Research Article

In vitro Antifungal Activity of the Ethanolic Extract of Certain Indian Medicinal Plants.

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ABSTRACT

Natural resources have since antiquity been extensively exploited for medicinal purposes. Numerous plant materials have been employed in folk medicine in attempts to control diseases. In many countries around the world the use of medicinal plants still contributes significantly in primary health care. Although there is currently little scientific evidence regarding the efficacy of such treatments, traditional medicine is still widely practiced by people living all over the world. The present study was designed to evaluate the antifungal activity of ethanolic extract of various plant species obtained from various regions and were assayed for *in-vitro* antifungal activity against human dermatophytes viz: *Microsporum gypseum* and *Trichophyton mentragrophytes.* The solvent extract of the plant material was made through Soxehlet Apparatus and was run up to 48 hrs and was tested in two opposite concentrations. Results obtained reveal that the 1:5 concentration of plant material to that of nutrient Broth medium was more effective against dermatophytes as compared to 2:5 ratio. The maximum inhibition was shown by *Lindenbergia indica* (46.51 %) against *Microsporum gypseum* in the ratio 1:5, whereas against *Trichophyton mentragrophytes* was shown by *Achyranthus aspera* (63.15 %) at the same concentration. However maximum inhibition in the ratio 2:5 against *Microsporum gypseum* and *Trichophyton mentragrophytes* was shown by *Parthenium hysterophorus* (37.20 %) and *Lindenbergia indica* (52.63 %) respectively.

Keywords: Antifungal activity, human dermatophytes, indian medicinal plants & soxhlet apparatus

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INTRODUCTION

Dermatophytoses are among the world's common diseases and constitute an important public health problem as yet unresolved [1]. Because dermatophytes require keratin for growth, they are commonly restricted to hairs, nails and superficial skin. The prevalence of fungal infections in humans and the development of new antifungal agents have increased the interest in antifungal susceptibility testing for dermatophytes [2].

There is the knowledge, information and benefits of herbal drugs in our ancient literature of Ayurvedic and Unani medicine. One of the earliest treatises of Indian medicine, the *Charaka samhita* (1000 B.C) mentions the use of over 2000 herbs for medicinal purpose. According to the WHO survey 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. Exploration of the chemical constituents of the plants and pharmacological screening may provide us the basis for developing the leads for development of novel agents [3].

In addition herbs have provided us some of the very important life saving drugs used in the armamentarium of modern medicine. However, among the estimated 250,000 to 400,000 plant species, only 6% have been studied for biological activity and about 15 % have been investigated phytochemically. This showed a need for planned activity guided phyto-pharmacological evaluation of herbal drugs [4-5].

Medicinal herbs have been an essential part of human society since the civilization started. Medicinal plants are boon of nature to cure a number of ailments of human beings. Practitioners of Ayurveda and Unani system of medicine regularly employ a large number of Indian medicinal plants as antibiotic agents. In many parts of the world medicinal plants are used against bacterial, viral and fungal infections. Our country represents a source of genetic diversity of plants [6].

In recent years multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. In addition to this, antibiotics are sometimes associated with adverse effects on the host like hypersensitivity [7]. Hence in the current study, the *In-vitro* antifungal activity of certain Indian medicinal plants was carried out to overcome the problem of drug resistance due to their indiscriminate use against dermatophytic fungi i.e, *Microsporum gypseum* and *Trichophyton mentagrophytes.*

MATERIALS AND METHODS

Plant Materials

Some of the common medicinal plants like *Achyranthes aspera* (Amaranthaceae), *Acalypha indica* (Euphorbiaceae), *Euphorbia hirta* (Euphorbiaceae), *Lindenbergia indica* (Scrophulariaceae), *Parthenium hysterophorus* (Compositae) and *Peristrophe bicalyculata* (Acanthaceae) were collected in the morning from different localities of Agra city. Fresh plant materials were washed under running tap water and then with distilled water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preparation of Extracts

For the preparation of solvent extract, 25 g of air-dried powder of the medicinal plants were taken separately with 150 ml of organic solvents (Ethanol) and were taken into the soxhlet apparatus which was run up to 48 hrs till the green colour of the plant material disappeared. After which the extracts were collected and stored at 4^{o}C in airtight bottles.

Preparation of Medium

Sabouraud Dextrose Broth medium (SDB) was prepared by dissolving Dextrose and Peptone in the ratio 40:10 in distilled water (1000 ml), the pH of the solution was adjusted to 7.4 and then sterilized for 15 min at 15 lb pressure in an autoclave.

Microorganisms used

The two human dermatophytic fungi viz *Microsporum gypseum* and *Trichophyton mentragrophytes* were taken as experimental organisms for studying antifungal nature of the plant extracts.

Antifungal Assay [8]

The Dry Mycelial weight method as described by Chandrasekaran (2004), was adapted for evaluating antifungal nature of selected plant extracts, during antifungal assay.

Screening for antifungal activity

The *in vitro* tests were carried out to measure the effects of the leaf extracts on mycelial growth of experimental organisms. To every 25 ml of sterile Sabouraud dextrose Broth medium in Erlenmeyer flasks, 5 ml (low concentration ratio, 1:5) and 10 ml (high concentration ratio, 2:5) of solvent extract of each plant were added separately. The solution in each flask was gently swirled and was again sterilized in an Autoclave. The flasks were inoculated with 6mm inoculum-disc of each experimental organism and incubated at 28±1°C for 14 days. The medium with inoculum disc but without any extract served as control.

RESULTS AND DISCUSSION

The antifungal activity of selected Indian medicinal plants due to Ethanolic extract revealed against experimental organisms is represented in **(Tables 1 & 2)**. The tables depicts that low concentration ratio (1:5) of the plant extract seems to be better than high concentration ratio (2:5) in all the tested plants except in that of *Parthenium hysterophorus* and *Acalypha indica,* where high concentration ratio effected more percentage inhibition of mycelial weight against human dermatophytic fungi.

Among the plants *Achyranthes aspera* shows maximum inhibition of 63.15% due to low concentration ratio, where as 52.63 % inhibition due to high concentration of *Lindenbergia indica* has been observed in mycelial growth of *Trichophyton mentagrophytes.* While the low concentration ratio of *Lindenbergia indica* affected maximum inhibition of 46.15 % in that of *Microsporum gypseum.*

Table 1: Antifungal activity of various medicinal plants against *Microsporum gypseum.*

Sr. No.	Plant Name	Concentration of plant extract	Control weight in mgs.	Dry mycelial Weight	Percentage loss/inhibition (C-T/C × 100)
1	*Achyranthes*	Low conc. (1:5)	43	28	34.88
	Aspera	High conc. (2:5)	43	31	27.90
2	*Acalypha*	Low conc. (1:5)	43	30	30.32
	Indica	High conc. (2:5)	43	28	34.88
3	*Euphorbia*	Low conc. (1:5)	43	26	39.53
	Hirta	High conc. (2:5)	43	29	32.55
4	*Lindenbergia*	Low conc. (1:5)	43	23	46.51
	indica	High conc. (2:5)	43	29	32.55
5	*Parthenium*	Low conc. (1:5)	43	31	27.90
	hysterophorus	High conc. (2:5)	43	27	37.20
6	*Peristrophe*	Low conc. (1:5)	43	29	32.55
	bicalyculata	High conc. (2:5)	43	32	25.58

Note: Each datum is the average of three independent determinations

Among the plants *Achyranthes aspera* shows maximum inhibition of 63.15 % due to low concentration ratio, where as 52.63% inhibition due to high concentration of *Lindenbergia indica* has been observed in mycelial growth of *Trichophyton mentagrophytes.* While the low concentration ratio of *Lindenbergia indica* affected maximum inhibition of 46.15% in that of *Microsporum gypseum.*

Table 2: Antifungal activity of various medicinal plants against *Trichophyton mentagrophytes.*

Sr. No.	Plant Name	Concentration of plant extract	Control weight in mgs.	Dry mycelial Weight	Percentage loss/inhibition (C-T/C × 100)
1	*Achyranthes*	Low conc. (1:5)	38	14	63.15
	Aspera	High conc. (2:5)	38	26	31.57
2	*Acalypha*	Low conc. (1:5)	38	20	47.36
	Indica	High conc. (2:5)	38	19	50.00
3	*Euphorbia*	Low conc. (1:5)	38	26	31.57
	Hirta	High conc. (2:5)	38	24	36.84
4	*Lindenbergia*	Low conc. (1:5)	38	20	47.36
	indica	High conc. (2:5)	38	18	52.63
5	*Parthenium*	Low conc. (1:5)	38	23	39.47
	hysterophorus	High conc. (2:5)	38	30	21.05
6	*Peristrophe*	Low conc. (1:5)	38	27	28.94
	bicalyculata	High conc. (2:5)	38	22	42.10

Note: Each datum is the average of three independent determinations.

Between the experimental organisms tried *Trichophyton mentagrophytes* seemed to be more susceptible than *Microsporum gypseum* towards all plant extracts tested except in that of *Peristrophe bicalyculata* based on percentage inhibition affected in both experimental organisms. The order of plant extracts affecting percentage inhibition is shown to be in descending manner:

In *Microsporum gypseum* at low ratio:

Lindenbergia indica > Euphorbia hirta > Achyranthes aspera > Peristrophe bicalyculata > Acalypha indica > Parthenium hysterophorus.

In *Microsporum gypseum* at high ratio:

Parthenium hysterophorus > Acalypha indica > Lindenbergia indica = Euphorbia hirta > Achyranthes aspera > Peristrophe bicalyculata.

In *Trichophyton mentagrophytes* at low ratio:

Achyranthes aspera > Lindenbergia indica = Acalypha indica > Parthenium hysterophorus > Euphorbia hirta > Peristrophe bicalyculata.

In *Trichophyton mentagrophytes* at high ratio:

Lindenbergia indica > Acalypha indica > Peristrophe bicalyculata > Euphorbia hirta > Achyranthes aspera > Parthenium hysterophorus.

Among the plants *Achyranthes aspera* shows maximum inhibition of 63.15 % due to low concentration ratio, where as 52.63 % inhibition due to high concentration of *Lindenbergia indica* against *Trichophyton mentagrophytes* has been observed. While the low concentration ratio of *Lindenbergia indica* affected maximum inhibition of 46.15 % in that of *Microsporum gypseum.* On the other hand minimum inhibitory effects of 25.58 % due to *Peristrophe bicalyculata* in *Microsporum gypseum,* 21.05 % by *Parthenium hysterophorus* in *Trichophyton mentagrophytes* at high ratio had been observed.

Further the degree of susceptibility of experimental organisms towards various plant extracts tested was found to be variable; the results indicated that maximum fungicidal effects were obtained at lower concentration, which suggested that the antifungal substances contained in the extracts were fungicidal at lower concentrations.

Fig 1: Percentage inhibition showed by plants in two different concentrations against *Microsporum gypseum.*

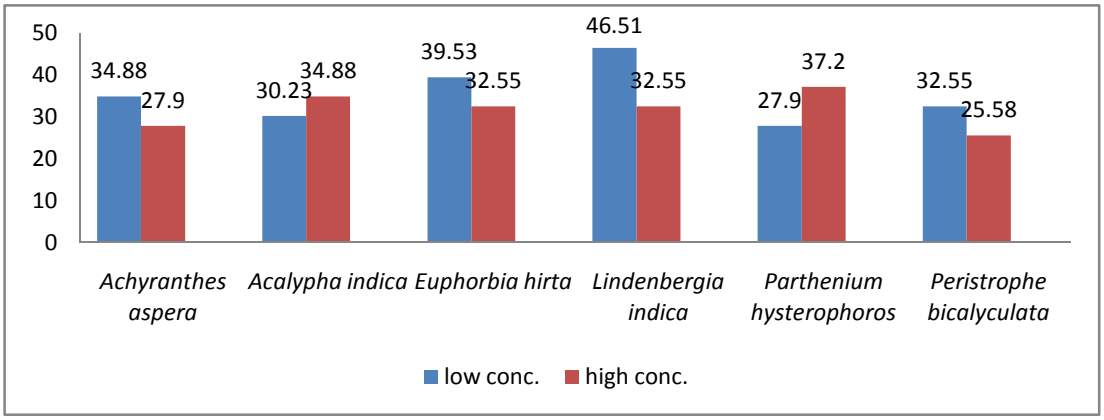
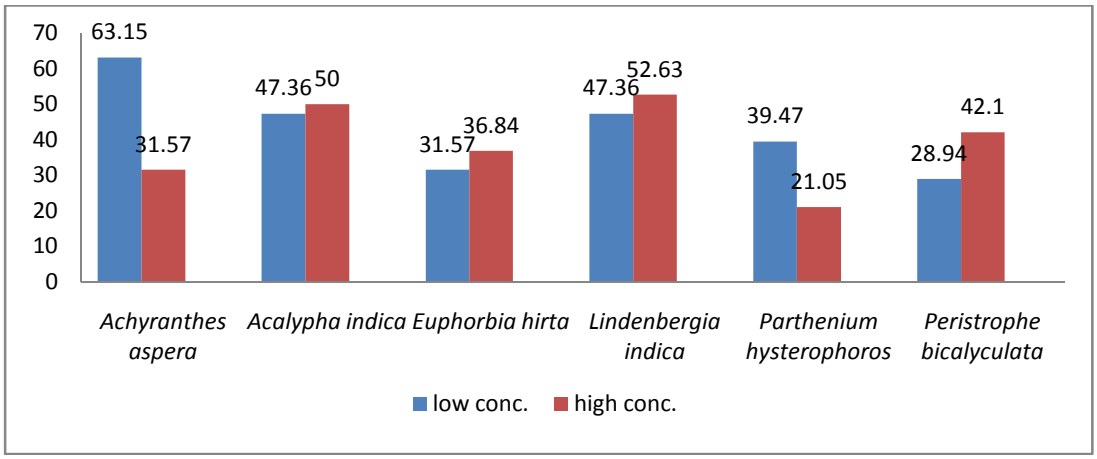


Fig 2: Percentage inhibition showed by plants in two different concentrations against *Trichophyton mentagrophytes.*



The tested plants provided promising sources of their substances with antifungal activity. It was noticed that due to ethanolic extraction, more phytocompounds are being present in the various plant species tested which are of pharmacognostic in nature [9-10].

The maximum inhibitory effects due to *Achyranthes aspera* is correlated to various primary and secondary metabolites such as Alkaloids, Saponins, Glycosides, Phenols, Flavonoids, Anthraquinones, Terpenoids & Steroids which can be quantified for application in Pharmaceutical industry, while other plant species also showed promising results of antifungal nature.

CONCLUSION

These medicinal plants particularly *Achyranthes aspera* is commonly found as road side and at waste places throughout India. The plant is used in treating various ailments including eruptions. It is reported to contain Alkaloids, Saponins, Glycosides, Phenols, Flavonoids, Anthraquinones, Terpenoids and Steroids. The pharmacological studies made earlier confirmed the therapeutic value of *Achyranthes aspera* [11]. Presence of wide range of chemical compounds to this plant could serve as lead for the development novel agents. The present study further confirms the antifungal activity of *Achyranthes aspera* as well as other medicinal plant species employed. Further studies including standardization of the extracts, identification, isolation of active principles and Pharmacological studies of isolated compounds may serve for the purpose of use of specific extract in specific herbal formulation in treating various skin diseases due to dermatophytic fungi.

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