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Effect of plant extracts on the growth of *Microsporum gypseum*

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Abstract
The aqueous extracts at two different concentrations (5% and 10%) of *Azadirachta indica, Lawsonia inermis, Allium sativum, Murraya koenigii, Ocimum sanctum* were used to test their antifungal properties against the keratinophilic fungus *Microsporum gypseum* The present study revealed that *Allium sativum* and *Ocimum sanctum* at 10% conc. were more pronounced compared to all the other extracts followed by *Azadirachta indica, Lawsonia inermis* and *Murraya koenigii.*

Keywords: *Allium sativum*, *Azadirachta indica*, *Lawsonia inermis*, *Microsporum gypseum, Murraya koenigii, Ocimum sanctum*

INTRODUCTION

Different plant parts have been widely used for the preparation of folk remedies. Apart from leaves, fruits and seeds, inflorescence has also been screened for their antimicrobial properties. Green plants because of their vast diversities contain a wide spectrum of plant defense chemicals, most of which make vital contribution to the list of medicines for human even today. At the time of 2500-600 BC, large number of plants has been reported in ayurvedic literature which possesses medicinal properties. The Egyptians, Greeks and Romans made use of materials of plant origin in human chemotherapy. The study of green plants for their anti-microbial activity had started since ancient times. Greeks and Romans used the juices of walnut shells against infectious fungal diseases of skin. Mixtures of certain vegetable oils were used by Egyptians for the preservation of mummies from protein decomposing bacteria.

In the recent past several attempts were made to screen various plants and plant products for their antifungal activity against the pathogenic fungi, in view of their low phytotoxicity and systemic activity Mahadevan [1] reported that several plants contain free formed chemicals, capable of inhibiting the germination and growth of pathogenic fungi. Therefore, in recent years attention has been paid by various researchers towards the screening of some higher plants for their fungitoxic properties. Allelochemicals are substances produced by higher plants that selectively inhibit the growth of microorganisms (virus, bacteria and fungi). Allelopathic agents encompass a wide array of chemical types, including volatile mono and sesqui terpenoids, phenyl propanoids, quinines, coumarins, flavonoids, tannins and other phenolics and cyanogenic glycosides.

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Antifungal activity of *Capillipedium foetidum* oil was studied by Garg and Jain [2] against nine human and plant pathogenic fungi. The oil showed excellent activity against most of the organisms. Garg and Jain [3] studied the biological activity of the essential oil of *Piper betle L.* and found it to be effective against keratinophilic fungi *Arthroderma benhamiae*, *Microsporum gypseum, Trichophyton mentagrophytes, Ctenomyces serratus.* Garg and Jain [4] studied the antigungal activity of *Luvunga scadens* against some keratinophiilic fungi using filter paper disc agar diffusion technique. The oil showed very good to moderate inhibitory effect against the fungi.

Jatisatienr *et al.* [5] studied the effect of the extract from eight species of medicinal plants on growth of selected plant pathogenic molds and dermatophytes of all the extracts, the extract obtained from *Acorus calamus* had a complete fungistatic effect on spore germination of all dermatophytes.

Kader *et al.* [6] studied the effect of aqueous extracts of *Allium sativum, Nigella sativa and Lawsonia inermis*. All the three plants inhibited the growth of dermatophytes. However, the aqueous extract of *Allium sativum* was found to be most effective. Kalemba [7] investigated a number of essential oils and their constituents for their antimicrobial properties against a series of bacteria and fungi. Some of the essential oils were found to possess strongest antimicrobial properties among those tested. Okunji *et al.* [8] reported an antifungal spirostanol saponin from fruit pulp of *Dracoena mani* against six pathogenic fungi and six dermatophytes of which it was strongly active towards viz., *Trichophyton mentagrophytes, T. tonsurans, T.soudanese, T.rubrum, M. audouinii, Cladosporium spp. and Geotrichum spp.* Qureshi *et al.* [9] evaluated the inhibitory nature of extracts of eighteen plant species against three keratinophilic fungi. Different extracts exhibited different rates of inhibition on these fungi.

Rai [10] evaluated the fungitoxic activity of crude extract of *Parthenium hysterophorus*. The experiments revealed that leaf extracts greatly inhibited the growth of *Epidermophyton floccosum, Trichophyton rubrum* and *Microsporum gypseum.* Singh [11] studied the efficacy of seed extracts of *Embelia robusta, Grevillea robusta, Ipomoea [Pharbitis] nil* and *Saraca indica* against dermatophytes. All extracts were fairly active against some of the test fungi. Singh and Singh [12] studied the antifungal activity of some plant extracts against dermatophytes and some related keratinophilic fungi; almost

all the plant extracts inhibited fungal mycelial growth, especially at 10% concentration. . Thus the utilization of plant resources appears to be an indigenous, non-toxic source of disease control. At present many works done on higher plants reported that the plant extracts possess antimicrobial activity which helps in controlling many diseases of plants, animals including the humans. In the present study, the effect aqueous extracts at two different concentrations (5% and 10%) of *Azadirachta indica, Lawsonia inermis, Allium sativum, Murraya koenigii, Ocimum sanctum* were used to test their antifungal properties against the keratinophilic fungus *Microsporum gypseum*.

MATERIALS AND METHODS

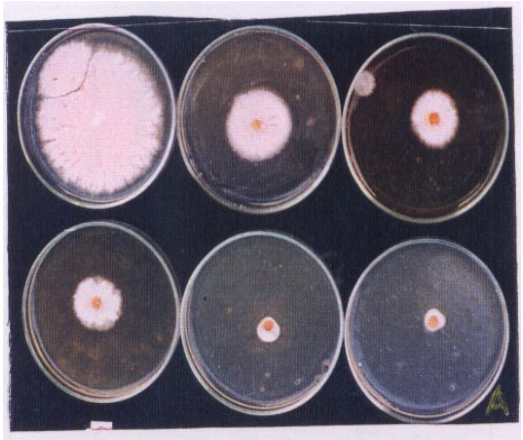
In the present study, plants (*Azadirachta indica, Lawsonia inermis, Allium sativum, Murraya koenigii, Ocimum sanctum*) have been used to test their antifungal properties against the keratinophilic fungus *Microsporum gypseum.* For crude extraction,ten grams of plant material was washed and crushed with the help of mortar and pestle by adding 10 ml of sterilized distilled water. The crude material was then filtered through double layered muslin cloth and filter paper. The filtrate obtained was further filtered through a milipore seitz filter for the purpose of sterilization. The filtrate thus obtained is used for further studies.

In vitro screening of the effect of plant extracts on the radial growth of Microsporum gypseum using poisoned food technique.

The effect of plant extracts on mycelial growth was studied in *in vitro* condition on Sabouraud's Glucose Agar medium (SGA). The medium supplemented with desired concentrations of plant extracts was poured in petriplates. These petriplates were inoculated with 5mm diameter mycelial disc taken from the margins of 8-10 day old colony raised on SGA. SGA without plant extract served as control. Three replicates of each concentration were maintained. The inoculated plates were incubated at 28±2^{0}C for seven days. The diameter of the colony was measured on 3rd, 5th and 7th days.

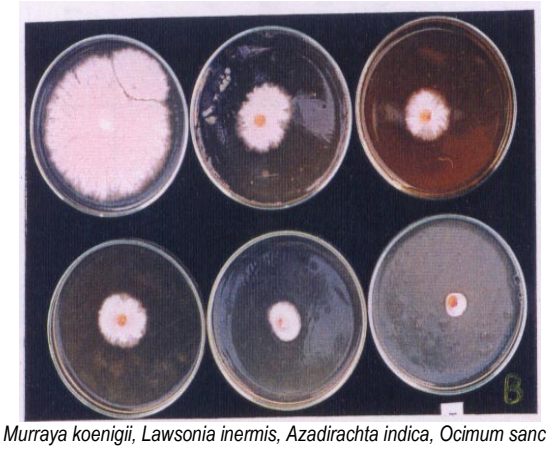
RESULTS

Aqueous extracts of leaves and bulbs of *Allium sativum*, aqueous leaf extracts of *Murraya koenigii, Azadirachta indica, Lawsonia inermis and Ocimum sanctum* at two different concentrations (5% and 10%) were screened for their antifungal properties against *Microsporum gypseum*. The results were recorded on 3rd, 5th and 7th days and are presented in the Tables (1-4) and figures (1, 2).



(Clock-wise – Control, *Murraya koenigii, Lawsonia inermis, Azadirachta indica, Ocimum sanctum and Allium sativum*)

Fig 1. Effect of 5% concentration of different plant extracts on the growth of *Microsporum gypseum*



(Clock-wise – Control, *Murraya koenigii, Lawsonia inermis, Azadirachta indica, Ocimum sanctum and Allium sativum*)

Fig 2. Effect of 10% concentration of different plant extracts on the growth of *Microsporum gypseum*

Table 1. Effect of different plant extracts on the growth (in cm)* of *Microsporum gypseum* (3rd day)

Plant Extracts	Concentration of plant extracts			
	C1	t-value	C2	t-value
Control	2.96 ± 0.08	-	2.96 ± 0.08	-
Murraya koenigii	2.20 ± 0.02	9.5***	1.58 ± 0.02	69.0 ***
Lawsonia inermis	1.80± 0.02	14.5 ***	1.45 ± 0.02	18.8 ***
Azadirachta indica	1.73 ± 0.01	15.3 ***	1.45 ± 0.02	18.8 ***
Ocimum sanctum	1.21 ± 0.037	20.58 ***	0.75 ± 0.02	27.6 ***
Allium sativum	0.65 ± 0.02	28.87 ***	-	-

C1= 5 % conc., C2= 10% conc.
# Mean ± S.E.
*, **, *** P ≤ 0.05, 0.025, 0.010 respectively

Table 2. Effect of different plant extracts on the growth (in cm)* of *Microsporum gypseum* (5th day)

Plant Extracts	Concentration of plant extracts			
	C1	t-value	C2	t-value
Control	4.2 ± 0.1	-	4.2 ± 0.1	-
Murraya koenigii	3.0 ± 0.05	10.9 ***	2.2 ± 0.017	20.5 ***
Lawsonia inermis	2.5 ± 0.05	13.6 ***	1.9 ± 0.028	22.3 ***
Azadirachta indica	2.2 ± 0.05	18.8 ***	1.84 ± 0.023	23.13 ***
Ocimum sanctum	1.6 ± 0.028	25.2 ***	1.10 ± 0.058	26.9 ***
Allium sativum	1.1 ± 0.05	28.18 ***	0.71 ± 0.016	34.55 ***

C1= 5 % conc., C2= 10% conc.
# Mean ± S.E.
*, **, *** P ≤ 0.05, 0.025, 0.010 respectively

Table 3. Effect of different plant extracts on the growth (in cm)* of *Microsporum gypseum* (7th day)

Plant Extracts	Concentration of plant extracts			
	C1	t-value	C2	t-value
Control	3.5 ± 0.11	-	3.5 ± 0.11	-
Murraya koenigii	2.75 ± 0.14	4.213 ***	1.9 ± 0.028	14.5 ***
Lawsonia inermis	2.45 ± 0.27	3.620 *	1.69 ± 0.02	16.4 ***
Azadirachta indica	1.80 ± 0.08	12.50 ***	1.6 ± 0.028	17.2 ***
Ocimum sanctum	1.40 ± 0.02	19.9 ***	0.9 ± 0.028	23.6 ***
Allium sativum	1.76 ± 0.016	24.90 ***	0.61 ± 0.037	24.91 ***

C1= 5 % conc., C2= 10% conc.
# Mean ± S.E.
*, **, *** P ≤ 0.05, 0.025, 0.010 respectively

Table -1 gives an account of the effect of different plant extracts on the growth of *Microsporum gypseum* recorded on 3rd day. It has been observed that of all the extracts employed in the present study maximum fungal growth inhibition was achieved by the aqueous extract from the bulbs of *Allium sativum* and no fungal growth was recorded at 10% concentration and a minimum growth was recorded at 5% concentration. This was followed by *Ocimum sanctum, Azadiracta indica, Lawsonia inermis* and *Murraya koenigii*. It has been observed that with the increase in concentration of the extract the inhibitory effect became more pronounced.

The effect of different plant extracts on the growth of *Microsporum gypseum* noted on 5th day is summarized in the Table - 2. As was observed on 3rd day maximum fungal growth inhibition was achieved by the aqueous extract of *Allium sativum* since a minimum amount of fungal growth was observed at 5% and 10% concentrations. This was followed by *Ocimum sanctum, Azadiracta indica, Lawsonia inermis* and *Murraya koenigii*. `It has been observed that effect of the aqueous extracts of *Allium sativum and Ocimum sanctum* on the growth *Microsporum gypseum* were more or less similar with a little difference.

Table – 3 gives an account of the effect of different plant extracts on the growth of *Microsporum gypseum* recorded on 7th day. The results obtained were similar to those observed on 3rd and 5th days. The effect of aqueous extracts of *Allium sativum* and Ocimum sanctum was more pronounced of all the extracts followed by *Azadiracta indica, Lawsonia inermis and Murraya koenigii.*

DISCUSSION

The present study revealed that amngst the five extracts tested, the extract of *Allium sativum* was found to be most effective almost completely checking the mycelial growth at 10% concentration showing 83.09% inhibition, followed by *Ocimum sanctum, Azadirachta indica, Lawsonia inermis* and *Murraya koenigii*. The results revealed that all the plant extracts were inhibitory to the mycelial growth. As the concentration of extracts increased in the medium, maximum growth inhibition of the test fungus was recorded.

The antifungal properties of the plant extracts may be due to their antimicrobial substances present in the extract. Cavallito *et al.* [13] reported that *Allium sativum* contains allyl compounds. Allicin (diallyl disulphide) is the active principle of *Allium sativum.* Besides allicin, other active compounds reported in Allium sativum

are allisatin I, allisatin II, garlicm and garlic phytoncide etc. It is still enigmatic that these compounds are allicin [14]. Garcia *et al.* [15] compared the effect of Allium extract on *Aspergillus fumigatus, Aspergillus niger, Candida albicans, Trichophyton mentagrophytes* and *Microsporum gypseum* and with that of several antifungal drugs. Gherbawy [16, 17] studied the response of keratinolytic and keratinophilic fungi to garlic extract and onion oil treatments revealed that all keratinophilic fungi were sensitive to garlic extract and onion oil. Guevara *et al.* [18] studied minimal inhibitory concentration of *Allium sativum* on *Microsporum gypseum*, *M. canis, Trichophyton mentagrophytes* and *Trichophyton rubrum*, and reported varying degree of reaction of these extracts towards different organisms.

Kader *et al.* [6] studied the effect of some medicinal plants on the growth of some dermatophytes. Of all the extracts the extract *Allium sativum* inhibited the growth by 47.5-100%, *Nigella sativa* inhibited growth by 35.13-100% and *Lawsonia alba* inhibited growth by 21.87-100%. Khan *et al.* [19] studied the effect of raw material, from neem tree, neem oil and neem leaves extract on fungi pathogenic to man. Dried plant parts of neem aqueous extracts and eluotropic solvent were tested in agar diffusion test. Different inhibitory effects on *Trichophyton rubrum, T. violaceum, T.mentagrophytes, Epidermophyton floccosum, Microsporum canis, Candida albicans, Fusarium spp*. and *Scopulariopsis brevicaulis* was observed.

Singh and Pandey [20] have conducted fungitoxic studies on bark extract of *Lawsonia inermis* against ringworm fungi. It exhibited absolute toxicity against *Microsporum gypseum* and *Trichophyton mentagrophytes.* Furthermore, the fungitoxicity of the extract remained unaltered at high temperature, on autoclaving and after long storage. This clearly indicates that higher plants are untapped reservoirs of various valuable chemicals. These antipathogenic, antimicrobial chemicals are widely distributed in higher plants belonging to diverse families, genera and species. These may be distributed through out the plant (or) may be localized in certain parts of a plant (or) in its special tissues.

A wide range of plants are still unexplored for their antimicrobial activity, medicine and agriculture. It needs to be demonstrated that the plants with strong antifungal activity may be effectively and beneficially exploited in the control of keratinophiles. Hence, the present study becomes important aspect as the plant extracts employed in the present study have exhibited antifungal properties.

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