PHYTOCHEMICAL AND ANTIDERMATOPHYTIC ACTIVITY OF METHANOLIC EXTRACT OF ANOGEISSUS ACCUMINATA LEAVES.

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Summary

The antidermatophytic activity of methanol extract of the leaf of *Anogeissus accuminata* was evaluated against three species of dermatophytes viz. *Trichophyton mentagrophytes* (MTCC8476), *T. Rubrum* (MTAA8477) and *Microsporum gypseum* (MTCC8469) by minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) study.The extract shows a varying degree of potential inhibitory effect against the tested pathogens. *T. mentagrophytes* was found to be the most sensitive while *M.gypseum* was the least sensitive among the tested pathogens.Important bioactive constituents like *tannin* and *flavonoids* are detected in the methanol extract of leaf which may be responsible for the observed antimicrobial activity of the plant.

Keywords: Antidermatophytic Activity, Anogeissus Acuminate, Methanolic Extract

Introduction

Emergence of multidrug resistant strain to commercial antifungals and the harmful effect of synthetic, semisynthetic drugs have turned the majority of scientific communities towards the traditional system of medicine. Some azole group of antifungal drugs, like itraconazole, fluconazole are presently used in antifungal therapies. However the question of new generation of antifungal compound arises due to its limitation[1]. Plant are considered as a major source of medicine due to its wide range of chemical diversity. India is rich in unmatched plant diversity due to different agro climatic zone and biodiversity hot spot. In the traditional health care system medicinal plants play a paramount role in the control of various diseases.The main lacuna of traditional system of medicine is the lack of authentic data and standardization techniques. Current research on natural molecules,primarily focuses on plants since they can be sourced more easily and be selected on the basis of their ethnomedicinal uses[2].The active component of many drugs found in plants are secondary metabolites[3]. Therefore basic phytochemical analysis of the extract for their main phyto compound is very vital[4]. Antimicrobial compounds of plant origin may occur in stems, roots, leaves, bark, flowers and fruits of plant[5]. To establish scientific rationale for its use as drug with less dose limiting toxicity and to explore new effective chemical to replace those currently in use, scientific research is an utmost need of the day.

The genus Anogeissus belonging to combretaceae family comprising of 8 species most of the species of Anogeissus are used in folk medicine for the treatment of several disorders. It is also used for treating wounds and burns[6]. It is know for its ornamental, chemical and pharmaceutical potential. The genus Anogeissus is mainly enriched with volatile oil, tannins and flavonoids[7].It is an evergreen large deciduous tree. The leaves are elliptic or lanceolate with greenish flowers. The methanolic extract of the leaves is choosen to evaluate antidermatophytic property relevant to 3 species of dermatophytes.

Material And Methods

Plant Material

The leaves of Anogeissus accuminata were collected from Tirupathi in May 2009. The Plant was identified by Dr. Madhavachetty of S.V.university. Fresh plant materials were washed under running water and dried at room tempertire (20-23^{0}C). The dried plant material was then pulverized and stored in polythene bag at room temperature until required.

Preparation of extract:

The dried plant material (200g) was extracted twice with 800ml of 100% methanol for 48h at room temperature.The crude extract was filtered by using whatman filter paper no.1. The filtrates were pooled and evaporated to dryness with rotary evaporator under reduced pressure and kept in the refrigerator at 4^0 until use. The graded concentrations (70mg/ml, 50mg/ml, 100mg/ml, 150mg/ml) were prepared for bioassay by using dimethyle sulfoxide(DMSO) as solvent.

Preparation of inoculum:

All the fungal cultures were maintained on Sabouraud Dextrose agar slant. One loopful surface growth of spores and hyphae were scraped off with a sterile wire loop (2mm dia)and inoculated in 50ml Sabouraud Dextrose Broth(SDB). Then it is incubated at 28 ± 2^0 for 48 h. This was used as inoculum for the study.

Bioassay

The efficacy of plant extracts can be determined by inhibition of growth of the test organisms that are placed in contact with the extract. Here qualitative antifungal screening was carried out using the agarwell diffusion assay[8].

Inhibitory zone estimation

Twenty mL of sterilized Sabouraud Dextrose Agar(SDA) medium was poured into 80cm sterile petriplates and allowed to solidify.After solidification of the medium the broths were vortexed and 100mcL of inoculum suspension of each test organism was distributed evenly over the surface of the agar plates.A well of 6mm diameter was made in the center of each plate using a sterile cup borer. The cut agar discs were carefully removed by a sterilized forceps. The graded concentrations of test extract were sterilized by filtering through milipore filter (0.2mcm pore size).

Fifty mcL of different concentrations of test extract of Anogeissus accuminata were introduced into the wells.control experiments comprising of inoculum without plant extract were also setup.The plates were incubated for five days at 28± 2ºC .Results of the qualitative screening were recorded as the average diameter of the inhibition zone surrounding the wells containing the test solution.The experiment was replicated five times. Mean and ± SD (standard deviation)values were written in table1.Results were compared with standard drug clotrimazolc(1000mcg/ml).

Determination Of Mic

The minimum inhibitory concentration was recorded as the lowest concentration,which inhibit the growth of micro organisms producing a visible zone of inhibition.Decreasing concentrations of crude extract were for testing.

Activity index determination

The activity index of the extracts was determined by the following formula[9].

$$\text{Activity index} = \frac{\text{Zone of inhibition of extracts}}{\text{Zone of inhibition of standard}}$$

Analysis of percent of inhibition

Percent of inhibition was calculated according to following formula[10].

$$\% \text{ inhibition} = \frac{\text{inhibition zone in mm}}{\text{Control}} \times \mathbf{100}$$

Growth zone is equal to plate diameter i.e.80 mm as growth occurs all over the agar plate.

Phytochemical screening

The photochemical analysis of the plant extracts was performed according to the method described by Odebiyi O.O. 1978 and Kapoor L.D. 1969[11].

Results and Discussion

Results obtained from the agar well diffusion method are summarized in the TableI.After three days of incubation it was possible to read the diameter of inhibition zone of all *M.gypseum* and *T.mentagrophytes* and full growth was obtained in SDA control plates.Where as *T.rubrum* required longer incubation period i.e., 5 days

The zone of inhibition of the tested organisms indicated their susceptibility to the leaf extract used in the study.Itwas observed that zone of inhibition varies from one organism to another at different concentrations (Table I).The efficacy of antimicrobial agent is concentration dependent[11].All the tested concentrations were found effective against the tested pathogens.Highest sensitivity was shown by *T.mentagrophytes* at all the concentrations with the highest percent of inhibition of 64.25 at 150mg/ml.

Whereas incase of *T.rubrum*,and *M.gypseum* it was 55.5,43,35 respectively at the same concentration (Table1).The activity of the standard drug was found comparatively higher than test extract except in case of *T.mentagrophytes* where it was slightly higher (1.05) at the concentration of 150mg/ml(Table1)The zone of inhibition shown by plant extract at different concentrations was statistically evaluated. The calculation was made by one way analysis of variance(ANOVA)and was found that variance ratios (F-ratios)were highly significant at 1% level of significance.By comparing values of treatment difference with the LSD (least significant difference),it is found *T.mentagrophytes* is more sensitive to the extract than other tested organisms.The antifungal efficacy of the extract was found to be decreased in the following order among the tested organisms.

Table 1 : Effect of gradedconcentrations of A.accuminata extract on clinically Important fungal strains

Organisms	**Conc. (mg/ml)**	**Inhibition zone(mm)**	**% Inhibition**	**Activity index**	**SE**	**F-Ratio**	**LSD**
T.mentagrophytes	10 50 100 150	36.2±3.46 40±3.16 48±3.95 51.4±4.54	45.25 50 60 64.25	0.74 0.82 0.98 1.05	1.605	54.011	3.403
T.rubrum	10 50 100 150	25.4±1.63 30±2.83 37.6±2.33 44.4±2.33	31.75 37.5 47 55.5	0.46 0.55 0.68 0.80	1.844	40.539	3.909
M.gypseum	10 50 100 150	16±2.19 20±2.28 25±2 28±1.79	20 25 31.25 35	0.38 0.48 0.59 0.66	2.131	47.558	4.517

Significant at 1% level

T.mentagrophytes> T.rubrum>M.gypseum.

The MIC study of the extract revealed 0.2mg/ml against *T.mentagrophytes* and *m.gypseum* it was 0.5mg/ml.

The crude extract was subjected to various phytochemical tests.Preliminary phytochemical screening reveals the presence of tannin and flavonoids.

Several phenolic compounds like tannins present in the cells of plant or potential inhibitors of hydrolytic enzymes. Other compounds like saponins also have anti fungal properties[13].Many plants contain non-toxic glycosides that can get hydrolysed to release phenolics that are toxic to microbial pathogen.Therefore the compounds detected may be responsible for the antifungal activity against the tested pathogens.

The findings of the present study may lead to the discovery of alternate sources of molecules for consideration,with better efficacy,lower toxicity and higher activity against resistant microorganisims.

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