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# ANTI DERMATOPHYTIC ACTIVITY ON ETHNOMEDICAL PLANTS USED BY A PRIMITIVE TRIBE "GADABAS" OF PADERU

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## ABSTRACT

An attempt has been made to report antidermatophytic active on methanolic and ethyl acetate extracts of seven ethnomedicinal plants Adhatoda zeylanica medic (leaf), Argyrea nervosa Burn.F (fruit), Diplocyclos palmatus Linn. Jeffrey (fruit), Hoya pendula White & Arn (leaf), Paracalyx scariosa Roxb (Twig), Ricinus communis Linn (Root), Stemona Tuberosa Lour (Tuber) Used by Gadabas, a primitive group confining to Paderu, Visakhapatnam Dist, A.P. Trichophyton rubrum is most prevalent species of Dermatophytes causing ring worm or tinea in and around Visakhapatnam out of all the plants tested Hoya pendula methanol extract, ethyl acetate extract showed significant zone of inhibition 27 mm and 28mm at 50µl concentration and showed minimal inhabitation concentration (MIC) at 100µg in crude extracts. Further investigation is needed to identify the responsible compound which is present in Hoya pendula Leaf to develop a novel drug.

**KEY WORDS:** **Antidermatophytic, MIC, Gadabas, Trichophyton rubrum**

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## INTRODUCTION

Many plants are known to possess fungicidal substances and much work has been carried out on the effects of medicinal and aromatic plant extracts against various fungi[1] but their effect upon Dermatophytes is unexplored. Dermatophytes are known to cause superficial skin infections like scabies[2]. Dermatophytosis is one of the most common diseases in the world and it's prevalence in recent years has increased by 20-25% of the world's population. Some of the contributing factors like community living, use of antibiotics increase the risk of infection towards Dermatophytes[3]. Dermatophytes are a unique group of fungi that infect keratinous tissue and the most common sites of infection are skin, hair and nail. The disease commonly known as ring worm (Tinea). In concern to drawbacks of conventional medicine, the use of natural products as an alternate to the conventional treatment in the healing and treatment of various diseases has increased in the last few decades[4]. Currently several plants have been screened and discovered to possess significant antimycotic activities[5,6]. The present research sought to validate ethnomedicinal (antidermatophytic) use of the following plants (Fig-1)

**Figure 1**

***1. Adhatoda zeylanica medic*:** Commonly known as Malabar nut, belongs to Acanthaceae is a versatile plant with wide spectrum medicinal activities[7] like anti - tubercular, anti-ulcer, anti-bacterial, anti-inflammatory, Hepato & Cardiovascular protective.

***2. Argyrea nervosa Burn.F*:** Is a perennial climbing vine, belongs to Convolvulaceae, commonly known as Elephant creeper. The root is used as an alternative tonic in case of Rheumatism and neurological disorders. It also possesses various traditional and tribal uses for cure of human ailments.

***3. Diplocyclos palmatus Linn. Jeffrey*:** Is a vine of the Cucurbitaceae, commonly known as native bryony or striped cucumber, fruits having an important use in the area of reproductive medicine & roots are used in tooth decay[8].

***4. Hoya pendula White & Arn:*** Belongs to Asclepiadaceae, commonly known as pendulus wax flower, is a slender fleshy, twining epiphyte, leaves oblong, flowers white waxy & in pendulus cluster. The plant is emetic and alexi pharmic[9]. Leaf and root used for eye infection, prolapse of uterus, heart pain[10].

***5. Paracalyx scariosa Roxb:*** Is commonly known as Ranghevada, a climbing shrub which belongs to Fabaceae known for its nutritive values[11] .

***6. Ricinus communis Linn:*** Is a flowering plant commonly known as Castor oil plant, which belongs to Euphorbiaceae. It is used for expelling worms, colds, colic, convulsion, fever, gout, nerve pain and warts[12].

***7. Stemona Tuberosa Lour*:** It is a flowering plant belongs to Stemonaceae, commonly known as wild Asparagus (Bai Bu in Chinese). Tubers of the plant contain an alkaloid called stemonine[13].

# MATERIALS AND METHODS

### *Collection of plant material*

Adhatoda zeylanica medic (leaf), Argyrea nervosa Burn.F (fruit), Diplocyclos palmatus Linn. Jeffrey (fruit), Hoya pendula White & Arn (leaf), Paracalyx scariosa Roxb (Twig), Ricinus communis Linn (Root), Stemona Tuberosa Lour (Tuber) were used for the study. These plants were collected from the forests of Paderu, a tribal area near Visakhapatnam. Plants are identified and Authenticated by plant Taxonomists Department of Botany, Andhra University, Visakhapatnam, A.P.

### *Collection and isolation of test dermatophyte (Trychophyton rubrum) from patients*

Skin scales, hair, and nail specimens were collected from patients with suspected dermatophytosis , who is attending out patient department of dermatology, King George Hospital of Andhra Medical College, Visakhapatnam India. The affected lesion was thoroughly washed with 70% alcohol and samples collected in sterilized Whatman filter paper-1[14]. Samples were cultured on duplicate plates of Sabourauds Dextrose Agar (Himedia) and Dermatophyte Test Medium (Himedia) prepared according to manufacturers instruction. The plates were inoculated with finely divided pieces of each sample and incubated at 28°C in BOD incubator (Remi) for 10-20 days for the recovery. The cultures were identified on the basis of their Macro and Micro conidial features[15] and Urease test[16]. 58% of the samples collected having Tineacorporis out of which Trychophyton rubrum is the most prevalent dermatophyte.

### *Prepration of plant extracts*

All seven plant parts were air dried and then ground into powder, which was dissolved in methanol (SRL) and ethyl acetate (SRL) so as to make 40% solvent extract. The extract was kept in an orbital shaking incubator for three days and centrifuged to remove debris. Finally clear solvent extracts were collected and then the solvent was evaporated by using

rotavapour (Buchi, India) to powder. This was appropriately dissolved in DMSO (SRL) and tested for antidermatophytic activity.

### *Preperation of inoculum*

Twenty one days old grown culture of T.rubrum was scraped with sterile needle and dissolved in 0.9 % sterile saline solution to make different dilutions. One of the diluted suspensions was used as inoculum which had an absorbance of 0.600 at 450 nm, determined spectroscopically (Electronics India)[17,18]

### *Agar well diffusion method*

Antifungal screening was carried out using the agar well diffusion assay. 20 ml of sterilized SDA medium poured into a 15cm Petri dish. 20µl of inoculum suspension of T.rubrum. was distributed evenly over the surface, a 6mm well was cut in the center of each plate using a sterilized cork borer. Different plant extracts, extracted in methanol and ethyl acetate were placed into the wells. The plates were incubated for 7-14 days at 28°C pure methanol and ethyl acetate solvents were used as controls and results were determined based on the size of the inhibitory zone(mm) surrounding the wells containing the test solution. The diameter of zones of inhibition was measured in mm using zone reader [19,20] (Himedia). Clotrimazole (HiMedia) disks used as standard for comparison .

### *Determination of MIC by Broth Dilution Assay*

The minimum inhibitory concentration (MIC) of the plant extracts was determined by Broth Dilution Assay (NCCL 38A, CLSI) [21,22] the medium containing different concentrations of plant extracts viz., 1000mg-10µg per ml prepared by serial dilution ($10^{-1}$ dilution). After inoculation of culture (20µl) 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 (E.I) at 520 nm and compared the result with those of the non-inoculated broth used as blank.

## RESULTS

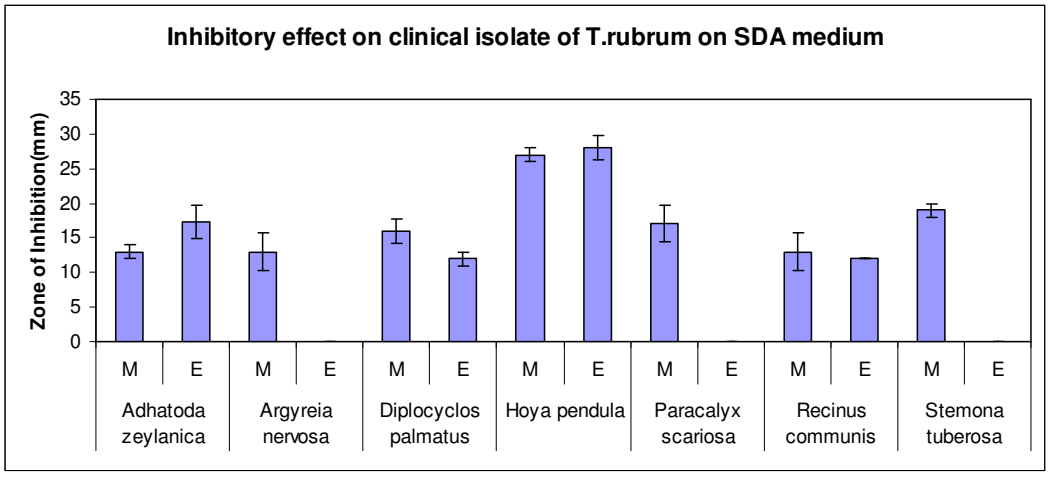
Samples from patients attending Dept. of Dermatology, K.G.H, A.M.C cultured on SDA and DTM (Himedia). T.rubrum colonies are white, fluffy, with wine red coloured pigmentation on reverse. Microscopically showed pyriform microconidia along the hyphae and pencil shaped macro conidia. Urease test negative, in-vitro hair perforation test was negative, which was further confirmed as T.rubrum. It was subcultured and used in the study after 21 days. Table-1 shows the antidermatophytic activity of different solvent extracts of various plant parts. In which methanolic extracts of Hoya pendula, Stemona tuberosa, Para calyx scariosa Roxb & Diplocyclos palmatus Linn. showed inhibitory activity against T.rubrum while methanolic extracts of Adhatoda zeylanica medic, Argyrea nervosa Burn & Ricinus communis Linn showed slight activity. Ethyl acetate extracts of Hoya pendula showed highest inhibitory activity followed by Adhatoda zeylanica medic, Diplocyclos palmatus Linn.Jeffrey, Ricinus communis Linn, while Argyrea nervosa Burn, Para calyx scariosa Roxb, Stemona tuberosa did not showed any activity. Table-1 contains zone of inhibition and MIC of different solvent extracts and different plant parts Hoya pendula leaves showed zone of inhibition both in methanol (27mm) and ethyl acetate (28mm) & MIC was found to be 100µg/ml. Stemona tuberosa methanol extract, Adhatoda zeylanica ethyl acetate extract showed statistical significant zones of inhibition (Fig-2), however their MIC are 1000µg/ml.

**Table 1**

| T.rubrum inhibitory effect | | | | |
|---|---|---|---|---|
| **Plant Species** | **Plant Part** | **Type of Solvent*** | **Zone of Inhibition (mm)** | **MIC (µg)** |
| Adhatoda zeylanica | Leaf | Methanol | 13 | - |
| | | Ethylacetate | 17.3 | 1000 |
| Argyreia nervosa | Fruit | Methanol | 13 | - |
| | | Ethylacetate | 0 | - |
| Diplocyclos palmatus | Fruit | Methanol | 16 | - |
| | | Ethylacetate | 12 | - |
| Hoya pendula | Leaf | Methanol | 27 | 100 |
| | | Ethylacetate | 28 | 100 |
| Paracalyx scariosa | Twig | Methanol | 17 | - |
| | | Ethylacetate | 0 | - |
| Ricinus communis | Root | Methanol | 13 | - |
| | | Ethylacetate | 12 | - |
| Stemona tuberosa | Tuber | Methanol | 19 | 1000 |
| | | Ethylacetate | 0 | - |
| Standard | Disc | Clotrimazole | 32 | 10 |
| -ve control | - | Methanol | 0 | - |
| +ve control | - | Ethylacetate | 0 | - |

* 50 µl of plant extract per well

**Figure 2**




# DISCUSSION

The most common etiologic agent T.rubrum was isolated from the patients with suspected dermatophytosis, in and around Visakhapatnam, who are attending K.G.H during January to May 2013. Methanol & ethylacetate extracts of several plants were tested. Hoya pendula(M) extracts showed highest activity followed by Paracalyx scariosa Roxb and Diplocyclos palmatus Linn.Jeffrey, results compared with standard drug. Hoya pendula(EA) showed highest zone of inhibition followed by Adhatoda zeylanica medic, Diplocyclos palmatus Linn. Jeffrey & Ricinus communis Linn while other plants showed

negative results compared with standard drug. A number of reports are available related to in vitro and in vivo efficacy of plant extracts on plant and human pathogens causing fungal infection. Nagamalleswari reported antifungal studies of Pittosporum floribundum[23]. The activity of plant extracts against Dermatophytosis can be visualized from the reports of Venugopal and Venugopal [24]. Natarajan reported effect of Azadirachta indica (neem) on the growth of Dermato phytes. Balakumar reported that the antifungal activity of Aegle marmelos leaf extracts on the clinical isolates of dermatohytic fungi. Euginol, a phenolic compound the most antimicrobial compound found in many plant extracts[25]. The significance of phytochemicals with respect to the plants in traditional medicine and their phytochemical analysis was previously reported[26]. A number of reports are there on phytochemical analysis of ethno medicinal plants which are used in this analysis. Adhatoda zeylanica contains active constituents like vasicine and Vasicinone[27]. Argyria nervosa contain a number of compounds like alkoloids, steroids and important source of compounds like 1- tria contanol, β - sistosterol, chanoclavin -1[28]. A wide range phytochemicals like Ergoline alkaloids & Lysergic acid amid ergine[29] has been isolated. Diplocyclos palmatus fruit contains a number of alkaloids, triterpinoids, flavonoids, saponins, steroids and proteins[30]. Stemona tuberosa contains alkaloid tuberostemone, isotuberostemonine, stemonidine, sino stemonine, glucocides lipids, proteins, organic acids reported by W.H.O[31]. Scariosin was isolated from Paracalyx scariosa and two flavanoids are isolated from the same plant[32]. Recin a toxin isolated from the castor bean plant, Ricinus communis reported by Cornell University college of Agriculture and Life Sciences, the structure of recin was reported[33]. Hardly any work was reported against Hoya pendula Wight & Arn syn (Hoya alexicaca (N.J.Jacq) Moon, Hoya iconum Santapau). In our study the plant crude leaf extract showed significant zone of inhibition on par with purified Clotrimazole suggesting it may be a potent cure for Tinea. This investigation has demonstrated that Hoya pendula both extracts showed potent inhibitory activity against T.rubrum. Further investigation is needed to identify the responsible Antidermatophytic compound which is present in the above plant.

## CONCLUSION

From the present study it was concluded that Tinea or Ring worm was predominantly caused by T.rubrum. Hoya pendula, methanolic and ethylacetate extracts showed potential inhibitory activity than other tested methanolic extracts of Stemona tuberosa and ethyl acetate extract of Adhatoda zeylanica.

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### CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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