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Research Article

Antimycotic activity of the componenets of *Abutilon indicum* (Malvaceae)

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ABSTRACT

The search for novel antifungal agents relies in great part on ethnobotanical information and ethnopharmacologic exploration. Methanol extracts provide a more complete extraction, including less polar compounds, and many of these extracts have been found to possess antifungal properties. Methanolic extracts of various parts of *Abutilon indicum* were tested for their ability to inhibit the aetiological agents of dermal fungal infections in humans. The screening for the antimycotic activity was performed by testing Minimum Inhibitory concentration and Disc diffusion method. Thin layer chromatographic analysis of plant extract used to purify the flavonoid content of plant parts. Quercetin present in these extracts was separated. Methanolic extract of leaves of *Abutilon indicum* shows remarkable antifungal activity against *Trichophyton rubrum*. This study provides a sample large enough to determine the antimycotic properties of *A. indicum* and suggest further studies for a possible therapeutic use.

Keywords: *Abutilon indicum*, antidermatophytic, flavonoids, quercetin, TLC

INTRODUCTION

Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices of for other purposes that suggested potentially useful biological activity¹. Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we could currently characterize as antimicrobial principles, as well accepted². Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases³.

Fungi are ubiquitous in the environment and infection due to fungal pathogens has become more frequent⁴. Skin infection due to dermatophytes has become a significant health problem affecting children, adolescents and adults⁵. Over the past 20 years, there has been a lot of investigation on plants as sources of new antifungal agents. But still there is an immediate need to identify novel sub-

stances active towards pathogens with high resistance⁶. Crude extracts of plants also shown significant antimicrobial activity. So the quality control and quantitative analysis of compounds present in traditional medicine have become a necessary undertaking in research.

One of the most abundant groups of polyphenolic compounds in plants is the flavonoids. Over 4000 different flavonoids occurring in plants have been described, and these compounds have various pharmacological properties such as anti oxidative, anti-inflammatory, diuretic, and antimicrobial activity^{7,8}. Quercetin and other flavonoids have been shown to modify eicosanoid biosynthesis, prevent atherosclerotic plaque formation, antihypertensive, antiarrhythmic effect and act as a prophylactic agent⁹.

Abutilon indicum of family Malvaceae is found throughout tropical and sub tropical region in India, is known as, Atibal in sanskrit. The various parts of plant have claimed to have several traditional medicinal properties. *A.indicum* showed hepatoprotective¹⁰, larvicidal¹¹, hypoglycemic¹², analgesic¹³ and wound healing activity¹⁴. *A. indicum* has been reported best antimicrobial activity against *Bacillus subtilis* and *Chromobacterium* but not effective against *Candida albicans*¹⁵. This paper present the first detailed investigation of the antimycotic properties of this plant aerial part using a large collection of pathogenic strains, belonging to different genera and clinical isolates.

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MATERIALS AND METHODS

Preparation of Methanolic extract

The methanolic extracts of leaves, stem and flowers of *A. indicum* were obtained by maceration method. The above parts of the plant were collected from Vivekanandha college campus, Tiruchengode and identified by the Department of Botany, Vivekanandha college of Arts and Sciences for women, Tiruchengode and placed in the Herbarium for future reference (Voucher No. RUBL- 19910). Quercetin (RM 6191) and Ketaconazole (RM 4322) and DMSO were purchased from Himedia Laboratories (Mumbai, India). *A.indicum* leaves, stem and flowers were collected, separately dried, and powdered. 5g of each plant part was macerated individually with 95% methanol for 16h, percolate was evaporated and residues were collected and named as AIL (*A.indicum* leaf extract), AIS (*A.indicum* stem extract) and AIF (*A.indicum* flower extract).

Thinlayer chromatographic Analysis

Silica gel plates were prepared to 2mm thickness, activated at 100°C for 20 minutes. Toluene: acetic acid (40:20, V/V) was used as the mobile phase for separation of quercetin from plant samples.

Microorganisms

A total of eleven fungal strains were used. Clinical isolates from vaginal swabs, blood, urine, onychomycosis and sputum: *Aspergillus niger*, *A.flavus*, *A.fumigatus*, *Candida albicans*, *C.utilis*, *Fusarium oxysporum*, *F.solani*, *Microsporium gypseum*, *Trichophyton metagrophytes*, *Epidermophyton floccosum*, and *Trichophyton rubrum*. strains were identified according to morphological and biochemical procedures. The strains were cultured in Sabouraud broth or agar and stored in glycerol at 80°C.

Minimum Inhibitory Concentration

MICs were determined by broth dilution method as described earlier¹⁶. Duplicates of serial dilutions of broth and crude extract of *A. indicum* were made. Sabouraud's broth and Potato Dextrose Broth were used for cultivation of yeast and fungus respectively. The MICs were determined against 1×10^9 cells of each culture, as the lowest concentration of extract that reduced the growth of these microbes.

Disc diffusion method

Yeast and fungal broth cultures aliquots were adjusted to ca. 5×10^4 CFU ml⁻¹ were added to respective agar medium and spreaded uniformly. Sterile paper discs (8 mm, Whatmann filterpaper) were impregnated with 50 µl of 25% (v/v, 12.5µg) or 50% (v/v, 25µg) ethanol extracts of plant leaves, stem and flowers and antifungal solution and placed on the culture plates after removing solvent by evaporation. The diameter of the zone of inhibition (mm) around the disk was measured after cultivation of at 24-28°C after 48hours. Quercetin and Ketaconazole were used as positive control and DMSO as Negative control. The values are the means of tests performed in triplicates.

RESULTS AND DISCUSSION

Thin layer chromatographic analysis for Quercetin

200 mg/ml of AIL, AIS and AIF were prepared by dissolving in ethyl acetate were subjected to TLC. 500 microlitre of each sample and standard quercetin were spotted on silica gel. After the evaporation of solvent, plate was placed in the solvent for development. The chromatogram was evaluated under UV light at 254nm and 365nm. Yellowish brown spot formed from each sample was compared to quercetin spot and scrapped, dissolved from the preparative TLC chromatogram and final concentration of each extract was adjusted to 0.1% of flavonoids by addition of the sterile DMSO, as a solvent. These samples were used for Disc Diffusion Method. Separated flavonoids were prepared in four different concentration (2.5, 5.0, 10.0 and 20.0mg/ml) for MICs.

Minimum Inhibitory Concentration (MICs)

Despite advances in antifungal therapies, many problems related to drug resistance and toxicity remains to be solved. This situation highlights the need for advent of safe, novel and effective fungal agents¹⁷. Antimicrobials of plant origin have enormous therapeutic potential. They were effective in the treatment of infectious diseases while simultaneously mitigating many side effects that are associated with synthetic antimicrobials¹⁸. The present investigation reports for the first time the active antimycotic activity of *A.indicum*.

Methanol extract of various parts of the selected plant showed antifungal activities at varying concentration against all eleven tested fungi. Ketaconazole and extracts of *A. indicum* showed fungicidal activity against all the eleven tested fungi were listed in Table 1. Ketaconazole showed strongest activity against *A.niger*, *A.flavus*, *F.oxysporum*, and *F. solani* with MICs of 12.5 µg ml⁻¹. MICs of the plant extracts were higher than those of standard Quercetin for most of the fungi. But the activity was mainly based on the Quercetin content of the *A.indicum* extracts.

Table1. In vitro susceptibilities of fungi to extracts of *Abutilon indicum* Quercetin, and Ketaconazole by broth microdilution method.

Fungi	Quercetin mg ml ⁻¹	AIL* mg ml ⁻¹	AIS** mg ml ⁻¹	AIF*** mg ml ⁻¹	Ketaconazole mg ml ⁻¹
<i>Aspergillus niger</i>	2.5	2.5	5.0	5.0	12.5
<i>Aspergillus flavus</i>	2.5	2.5	5.0	5.0	12.5
<i>Aspergillus fumigatus</i>	2.5	2.5	5.0	5.0	12.5
<i>Candida albicans</i>	10.0	10.0	10.0	10.0	12.5
<i>Candida utilis</i>	5.0	10.0	10.0	5.0	25.0
<i>Fusarium oxysporum</i>	5.0	10.0	5.0	5.0	25.0
<i>Fusarium solani</i>	2.5	10.0	5.0	10.0	12.5
<i>Microsporium gypseum</i>	5.0	10.0	10.0	10.0	25.0
<i>Trichophyton metagrophytes</i>	5.0	10.0	10.0	20.0	25.0
<i>Epidermophyton floccosum</i>	5.0	10.0	20.0	20.0	12.5
<i>Trichophyton rubrum</i>	5.0	20.0	10.0	20.0	25.0

* MICs (mg ml⁻¹) of Ethanolic extract of *A. indicum* Leaves

** MICs (mg ml⁻¹) of Ethanolic extract of *A. indicum* stem

*** MICs (mg ml⁻¹) of Ethanolic extract of *A. indicum* flowers

Table 2. Antimycotic activity of Quercetin and the plant extracts of *Abutilon indicum* estimated by disc diffusion method.

Fungi	Zone of Inhibition (mm)							
	Quercetin		AIL*		AIS**		AIF***	
Concentration (microgram ml ⁻¹)	12.5	25	12.5	25	12.5	25	12.5	25
<i>Aspergillus niger</i>	8.3±0.5	11.3±0.5	3.75±0.5	5.75±0.9	3.75±0.5	6.0±1.1	3.25±0.5	5.5±0.5
<i>Aspergillus flavus</i>	9.5±0.4	11.5±1.5	3.6±0.5	6±1.0	3.6±1.0	6.3±1.1	3.3±0.5	5.6±1.5
<i>Aspergillus fumigatus</i>	6.6±0.5	7.6±1.5	3.75±0.5	6.0±1.1	3.25±0.5	5.75±0.9	3±0.0	5.5±0.5
<i>Candida albicans</i>	7±1.0	9.6±0.5	3.25±0.5	5.7±0.9	3±0.8	5.25±0.9	2.75±0.5	5.75±0.5
<i>Candida utilis</i>	6.3±0.5	8.6±0.4	4	7	2.75±0.5	5±0.8	3	5.25±0.5
<i>Fusarium oxysporum</i>	7±0.0	8.6±0.5	3	4.3±0.5	4±1.0	5	2.6±1.0	4.3±0.5
<i>Fusarium solani</i>	6.3±0.5	10.6±1.5	2.75±0.5	4.5±0.5	2.5±1.0	5.75±0.8	2.5±0.5	5.25±0.9
<i>Microsporum gypseum</i>	5.3±0.0	6.3±0.5	2.75±0.5	4.5±0.5	2.5±1.0	4.25±0.5	2.5±0.5	4.25±0.9
<i>Trichophyton metagraphytes</i>	5.2±0.5	6.0±1.0	2.6±0.5	4.3±0.5	2.6±1.1	4.3±1.1	2.6±0.5	4±1.0
<i>Epidermophyton floccosum</i>	7.3±0.5	9.6±1.5	2.3±0.5	4±1.0	2.3±0.5	4	2.6±0.5	4±1.0
<i>Trichophyton rubrum</i>	7.6±0.7	12.3±1.5	2.5±0.7	4.5±0.7	4±1.0	4±1.4	2.5±0.5	4.5±0.7

Note: Values are mean ±SD n=3 in each group

* (microgram ml⁻¹) of Ethanolic extract of *A. indicum* Leaves

** (microgram ml⁻¹) of Ethanolic extract of *A. indicum* stem

*** (microgram ml⁻¹) of Ethanolic extract of *A. indicum* flower

Out of the eleven tested fungi, *C. albicans* was found to be more resistant at lower concentrations of Quercetin and plant extracts. Leaf extract inhibited growth of *Aspergillus* sp.. at low concentration 2.5 mg/ml. The fungicidal activity of *A. indicum* extracts reported only at higher concentration (10mg/ml) against *M. gypseum*, *T. Metagraphytes*, *E. floccosum*, and *T. rubrum*.

Disc Diffusion Method

Flavonoids are ubiquitous in photosynthesizing cells; preparations containing these compounds have been used to treat human disease. Fungitoxic effect of flavonoids on the mycelial growth was also studied¹⁹. *Abutilon indicum* methanol extract of leaves, stem and flowers were tested for antifungal activity using Disc diffusion method represented in Table.2. Quercetin, Ketoconazole, and *A. indicum* extract significantly and dose dependently inhibited the growth of all the fungi. Leaf extracts was particularly active against *C. utilis* and *A. fumigatus*. Leaf and stem extracts were more effective than Flower extracts at both the concentrations (12.5 & 25 microgram /ml). All the three extracts were less active against *C. albicans* than standards. They produced zones of inhibition of 3-5 mm diameter only.

CONCLUSION

Standardizing the method for determining MIC is important when evaluating the antimicrobial properties of plant extracts, in order to permit comparison of the data generated by different authors. Thin layer chromatographic separation of flavonoids present in aerial parts of *Abutilon indicum* revealed the presence of quercetin. Further studies are under progress to find type quercetin glycoside and mechanism of action. The present study therefore provides some scientific justification for the utilization of extracts from this plant to treat skin disease and useful in the search of novel antifungal agents.

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