

Bioprospecting of some medicinal plants explored for antifungal activity

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ABSTRACT

In the present study, evaluation of different plant parts of fifteen medicinal plants belonging to different families have been screened for *in vitro* efficacy of antifungal activity against phyto pathogenic (*Aspergillus* and *Fusarium* species) as well as human pathogenic fungi (*Candida albicans* and *Microsporum* species) using agar well diffusion assay. The results showed that among fifteen medicinal plants, crude extracts of different solvents viz., petroleum ether, chloroform, ethyl acetate and methanol tested for antifungal activity, twelve plants were found to be effective against one or the other test fungi, among these plants, solvent extracts of *Callistemon lanceolatus* showed significant activity against *C. albicans*, *Microsporum gypseum*, *Cordia dichrotoma* leaves extracts exhibited significant activity against *A. niger*, *A. flavus* and *C. albicans*. *Sphaeranthus indicus* L. whole plant extracts exhibited significant activity against *Aspergillus* spp., *C. albicans* and *Microsporum canis*. Leaves extracts of *Vitex negundo* exhibited significant activity against *A. niger*, *A. flavus*, *F. verticillioides*, *C. albicans* and moderate activity against *F. crookwellense*. Extracts of

Butea monosperma exhibited significant to moderate activity against all test pathogens except *C. albicans*. The obtained results imparts a preliminary piece of significant information regarding the antifungal potentiality of screened medicinal plants with crude solvent extracts, which could be exploit for further isolation and investigation of antifungal agents for crop diseases management and human health.

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INTRODUCTION

Plants are persistently exposed and threatened by diverse pathogenic microorganisms such as fungi, bacteria, nematodes, viruses etc, present in their environments. More than 800 million people in developing countries do not have adequate food supplies and at least 10% of food is lost due to plant diseases. During food grains storage, nearly up to 30% loss is caused grains due to microbial bio-deterioration.¹ The World's annual crop losses as a consequence of disease have been estimated at 25,000 million US dollars; of this fungi carried through seeds forms the major pathogen.²⁻³ Compared to other parasites, fungi are significant destroyers of foodstuffs and grains during storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins.⁴⁻⁵ resulting in terrible impact with regard to diseases and crop production losses.⁶ Till date, more than 100,000 fungal species are considered as natural contaminant of agricultural and food products.⁷ Even though, effective management of plant diseases caused by seed borne fungi can be achieved by the application of fungicides, the same does not holds effective for grains due to pesticide toxicity.⁸ As a result, the existence of microbial resistance has stipulated as a serious problem in agricultural fields⁹ along with many more side effects. The toxicity of synthetics can only be reduced by persistent search for effective, new and safer green fungicides, pesticides as for human and environmental concern.¹⁰

Fungi are also responsible for causing severe threat to human mankind and form one of the major causes of morbidity and mortality.¹¹ In recent decades, fungal infections in human beings, ranges from superficial to deeply invasive or disseminated, and have been increased fiercely. The humid weather, over population and poor hygienic conditions are ideally favors for the fungal growth and these factors are more important in a tropical country like India. As a part and parcel, increased fungal infections can be observed in an immune-compromised population such as organ transplant recipients, cancer and HIV/AIDS patients due prolonged use of drugs, resistance and its toxicity. This will again render the development of multi-drug resistant strains, which has become global menace. The production and availability of antifungal drugs in a market

is less in comparable with antibacterial drugs. Therefore, there is a need to search for new antifungal drugs. The ramification of use of synthetic antifungal agents against plant and human diseases has created awareness among the researchers worldwide, and thus much of the research areas are focused on development of novel, potent and green principle based antifungal agents using medicinal plants, owing to their different mode of action, different target sites with minimized side effects than the conventional available drugs. Though, plants represent a natural repository consists of chemotherapeutants with myriad biological potentiality can provide valuable sources of natural antimicrobial agents, and also by knowing the facts that plant can synthesize inexhaustible bioactive compounds with bearing characteristic chemical structures. Screening of crude plant extracts is a preliminary step to check the biological efficacy rather than screening of pure compounds isolated from plants. Thus, the present study designed to evaluate the *in vitro* antifungal activity of fifteen medicinal plants crude extracts against both plant and human pathogenic fungi.

MATERIALS AND METHODS

Plant sample collection

Fifteen medicinal plant species were collected in and around Mysore district of Karnataka, India. The Voucher specimen of plant material has been deposited and authenticated at the National Ayurveda Dietetics Research Institute, Bangalore. The collected healthy and mature plant materials were washed thoroughly under running tap water for 2-3 times and once with distilled water and dried at room temperature. The dried plant materials were grounded to a coarse powder and then used for extraction using cold extraction method.

Preparation of plant extracts

Fifty grams of shade-dried and coarsely powdered plant material was extracted successively with 250 ml each of petroleum ether, chloroform, ethyl acetate and methanol in the increasing order of their polarity and

kept in a shaker for 24- 48 h. The extracts thus obtained were filtered with Whatman No. 1 filter paper and the filtrate was evaporated to dryness. After complete solvent evaporation, extracts were stored at 4°C in airtight amber bottles for further use.¹²

Test pathogens

Plant pathogenic fungi includes *Aspergillus* spp. such as *A. flavus oryzae*, *A. niger* and *Fusarium* spp. such as *F. crookwellense*, *F. sporotrichioides*, *F. verticillioides* were selected. The cultures were maintained on Potato-Dextrose Agar (PDA) medium. Human pathogenic fungi such as *Candida albicans*, *Microsporum canis* and *Microsporum gypsum* were preserved on Sabouraud's agar (SA) medium employed for the assay.

Antifungal assay by Agar well diffusion method

Antifungal activity of different test solvent extracts (petroleum ether, chloroform, ethyl acetate and methanol) was determined by agar well diffusion method.¹³ An aliquot of 100 µl of each fungal spores suspension to be tested containing approximately 10⁶ spores/ml, was spread with a sterile swab on Potato dextrose agar and Sabouraud's agar plates for plant and human pathogenic fungi respectively. Wells of 8 mm diameter were made on agar medium using sterile cork borer and the test solvent extracts were added into the wells and allowed to diffuse at refrigerator for 2h. The plates were then incubated at in the upright position at room temperature for 3-4 days. After incubation, the diameter of the inhibition zone (mm) was measured. Triplicates were maintained and the data were expressed as mean± standard error. Bavistin and miconazole serves as reference antibiotics.

RESULTS

In the present investigation, antifungal activity of fifteen medicinal plants of different plant parts (Table 1) with different solvents by cold extraction method was evaluated by agar well diffusion method at 100 µg/ml concentration against plant pathogenic fungi such as *Aspergillus* and *Fusarium* species and human pathogenic fungi such as *Candida albicans*, *Microsporum canis* and *Microsporum gypsum* (Table 2). Among fifteen medicinal plants screened for antifungal activity against five plant pathogenic and three human pathogenic fungi with four different solvent viz., petroleum ether, chloroform, ethyl acetate and methanol crude extracts, twelve plants were found to bear antifungal potential and were effective against one or the other test fungi, wherein remaining test plants were completely ineffective against all test fungi.

Petroleum ether leaves extracts of *Andrographis paniculata* (Burm. F.) Wall. Ex. Nees. showed least activity against *F. crookwellense*, *F. sporotrichioides* and moderate activity with chloroform, ethyl acetate and methanol extracts. Whereas petroleum ether, chloroform extracts showed least activity and ethyl acetate, methanol extracts showed significant activity against *A. niger*. All the test solvent extracts did not show any inhibition against *A. flavus*, *F. verticillioides*, *Candida albicans*, *Microsporum canis* and *M. gypseum*. Only ethyl acetate and methanol seeds extracts of *Annona squamosa* L. showed least activity against *F. verticillioides* whereas remaining test solvent extracts did not show any antifungal activity against test fungi. Petroleum ether flower extract of *Butea monosperma* (Lam.) Tuberf. showed moderate activity against *A. niger*, *A. flavus*, *F. crookwellense*, *F. sporotrichioides* but there was no zone of inhibition against *F. verticillioides*, *Candida albicans*, *Microsporum canis* and *M. gypseum*. Chloroform extract showed significant activity against *A. niger*, *A. flavus*, *F. crookwellense*, *F. verticillioides*, *Microsporum canis*, *M. gypseum* and moderate activity against *F. sporotrichioides*, but no activity was found for *Candida albicans*. Ethyl acetate extract showed moderate activity against *A. flavus*, *F. crookwellense*, *F. sporotrichioides* and *M. canis* but no activity was found against *A. niger*, *F. verticillioides*, *C. albicans* and *M. gypseum*. Methanol extract showed significant activity

Table 1: Test medicinal plants employed for antimicrobial activity

Medicinal plants	Family	Plant part used
<i>Andrographis paniculata</i> (Burm. F.) Wall. Ex. Nees.	Acanthaceae	Leaves
<i>Annona squamosa</i> L.	Annonaceae	Seed
<i>Argyrea nervosa</i> (Burm.f.) Bojer	Convolvulaceae	Flower and twig
<i>Asclepias curassavica</i> L.	Asclepiadaceae	Whole plant
<i>Butea monosperma</i> (Lam) Tuberf.	Fabaceae	Flower
<i>Callistemon lanceolatus</i> DC.	Myrtaceae	Leaves
<i>Canthium parvilorum</i> Lam.	Rubiaceae	Leaves
<i>Cissus quadrangularis</i> Linn.	Vitaceae	Leaves
<i>Cordia dichrotoma</i> Forster F.	Boraginaceae	Leaves
<i>Ficus religiosa</i> Linn.	Moraceae	Leaves
<i>Helicteres isora</i> L.	Sterculiaceae	Leaves
<i>Moringa oleifera</i> Lam.	Moringaceae	Leaves
<i>Sphaeranthus indicus</i> Linn.	Asteraceae	Whole plant
<i>Streblus asper</i> Lour.	Moraceae	Leaves
<i>Vitex negundo</i> L.	Verbenaceae	Leaves

against *F. verticillioides* and *M. gypseum*, moderate activity against *A. niger*, *F. crookwellense*, *F. sporotrichioides* whereas no activity was observed against *A. flavus*, *C. albicans* and *M. canis* when compared to reference antibiotics.

None of the test solvent leaves extracts of *Callistemon lanceolatus* DC. showed antifungal activity against plant pathogenic fungi whereas significant activity was observed with all the test solvent extracts against *C. albicans* and chloroform, ethyl acetate extracts showed significant and moderate activity against *M. canis* respectively but no activity was observed against *M. gypseum* with the test solvent extracts. Petroleum ether leaves extract of *Canthium parvilorum* Lam. showed least activity against *A. flavus* only, but no activity was found against other test pathogens. Chloroform extract displayed no antifungal activity against the test pathogenic fungi. Ethyl acetate showed least activity against *F. crookwellense*, whereas the other test pathogens were found to be ineffective. Methanol extracts showed least activity against *A. niger*, *A. flavus* and *F. crookwellense* but the extract was ineffective against rest of the test pathogens.

Leaves extracts of *Cissus quadrangularis* L. showed least activity against *A. flavus* compared to reference antibiotic whereas the test solvent extracts were found to be ineffective against other test plant and human pathogenic fungi. Petroleum ether, chloroform and methanol leaves extracts of *Cordia dichotoma* Forster F. exhibited moderate to significant activity against *A. niger* whereas ethyl acetate extract did not show any antifungal activity. Petroleum ether, chloroform extracts showed moderate and ethyl acetate and methanol extracts exhibited significant activity against *A. flavus*. Moderate activity was observed with the test solvent extracts against *F. crookwellense* whereas only ethyl acetate and methanol extracts was found to be significant against *F. sporotrichioides*. No activity was found against *F. verticillioides*, *M. canis* and *M. gypseum* whereas *C. albicans* was significantly inhibited with the test solvent extracts. Leaves extracts of *Ficus religiosa* L. did not show any antifungal activity against plant pathogenic fungi whereas only petroleum ether and methanol extracts showed moderate and significant antifungal activity against *C. albicans* whereas other test solvent extracts did not showed any inhibition zone. Also, *M. canis* and *M. gypseum* was found to be resistant to all test solvent extracts. Chloroform and ethyl acetate extracts of *Moringa oleifera* Lam. leaves exhibited significant antifungal activity against *A. niger*. Moderate activity was observed with petroleum ether, chloroform, ethyl acetate extracts against *F. crookwellense* and also moderate was exhibited against *F. sporotrichioides* with petroleum ether, chloroform and metha-

Table 2: Antifungal activity of cold extraction of successive solvent extracts of medicinal plants and standard on plant pathogenic fungi and dermatophytes at 100 µg/ml

Plant name	Parts used	Solvent extracts	Plant pathogenic bacteria								Human pathogenic fungi									
			1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8		
<i>Andrographis paniculata</i> (Burm. F.) Wall. Ex. Nees.	Leaves	P	16.00±1.00	0.00±0.00	8.66±0.57	14.33±0.57	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		C	11.66±1.52	0.00±0.00	20.33±0.57	20.33±0.57	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		E	20.00±0.00	0.00±0.00	26.00±0.00	21.00±1.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		M	21.00±1.00	0.00±0.00	15.66±0.57	22.00±1.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>Ammonia squamosa</i> L.	Seeds	P	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		E	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		M	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>Argyrea nervosa</i> (Burm.f.) Bojer	Flower and twig	P	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		E	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		M	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>Asclepias curassavica</i> L.	Whole plant	P	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		E	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		M	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>Butea monosperma</i> (Lam) Tuberf.	Flower	P	18.66±0.57	30.66±1.15	36.66±2.88	30.66±1.15	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		C	20.33±0.57	29.00±1.00	40.66±0.57	32.00±1.00	42.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		E	0.00±0.00	27.00±1.00	33.66±1.52	29.33±0.57	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		M	16.33±1.15	0.00±0.00	31.00±1.73	35.00±1.00	40.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>Callistemon lanceolatus</i> DC.	Leaves	P	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		E	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		M	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>Canthium parviflorum</i> Lam.	Leaves	P	0.00±0.00	15.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
		E	0.00±0.00	0.00±0.00	18.33±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		M	10.00±0.00	15.33±0.57	25.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

nol extracts. None of the test solvent extracts showed antifungal activity against *A. flavus*, *F. verticillioides*, *C. albicans*, *M. canis* and *M. gypseum*.

Sphaeranthus indicus Linn. whole plant petroleum ether, chloroform, ethyl acetate and methanol extracts exhibited significant activity and least activity with chloroform extract against *A. niger*. Moderate activity was observed against *A. flavus* with all the test solvent extracts. Only ethyl acetate extract showed significant activity against *C. albicans*. All the test solvent extracts exhibited significant activity against *Microsporum canis*, whereas none of the test solvent extracts showed antifungal activity against *F. crookwellense*, *F. sporotrichioides*, *F. verticillioides* and *M. gypseum*. *Streblus asper* Lour. leaves showed least antifungal activity against *Aspergillus niger* and moderate activity against *A. flavus*. Moderate zone of inhibition was observed with petroleum ether, chloroform and methanol extracts against *F. crookwellense*. Least and moderate antifungal activity was observed with petroleum ether, chloroform and ethyl acetate, methanol extracts respectively against *F. sporotrichioides*. Only petroleum ether and ethyl acetate extracts showed least activity against *M. canis* whereas no zone of inhibition was found against *F. verticillioides*, *C. albicans* and *M. gypseum*.

Leaves of *Vitex negundo* L. exhibited significant antifungal activity against *A. niger* and *A. flavus* with all the test solvent extracts, whereas moderate activity was found against *F. crookwellense* with chloroform, ethyl acetate and methanol extracts but there was no zone of inhibition with petroleum ether extract. All solvent extracts showed significant activity against *F. verticillioides* except ethyl acetate extract. Only ethyl acetate and methanol extracts showed significant activity against *C. albicans* in comparison with reference antibiotic. There was no antifungal activity against *F. sporotrichioides*, *M. canis* and *M. gypseum*. Four different test solvent extracts of *Argyrea nervosa* (Burm. F.) Bojer. flower & twig, whole plant of *Asclepias curassavica* L. and leaves of *Helicteres isora* L. were found to be ineffective against all the test pathogenic plant pathogenic and human pathogenic fungi.

DISCUSSION

In current scenario, there is wide search for new effective antimicrobial agents from natural sources. Numerous studies have been carried out with the various plants extracts for exploration of their antimicrobial activity against pathogenic microorganisms. Thus the present investigation indicates that the majority of the plants tested are an important source of antifungal compounds that may provide renewable sources of useful antifungal drugs against plant and human pathogenic fungi. In accordance with our obtained results, the investigation by Radha *et al.*,¹⁴ revealed significant inhibitory activity against *Candida albicans* and *Aspergillus flavus* with methanol leaf and acetone stem extracts of *Andrographis paniculata* respectively. Another study revealed antifungal against *Penicillium* sp and *A. flavus* but did not show activity against *C. albicans*, *A. niger*, *A. oryzae*.¹⁵ *Annona squamosa* seed extracts against test pathogenic fungi, exhibited scanty reports. Significant antifungal activity was reported against *Trichophyton rubrum*, *A. niger*, *A. flavus* and *Candida albicans*.¹⁶ Two fractions (CVP-1 and CVP-2) of roots and flowers of *Butea monosperma* (Lam.) were tested against *Alternaria*, *Fusarium* and *A. flavus*. Fraction CVP-1 was found significant activity against *Fusarium* followed by *Alternaria* and *A. flavus*, fraction CVP-2 exhibited significant activity against *Alternaria*, *Fusarium* and least activity against *A. flavus*.¹⁷ In comparable with our results with *Callistemon lanceolatus*, the moderate antifungal activity against *Candida albicans* and *A. niger* was found with petroleum ether, ethyl acetate but no activity was found with methanol extracts of *Callistemon citrines*.¹⁸ According to Merinal and Boi,¹⁹ showed that the ethanol leaf extract of *Cissus quadrangularis* with maximum inhibition against *Candida albicans* followed by *Aspergillus flavus* and *Fusarium* sp. Diethyl ether extract exhibited significant activ-

ity against *Aspergillus flavus* but there was no inhibitory effect on *Fusarium solani* and also the aqueous extract did not show any inhibitory zone against tested pathogens. The inhibitory activity against *Candida albicans* and *Candida tropicalis* have been reported.²⁰ by The use of solvents might have influenced the phyto-compounds extraction responsible for antifungal activity. Suman *et al.*,²¹ reported significant activity against *A. niger* and *C. albicans* with methanol leaf extract. Whereas, no inhibitory activity was recorded against *Aspergillus niger*, *Penicillium* spp., *Scytalidium* spp.²² with leaf extracts of *Cordia myxa* in comparison with our data using *C. dichotoma*. Aqueous leaf extract showed significant activity against *Aspergillus niger* and moderate activity against *Candida albicans*.²³ Ramakrishnaiah and Hariprasad,²⁴ reported least activity with methanol leaf extract against *A. niger* whereas no activity was observed with diethyl ether extract. In accordance with the obtained results in our study infers that solvent might have influenced in the inhibitory activity. Steam distillate of *Moringa oleifera* Lam, was evaluated for antifungal activity²⁵ resulted in zone of inhibition against *A. niger* followed by *A. oryzae*, *A. terreus* and *A. nidulans*. Devendra *et al.*,²⁶ reported antifungal activity against *Aspergillus niger* and *Candida albicans* with leaf extract. In another study,²⁷ the antifungal activity of methanol and ethanol leaf extracts against *Penicillium* sp and *Aspergillus* sp resulted in the growth was found to be decreased with increasing concentration of the extracts. Significant activity using ethanol leaves extract of *Sphaeranthus indicus* against *Candida albicans* was reported.²⁸ In earlier investigation,²⁹ showed significant activity against *Microsporum gypseum* compared to standard antibiotic with flower extract whereas moderated activity was observed against *A. fumigates*. Though all the parts of this plant has showed significant antifungal activity demonstrated from the earlier reports, whole plant extracts in our study was also successful in revealing the antifungal activity against test pathogens. Another study reported anti-candidal activity with leaf extract and exhibited potential controlling germ tube production by *C. albicans* adhesion.³⁰ The present study reports the moderate to least antifungal activity against *Aspergillus* spp. *Fusarium* spp. and *Microsporum* spp. is the first report as per the literature survey. Least antifungal activity was observed against *Candida albicans* but no activity was found against *A. flavus*, *A. niger* and *Trycophyton mentegrophyte*.³¹ The variation among the results may be due to the location and duration of plant sample collection.

In accordance with obtained results, Devi *et al.*,³² reported least activity against *Trichophyton rubrum* with methanol fruit extract of *Argyrea nervosa* whereas ethyl acetate extract did not show any activity. Badgular *et al.*,³³ reported significant activity against *Cryptococcus neoformans*, *Candida tropicalis*, *Trycophyton rubrum*, *Microsporum furfure*, *Epidermophyton floccosum* with methanol extract of stem bark of *Helicteres isora* whereas the petroleum ether extract showed weak antifungal activity and no reports on antifungal activity against test plant and human pathogenic fungi using leaves extracts of *Asclepias curassavica* and *Canthium parvilorum*. Since, Survey of literature revealed the insufficient research data of antifungal activity using these plants; we made an attempt to disclose the antifungal activity against test pathogenic fungi.

CONCLUSION

The reconnaissance of literature survey on antifungal activity indicates that variety of plant species have known to possess antifungal activity. From the present study it can be concluded that the exploitation of plant extract employed in our work as antifungal agents are fewer as per the literature survey. As the present investigation has been carried out using crude plant extract against panel of phyto and human pathogenic fungi, the results obtained serve as a preliminary data with paramount importance for the search of newer bioactive compounds. Though the importance of green pesticides, fungicides and also as antibiotics over

synthetic chemicals are gaining much interest in the current era, studies on plant extracts and its biologically active principles for antifungal activity has to be focused much in upcoming research that could serve in the maintenance of human and plant health.

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CONFLICTS OF INTEREST

The authors have declared that there is no conflict of interests.

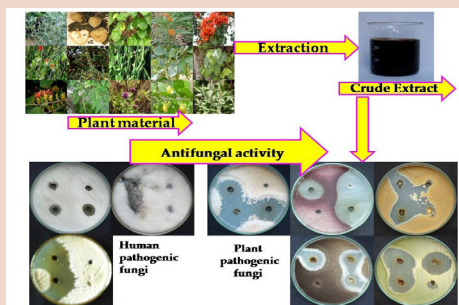
ABBREVIATION USED

C. albicans: Candida albicans, **A. niger:** *Aspergillus niger*, **A. flavus:** *Aspergillus flavus*, **F. verticillioides:** *Fusarium verticillioides*, **F. crookwellense:** *Fusarium crookwellense*, **ml:** millimeter, **h:** hours, **P:** Petroleum ether, **E:** Ethyl acetate, **M:** Methanol, **C:** Chloroform, **M. gypseum:** *Microsporium gypseum*, **µg:** micro gram.

REFERENCES

- Satish S, Raghvendra MP, Mohan DC, Raveesha KA. In-vitro evaluation of the antifungal potentiality of Polyalthia longifolia against some sorghum grain moulds. J Agric Technol. 2010; 6(1): 135-50.
- Agrios GN. Control of plant diseases. Plant pathology. 4th edition. 1997; California: Academic Press.
- Chandler J. Cost reduction in SIT programmes using exosect auto-dissemination as part of area wide integrated pest management. International Journal of Pest Control. 2005; 47(5): 257-60.
- Koirala P, Kumar S, Yadar BK, Premarajan KC. Occurrence of aflatoxin in some of the food and feed in Nepal. Indian Journal of Medical Sciences. 2005; 59(8): 331-6.
- Domijan A, Feraica M, Jurjevic Z, Ivil D, Cvjetkovic B. Fumonisin B1, fumonisin B2, Zearalenone and ochratoxin A contamination of maize in Croatia. Food additives and contaminants. 2005; 22(7): 677-80.
- Montesinos E. Antimicrobial peptides and plant disease control. FEMS Microbiology Letters. 2007; 1(270): 109-19.
- Sati SC, Joshi S. Aspects of antifungal potential of ethnobotanically known medicinal plants. Res J Med Plant. 2011; 5(4): 377-91.
- Harris CA, Renfrew MJ, Woolridge MW. Assessing the risk of pesticide residues to consumers: recent and future developments. Food additives and contamination. 2001; 18(12): 1124-9.
- Satish S, Mohana DC, Raghavendra MP, Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. Journal of Agricultural Technology. 2007; 3(1): 109-19.
- Kavitha HU, Satish S. Eco- friendly management of plant pathogens by some medicinal plant extracts. Journal of Agricultural Technology 2011; 7(2): 449-61.
- CSIR. Wealth of India, publications & information directory. New Delhi, India: CSIR; 1998; 164.
- Parekh J, Nair R, Chanda S. Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. Indian J. Pharmacol. 2005; 37(6): 408-9.
- Govindappa M, Sadananda TS, Channabasava R, Jeevitha MK, Pooja KS, Raghavendra VB. Antimicrobial, antioxidant activity and phytochemical screening of *Tecoma stans* (L.) Juss. Ex Kunth. Journal of Phytology. 2011; 3(3): 68-76.
- Radha R, Sermakkani M, Thangapandian V. Evaluation of phytochemical and antimicrobial activity of *Andrographis paniculata* Nees. (Acanthaceae) aerial parts. Int J Pharm & Life Sci (IJPLS). 2011; 2(2): 562-7.
- Rajalakshmi G, Aruna D, Bhuvanewari B, Venkatesan RS, Natarajan A, Jegatheesan K. Prophylactic effect of *Andrographis paniculata* extracts against fungal species. Journal of Applied Pharmaceutical Science 2012; 2(9): 058-60.
- Vidyasagar GM, Singh SP. A comparative antimicrobial activity of methanolic root, leaf, seed cotyledon extracts of *Annona squamosa* L. International Journal of Pharmacy & Pharmaceutical Sciences. 2012; 4(5): 289.
- Mehta JP, Pandya CV, Parmar PH, Golakiya BA. Isolation, characterization and antimicrobial activity of *Butea monosperma* (Lam.). Iranian Journal of Pharmacology & Therapeutics. 2011; 10(2): 76-81.
- Haque ME, Sultana A, Shibib BA, Islam MM. Antimicrobial, antioxidant and cytotoxic activities of *Callistemon citrinus* (Curtis) Skeels. Dhaka Univ J Pharm Sci. 2012; 11(1): 51-4.
- Merinal S, Boi VSG. In vitro screening of antimicrobial potentials of *Cissus quadrangularis* L. Asian Journal of Plant Science and Research. 2012; 2(1): 58-62.
- Saikia KKR, Borah VV, Kalita MC, Lahkar M. Comparative screening of antibacterial and antifungal activity of six ethno-medicinally important plants of Assam. Int J Pharm Bio Sci. 2013; 4(1): 890-8.
- Suman R, Ruchi S, Ankita S. Phytochemical study and antimicrobial activities of *Cordia dichotoma*. International research Journal of Pharmacy. 2013; 4(12): 53-6.
- Pandey B, Deshpande B, Singh S, Chandrakar V. Estimation of elemental contents of *Cordia myxa* and its antimicrobial activity against various pathogenic microorganisms. Indian J Sci Res. 2014; 4(1): 39-44.
- Rajiv P, Sivara R. Screening for phytochemicals and antimicrobial activity of aqueous extract of *Ficus religiosa* Linn. Int J Pharm Pharm Sci. 2012; 4(5): 207-9.
- Ramakrishnaiah G, Hariprasad T. In vitro antimicrobial activity of leaves and bark extracts of *Ficus religiosa* (Linn.). International Research Journal of pharmacy 2012; 3(9): 178-9.
- Kekuda TRP, Mallikarjun N, Swathi D, Nayana KV, Aiyar MB, Rohini TR. Antibacterial and antifungal efficacy of steam distillate of *Moringa oleifera* Lam. J Pharm Sci & Res. 2010; 2(1): 34-7.
- Devendra BN, Srinivas N, Prasad VSSL, Talluri, Swarnalatha P. Antimicrobial activity of *Moringa oleifera* Lam., leaf extract, against selected bacterial and fungal strains. International Journal of Pharma and Bio Sciences. 2011; 2(3): B13- 8.
- John SA, Selvi BTM. Preliminary phytochemical investigation and antimicrobial activity of *Sphaeranthus indicus* Linn. Asian Journal of Microbiology, Biotechnology & Environmental Sciences. 2011; 13(2): 251-6.
- Meher BR, Mahar S, Rath BG, Sahoo SK. Antimicrobial activity of ethanolic extracts of leaves of *Sphaeranthus indicus*. Der Pharmacia Lettre. 2013; 5(1): 8-10.
- Sharanya M, Oviya IR, Poornima V, Jeyam M. Antifungal susceptibility testing of few medicinal plant extracts against *Aspergillus* spp. and *Microsporium* sp. Journal of Applied Pharmaceutical Science. 2013; 3(8-S1): S12-6.
- Taweechaisupapong S, Choopan T, Singhara S, Chatrchaiwiwatana S, Wongkham. In vitro inhibitory effect of *Streblus asper* leaf extract on adhesion of *Candida albicans* to human buccal epithelial cells. J Ethnopharmacol. 2005; 96(1): 221-6.
- Gautam K, Kumar P. Extraction and pharmacological evaluation of some extracts of *Vitex negundo* Linn. Int J Pharm Pharm Sci. 2012; 4(2): 132-7.
- Devi TS, Padmaja IJ, Harshitha C, Kalyani CS, Lakshmi N, Bhavani AKD. Anti dermatophytic activity on ethnomedicinal plants used by a primitive tribe "Gadabas" of Paderu. Int J Pharm Bio Sci. 2014; 5(2): 292-9.
- Badgujar VB, Jain PS, Badgujar SV. Antifungal activity of stem bark of *Helicteres isora* Linn. Drug Invention Today. 2009; 1(2): 135.

PICTORIAL ABSTRACT



SUMMARY

- Efficacy of fifteen medicinal plants with crude extracts bearing antifungal activity against phyto pathogenic and human pathogenic fungi.
- Crude extracts of *Callistemon lanceolatus* displayed significant activity against *C. albicans* and *Microsporium gypseum*.
- *Cordia dichrotoma* leaves extracts exhibited significant activity against *A. niger*, *A. flavus* and *C. albicans*.
- *Sphaeranthus indicus* L. whole plant extracts exhibited significant activity against *Aspergillus* spp., *C. albicans* and *Microsporium canis*.
- Leaves extracts of *Vitex negundo* exhibited significant activity against *A. niger*, *A. flavus*, *F. verticillioides*, *C. albicans* and moderate activity against *F. crookwellense*.
- Extracts of *Butea monosperma* exhibited significant to moderate activity against all test pathogens except *C. albicans*.

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