail, and subcutaneous tissues in human and animal are subjected to infection by several organisms, including fungi mainly dermatophytes which cause dermatophytosis.

The disease is widely distributed all over the world in various degrees and more common in men than in women. Dermatophytosis is a trivial disease but has lot of psychological effect and a costly disease in terms of treatment. Though various species of dermatophytes

produce clinically characteristic lesions, a single species may produce variety of lesions depending upon site of infection. The dermatophytosis was grouped according to the infective location on the patients. Tinea corporis (Skin), tinea pedis (feet), tinea capitis (hair), tinea unguium (nails) and tinea cruris (groin). Tinea corporis was the most common dermatophytosis. There are three genera of moulds that cause dermatophytosis and they are *Trichophyton*, *Microsporium* and *Epidermophyton*. *Trichophyton rubrum* was commonly isolated etiologic agent [1]. Contagiousness among animal communities, high cost of treatment, difficulty of control and the public health consequences explain their great importance. A wide variety of dermatophytes have been isolated from humans and animals, but a few zoophilic species are responsible for the majority of the cases, viz. *Microsporium canis*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton equinum* and *Trichophyton verrucosum*, as also the geophilic species *Microsporium*

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gypseum [2]. Human beings are the main or only host for anthropophilic dermatophytes like *T. rubrum*[3].

Nowadays, natural products most likely continue to exist and grow to become even more valuable as sources of new drug leads. This is because of a broader degree of chemical diversity and novelty of molecules found in natural products than that from another source. Fungal diseases have historically been a difficult clinical entity to effectively deal with. The available drugs to treat fungal infections have been limited. Furthermore, in this armamentarium there are problems with side effects, the rapid development of resistance, drug interactions and fungistatic mechanism of action. Thus, there is need for the development of more efficacious antidermatophytic agents. Plants are the best source for identification of leads for the development of novel drug compounds [4].

Albizia lebbek (*A. lebbek*) belongs to Mimosaceae family. *A. lebbek* is a species of *Albizia*, native to tropical southern Asia, and widely cultivated and naturalised in other tropical and subtropical regions. Barks are used in toothache and diseases of the gum. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are used for piles and diarrhoea. Ethanolic extract of pods possesses antiprotozoal, hypoglycemic and anticancer properties. The methanolic extract of the pod was investigated for antifertility activity [5]. *A. lebbek* bark extract has antimicrobial activity. The active constituents of bark extract are anthraquinone glycosides. The main constituent from bark is active against aerobes and mechanism of action is that glycosides cause the leakage of the cytoplasmic constituents [6]. *Annona reticulate* (*A. reticulate*) is a small deciduous or semi-evergreen tree in the plant family Annonaceae. *H.M.Suresh* reported *In vitro* antiproliferative activity of *A. reticulata* roots on human cancer cell lines Padhi et al reported Anti microbial activity of leaf extracts of *A. reticulata* Because of the uniqueness of leaf property in curing of different ailments, this part was selected for the study[7,8]. *Cassia fistula* (*C. fistula*), known as the golden shower tree and other names, is a flowering plant in the family Fabaceae, native to southern Asia, from east of southern Pakistan through India to Myanmar and Sri Lanka. In Ayurvedic medicine, golden shower tree is known as aragvadhā, meaning “disease killer”. The root is considered a very strong purgative [9]. *Wrightia tinctoria* R. Br. (*W. tinctoria*) is considered to be very effective jaundice plant in Indian indigenous system of medicine. The juice of the tender leaves is used efficaciously in jaundice; also crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. In Siddha system of medicine, it is used for psoriasis and other skin diseases [10]. *Couroupita guianensis* (*C. guianensis*) is a tree belonging to the family Lecythidaceae. It is native to South India and Malaysia and commonly known as Nagalinga pushpam in Tamil. Various part of the tree have been reported to contain oils, keto steroids, glycosides, couroupitone, indirubin, isatin and phenolic substances and also reported to possess anti microbial activity [11].

The purpose of this study was to evaluate the clinical patterns and etiology of dermatophytosis and the anti dermatophytic potentiality of the some selected medicinal plants used by Tribal people in and around Visakhapatnam region, India.

2. Materials and methods

2.1. Collection of Samples

Skin scales, nail and hair Specimens were collected from patients with suspected dermatophytosis. A total of 51 cultures were obtained from 62 Patients with dermatophytosis, 36 males (70.5%) and 15 (29.5%) females, during May 2011– Jan 2012. All samples were obtained from patients attending the Dermatology outpatients department, King George Hospital of Andhra medical college, Visakhapatnam (India). *Albizia lebbek* bark, *Annona reticulate* bark and leaf, *Cassia fistula* leaf, *Wrightia tinctoria* bark and *Couroupita guianensis* bark which were used for this study. These plants were collected in the forests of Paaderu, a tribal area close to Visakhapatnam. Plants are identified and authenticated by plant taxonomists, Department of Botany, Andhra University, Visakhapatnam.

2.2. Isolation and identification of dermatophytes

The affected area was thoroughly cleaned with 70% alcohol to remove the surface contaminants. Whatman no.1 filter paper was used for collecting specimens [12]. After disinfection with alcohol skin lesions was scraped with a scalpel to collect epidermal scales. From the scalp hair was epilated with sterile forceps. Nails sample collected using nail clip. 10% KOH solution was used for skin & hair and for nail scrapings 20% KOH was used. All preparations were examined under low power and confirmed under high power. Samples are also cultured on duplicate plates of Sabouraud Dextrose Agar (Himedia, India) and Dermatophyte Test Medium (Himedia, India) prepared according to the manufacturer’s instructions. The plates were inoculated with finely divided pieces of each sample and incubated at 27 °C in BOD incubator (Remi, India) for recovery of dermatophytes or moulds. The dermatophyte test medium (DTM) is an alternative culture medium that suggests the presence of dermatophyte pathogens, even though it does not identify specific organisms macroscopically [13, 14], the ability to hydrolyze urea provides additional data that can be used to aid in the differentiation of *Trichophyton rubrum* (urease negative) from *Trichophyton mentagrophytes* (*T. mentagrophytes*) typically urease positive [15]. *In-vitro* hair perforation test states that the ability of *T. mentagrophytes* to penetrate the hair shaft but not *Trichophyton rubrum* (*T. rubrum*) was shown in. Similarly the cultures were identified on the basis of their macro and microscopic features [1, 3]. Standard culture of *Trichophyton rubrum* (MTCC 3272) was obtained from ITM, Chandigarh, India for comparative study with clinical isolate of *T. rubrum*.

2.3. Anti dermatophytic activity

2.3.1. Preparation of plant extracts

Plant material was removed and air dried then ground into powder which was dissolved in organic solvents i.e. hexane, ethyl acetate and methanol so as to make 40% respective solvent extract. The extract is kept in orbital shaking incubator for 3 days and then centrifuged to remove the debris. Finally clear solvent extracts were collected and then the solvent was evaporated by using rotavapour (BUCHI, India) to get the concentrated residue of the solvent,

which contains dissolved components of plant material. The concentrated residue of solvent extracts were appropriately dissolved in solvents and tested for anti dermatophytic assay.

2.3.2. Preparation of inoculum

21 days old grown culture of *T. rubrum* scraped with sterile scalpel and dissolved in sterile saline solution to make different dilutions. The diluted suspension which has the absorbance of 0.600 at 450nm determined spectroscopically (Electronics India) was used as inoculum^[16].

2.3.3. Agar well diffusion method

Antifungal screening was carried out using the agar well diffusion assay. Twenty ml of sterilized sabouraud dextrose agar medium poured into a 15 cm Petri dish. Twenty μ L of inoculum suspension of the *T. rubrum* was distributed evenly over the surface. A 6mm well was cut in the centre of each plate using a sterilized cork borer. 50 μ L of plant extracts and Griseofulvin (Dr.Reddy's Labs, India) were placed into the wells. The plates were incubated for 5 days at 28 °C. Pure organic solvents were used as control. Results were determined based on size of the inhibitory zone surrounding the wells containing the test solution. The diameter of zones of inhibition was measured in mm using HiMedia zone reader^[17, 18].

2.3.4. Determination the MIC by Broth Dilution Assay:

The minimum inhibitory concentration of the plant extract was determined using broth dilution assay. The medium containing different concentrations of plant extracts viz., 100mg – 1 μ g per ml prepared by serial dilution (10–1 dilution). After inoculation of culture, the tubes were incubated for 72 hours at 28 °C. The MIC of each sample was determined by measuring the optical density in the spectrophotometer (Electronics India) at 520nm and compared the result with those of the non-inoculated broth used as blank. Control was prepared using media and inoculum without plant extract^[19]. The experiment was conducted according to NCCLS standards (now called as CLSI)^[20, 21].

3. RESULTS

3.1. Isolation and identification of dermatophytes

51 out of 62 patients were positive (82.2%) by direct smear

and culture. Thirty-six (70.5%) out of 51 patients were males and 15 (29.5%) were females. The two dermatophyte species those were isolated and identified (Table 1). *T. rubrum* was the most frequent isolate (58.8%) followed by *T. mentagrophytes* (19.6%). Non dermatophyte fungi like *Candida albicans* (13.7%) and *Aspergillus niger* (7.8%) were also isolated in few cases. *T. rubrum* colonies were white, velvety to fluffy, occasionally powdery to granular with diffuse wine red coloured pigmentation on reverse and microscopically showed pyriform microconidia borne slightly along the hyphae and macroconidia which were pencil shaped and urease test was negative while *T. mentagrophytes* colonies were spherical microconidia in grape like clusters and macroconidia were thin walled creamytan, powdery to granular, flat surface with buff colour and microscopically which were abundant smooth cigar shaped. Sometimes spiral hyphae were seen, biochemical tests like urease & in-vitro hair perforation tests were positive. Figure 1 shows that the results were grouped according to the location of the dermatophytosis on the patients. Tinea corporis (60%) was the most common type of clinical presentation, followed by tinea pedis (15%), tinea capitis (12.5%), tinea unguium (7.5%), and tinea cruris (5%). 75% of tinea corporis was caused by *T. rubrum*. Tinea corporis was the most common dermatophytosis and *T. rubrum* (58.8%) was most commonly isolated etiologic agent followed by *T. mentagrophytes* (19.6%).

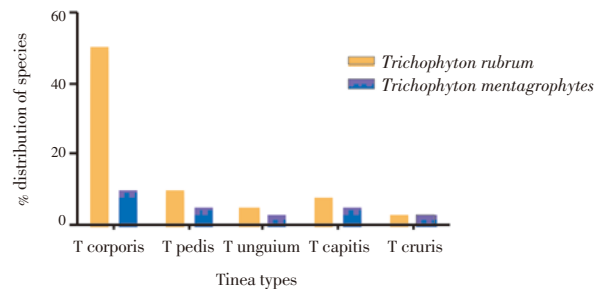


Figure 1. Percent distribution of dermatophytic species according to Clinical presentation.

3.2. Anti dermatophytic activity

Table 2 shows the anti dermatophytic activity of different solvent extracts of various plant parts in which *A. lebeck* bark, *A. reticulata* bark and leaf extracts showed inhibitory against *T. rubrum* while *C.fistula* leaf extracts did not show

Table 1.

Prevalence of isolated dermatophyte species according to site of infection and gender. (n=51)

| Species | Skin | Hair | Nails | Total | Male | Female |
|------------------------------------|-----------|--------|-----------|-----------|-----------|-----------|
| Dermatophytes (n= 40) | | | | | | |
| <i>Trichophyton rubrum</i> | 21 | 5 | 4 | 30(58.8%) | 21 | 9 |
| <i>Trichophyton mentagrophytes</i> | 6 | 1 | 3 | 10(19.6%) | 7 | 3 |
| Non dermatophytes (n=11) | | | | | | |
| <i>Candida albicans</i> | 4 | 1 | 2 | 7(13.7%) | 5 | 2 |
| <i>Aspergillus niger</i> | 2 | 1 | 1 | 4(7.8%) | 3 | 1 |
| Total | 33(64.7%) | 8(15%) | 10(19.6%) | 51(100%) | 36(70.5%) | 15(29.5%) |

(n) Number of samples.

significant inhibitory activity. *W. tinktoria* bark and *C. guianensis* leaf extracts did not show inhibitory activity.

Table 2.

Anti dermatophytic activity of selected medicinal plants against clinical isolate of *Trichophyton rubrum*.

| Name of the plant | Type of part | Type of extract | Anti dermatophytic activity |
|------------------------------|--------------|-----------------|-----------------------------|
| <i>Albizia lebbek</i> | Bark | Hexane | + |
| | | Ethyl acetate | + |
| | | Methanol | + |
| <i>Annona reticulata</i> | Bark | Hexane | + |
| | | Ethyl acetate | + |
| | | Methanol | + |
| <i>Annona reticulata</i> | Leaf | Hexane | + |
| | | Ethyl acetate | + |
| | | Methanol | + |
| <i>Cassia fistula</i> | Leaf | Hexane | NS |
| | | Ethyl acetate | NS |
| | | Methanol | NS |
| <i>Wrightia tinktoria</i> | Bark | Hexane | - |
| | | Ethyl acetate | - |
| | | Methanol | - |
| <i>Couroupita guianensis</i> | Leaf | Hexane | - |
| | | Ethyl acetate | - |
| | | Methanol | - |

(NS) Not significant, (+) Presence of inhibitory activity, (-) Absence of inhibitory activity.

Table 3 contains the zone of inhibitions and MIC of the different solvent extracts of *A. lebbek* bark, *A. reticulata* bark and leaf against *T. rubrum*. 20mm was the highest inhibitory zone showed by *A. reticulata* ethyl acetate leaf extract. Minimum inhibitory concentration was found to be in the range between 100 µg to 10mg/ml concentration. *A. lebbek* bark, *A. reticulata* bark and leaf ethyl acetate extracts showed comparable inhibitory activity (18, 19 & 20mm) with Griseofulvin (25mm) against clinical isolate of *Trichophyton rubrum*. All tested plant extracts showed similar results with standard *T. rubrum* culture (MTCC 3272).

Table 3.

Inhibitory effect of different organic solvent extracts of various plants on a clinical isolate of *Trichophyton rubrum*.

| Plant species | Type of plant part | Type of extract* | Zone of inhibition | MIC |
|-------------------------------|--------------------|------------------|--------------------|--------|
| <i>Albizia lebbek</i> | Bark | Hexane | 12 | 10mg |
| | | Ethyl acetate | 18 | 100 µg |
| | | Methanol | 17 | 100 µg |
| <i>Annona reticulata</i> Bark | Bark | Hexane | 13 | 10mg |
| | | Ethyl acetate | 19 | 100 µg |
| | | Methanol | 16 | 100 µg |
| <i>Annona reticulata</i> | Leaf | Hexane | 12 | 10mg |
| | | Ethyl acetate | 20 | 100 µg |
| | | Methanol | 18 | 100 µg |
| Pure solvents (Control) | | Hexane | 0 | ND |
| | | Ethyl acetate | 0 | ND |
| | | Methanol | 0 | ND |
| Griseofulvin (Standard) | | Sterile water | 25 | 1 µg |

* 50 µL of plant extract (1 µg/µL concentrated) per well (ND) Not determined

4. DISCUSSION

This survey on the prevalence and distribution of dermatophytes isolated from clinical samples at the Dermatology Outpatients department, King George Hospital of Andhra medical college, Visakhapatnam (India) during May 2011– Jan 2012 showed that the common dermatophytosis is tinea corporis (50%). The most commonly isolated etiologic agent was *T. rubrum* (58.8%) from dermatophytosis patients in and around Visakhapatnam. Hexane, ethyl acetate and methanol extracts of several plant parts were tested against *T. rubrum*, a common etiologic agent in and around Visakhapatnam. *A. lebbek* bark, *A. reticulata* leaf and bark extracts showed significant activity than *C. fistula* leaf extracts. *W. tinktoria* bark and *C. guianensis* leaf extracts were not showing inhibitory activity against *T. rubrum*. Ethyl acetate plant extracts showed significant inhibitory activity than methanolic plant extracts. Hexane plant extracts showed poor inhibitory activity against *T. rubrum*. All tested plant extracts showed similar results with standard culture of *T. rubrum* (MTCC 3272). The secondary metabolites like alkaloids, tannins, saponins and flavonoids are responsible for antimicrobial activity.

A number of reports are available related to in-vitro and in-vivo efficacy of plant extracts against plant and human pathogens causing fungal infections. The activity of plant extract against dermatophytosis i.e. the superficial infections of skin or keratinised tissue of man and animals can be very well visualized from the reports of Venugopal and Venugopal (1995) [22]. They reported the activity of plant extracts against 88 clinical isolates of dermatophytes which includes *Microsporum canis*, *M. audouinii*, *Trichophyton rubrum*, *T. mentagrophytes*, *T. violaceum*, *T. simii*, *T. verrucosum*, *T. erinacci* and *Epidermophyton floccosum* by agar dilution technique. Balakumar S reported that antifungal activity of *Aegle marmelos* leaf extracts and fractions on the clinical isolates of dermatophytic fungi like *Trichophyton mentagrophyte*, *Trichophyton rubrum*, *Microsporum canis*,

Microsporum gypseum and *Epidermophyton floccosum* [16]. All the above reports and many others have utilized plant extract, juice or oil for the in-vitro or in-vivo evaluation of the infections caused by various species of dermatophytes viz. *Trichophyton*, *Microsporum*, *Epidermophyton* and yeast/fungi of genera *Candida*, *Cryptococcus*, *Rhodotorula* and *Torulopsis trichosporon*. 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 important biologically active compound found in many plant extract [23].

From the present study it was concluded that tinea corporis was the common dermatophytosis in and Visakhapatnam region. *T. rubrum* was the most common etiologic agent. *A. lebbeck* bark, *A. reticulata* leaf and bark extracts showed potential inhibitory activity against *T. rubrum* than other tested ethno medicinal plant extracts of *C. fistula* leaf, *W. tinktoria* bark and *C. guianensis* leaf.

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Conflict of interest statement

We declare that we have no conflict of interest.

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