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Antifungal Activity Of Coccinia Indica Extracts Against Fungal Pathogens

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ABSTRACT: *In recent years there has been renewed interest in screening plants for novel biologically active products particularly to combat ailments, which have defied synthetics and antibiotics. Medicinal plants offer a vast source of novel natural compounds, often with exciting biological properties. Therefore, the systematic screening of plant extracts or plant derived substances remains an interesting strategy to find new lead compounds. The present study is about the evaluation of antifungal activity. The antifungal activity was tested using dermatophytes like Candida albicans, Microsporum gypsum and Microsporum canis and other fungi on Asthana and Hawker's and Sabourad's dextrose agar media. The organic extracts exhibited stronger antifungal activity than the corresponding aqueous extracts. Among all the extracts tested, petroleum ether and methanol extracts showed maximum activity. The gram positive bacteria were more susceptible compared to gram negative bacteria. The cultivated fruit extracts were most significant with larger zones of inhibition (antifungal: 3-8 mm) followed by leaf, root and stem extracts.*

KEY WORDS: *Coccinia indica,* Extracts, Solvents, Antifungal activity

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I INTRODUCTION

Antifungal drugs available today are not always successful in treating immunocompromised patients due to the ineffectiveness or toxicity that many of them have on the host and hence, there is a need for identification of novel antifungal agents. Plant extracts have been under investigation for their antifungal properties and many have been forced to possess activity (Feresin *et al*., 2001). They have become of interest due to their secondary metabolites exhibiting a wide range of antimicrobial activities.

Antifungal activity of methanol and ethyl acetate extract of mixture of stem and leaves of *Coccinia indica* was tested against *Candida albicans, Aspergillus fumigatus*, *Fusarium culmorum* and *Erwinia amylovora* by Hugo *et al*., (2005). They observed that methanol extract of *Coccinia* showed maximal antifungal activity against *Aspergillus fumigatus* and *Fusarium culmorum* and exhibited poor activity against other organisms. The ethyl acetate extract did not show any inhibition against *Aspergillus fumigatus* and *Candida albicans* but exhibited activity against *Erwinia amylovora* and *Fusarium culmorum*.

Dewanjee *et al*., (2007) tested the antifungal activity of *Coccinia indica* leaves when compared to two other plants using *Candida albicans, Aspergillus niger, Penicillium notatum* and *Penicillium funiculosum*. It was found that *Coccinia indica* was most effective against all the organisms tested. Against all fungal strains the extracts were found to be effective mostly at higher concentrations. *Candida albicans* showed highest sensitivity whilst *Penicillium species* were found least effective.

In addition to the bacterial drug resistance, there has been an increase in the resistance to antifungal agents in the past decade. Reasons being several, very little attention was paid to the study on fungal resistance that lagged behind the research on bacterial resistance. In addition to this, increase in fungal infections intensified the search for new, safe and more efficacious agents to combat serious fungal infections.

Caceres *et al*., (1991) screened 44 plant extracts for antimycotic activity, some of them belonging to cucurbitaceae in Guatemala for treatment of dermatophytic infections. Chandravadana *et al*., (1997) carried out work on antifungal activity of momordicines from *Momordica charantia*. Ashik and Ekramul (2003) reported the significant antimicrobial activity of goniothalamin isolated from *Bryonopsis laciniosa*. Dewanjee *et al*., (2007) worked on the antifungal activity of methanol extract of leaves of *Coccinia indica*. Alessandra *et al*., (2008) reported the antifungal activity of *M. charantia* seed essential oil. Hexane, dichloromethane, ethyl acetate and ethanol extracts of *M. charantia* were investigated against *C. albicans* by Jagessar *et al*., (2008), where it was found that prominent activity was shown by ethanol extracts. Belsem *et al*., (2009) investigated the anticandidal activity of Tunisian *Citrullus colocynthis*.

Material Methods:

Preparation of sample:
200 mg/ml concentration of each extract was prepared in DMSO.
Preparation of spore suspension
From the fresh cultures, spores were collected and transferred in a test tube containing sterilized distilled water (fungi) and Sabourad's dextrose broth (dermatophytes). The spore suspension thus obtained was used for testing antifungal activity.
Antifungal assay
The antifungal activity of petroleum ether, chloroform, ethyl acetate, acetone methanol and aqueous extracts was determined by agar well diffusion method as described by Magaldi *et al*., (2004). The culture plates inoculated with test organisms were allowed to solidify and punched with sterile cork borer (7.0 mm diameter) to make open wells. The open well were filled with 0.05ml or 50 µl of the extract. For normal fungi, the test was carried out on AH plates and for dermatophytes on SDA Plates and incubated at 30^oC and 22^oC respectively for 72 hrs. The zones of inhibition were measured and recorded.

II STATISTICAL ANALYSIS

All tests were conducted in triplicates. The data of all the parameters were statistically analysed and expressed as mean.
The present investigation evaluated the antifungal activity of crude extracts of fruit, leaf, stem and root prepared by using solvents in a soxhlet apparatus. The organisms used in the study were *Candida albicans, Microsporum gypscum, Microsporum canis* (dermatophytes), *Aspergillus fumigatus, Aspergillus flavus and normal fungal strains* i.e. *Cladosporium cladosporoides, Fusarium chlamydosporum, Curvularia lunata, Macrophomina phaseolina* and *Aspergillus niger*. The antifungal activity was analyzed using well diffusion method and the results were tabulated.

III RESULTS AND DISCUSSION

Antifungal activity of fruit

The cultivated variety fruit extracts were found to significantly inhibit almost all the organisms as evidenced by the zones of inhibition ranging from 3-7 mm followed by hybrid and wild variety fruit extracts (Table 1). Petroleum ether extract was more effective against all fungal strains but dermatophytes like *C. albicans, M. gypscum* and *M. canis* were found to be resistant with smaller inhibition zone (1-3 mm) or no inhibition. Acetone and petroleum ether extract showed highest activity against *Fusarium chlamydosporum* and *Macrophomina phaseolina*. Aqueous extracts showed least activity against fungal strains and no activity against dermatophytes. Methanol and chloroform extracts also showed considerable activity against fungi but moderate activity against dermatophytes. Ethyl acetate extract did not inhibit growth of *C. albicans, M. gypscum* and *M. canis* but showed moderate activity against *Cladosporium*, *Fusarium*, *Curvularia*, *Macrophomina* and *Aspergillus niger*.

Antifungal activity of leaf

The extracts from leaves of cultivated and wild varieties showed a range of 2-5 mm zone of inhibition indicating that the organisms were moderately sensitive to the extracts (Table 2). *C. albicans* was the exception as it was not inhibited by any of the extracts. Methanol extract of wild variety was the most active extract as it produced largest zone of inhibition (5 mm) against *Cladosporium* and *Fusarium*. Petroleum ether and acetone extracts were strongly effective against *Cladosporium* and *Fusarium*. Ethyl acetate, acetone, chloroform and aqueous extracts showed little activity against *M. canis* and *M. gypscum* but showed moderate activity against other fungi.

Antifungal activity of stem

Among the two varieties, cultivated variety extracts exhibited strong activity with inhibition zone ranging from 1-3 mm (Table 3). The order of extracts showing antifungal activity was methanol > petroleum ether > ethyl acetate > acetone > chloroform > aqueous extracts. Methanol, petroleum ether and ethyl acetate extracts showed maximum zone of inhibition against *Cladosporium*, *Fusarium* and *Curvularia* (1-3mm) whereas *C. albicans, M. canis, M. gypscum, Aspergillus flavus* and *Aspergillus fumigatus* were highly resistant to all the extracts. Chloroform and acetone extracts showed moderate activity against normal fungi but did not inhibit any of the dermatophytes or showed minimum activity against these organisms. Aqueous extracts of cultivated variety only inhibited *Cladosporium, Fusarium, Curvularia* and *Aspergillus niger* whereas aqueous extracts of wild variety did not inhibit any of the organisms.

Antifungal activity of root

Wild variety extracts produced larger zones of inhibition than the cultivated variety ranging from 1-4 mm (Table 4). Highest activity was shown by methanol extract of wild variety which was comparable to the standard followed by petroleum ether, acetone, ethyl acetate, chloroform and aqueous extracts. Maximum activity was seen against *Fusarium* followed by *Cladosporium*, *Macrophomina* and *Aspergillus niger*. Dermatophytes were highly resistant and inhibited to a lesser extent by methanol and petroleum ether extracts and produced very small zones of inhibition by other extracts.

Based on the results, it was found that fruit extracts were most significant with larger zones of inhibition and inhibiting a broad spectrum of fungi followed by leaf, root and stem extracts.

Similar work was done by other researchers on *Coccinia indica*. Hugo *et al*., (2005) investigated the methanol and ethyl acetate extracts of mixture of stem and leaves against *Aspergillus fumigatus, C. albicans, Erwinia amylovora* and *Fusarium culmorum* and found that they were effectively inhibited by *Coccinia indica* extracts. Methanol extract of leaves was investigated by Dewanjee *et al*., (2007) for antifungal activity against *C. albicans*, *Aspergillus niger, Penicillium notatum* and *Penicillium funiculosum*. Caceres *et al*., (1991) screened 44 plant extracts for antimycotic activity, some of them belonging to Cucurbitaceae in Guatemala for treatment of dermatophytic infections. Ashik and Ekramul (2003) reported the significant antimicrobial activity of goniothalamin isolated from *Bryonopsis laciniosa*. Antifungal activity of hexane, dichloromethane, ethyl acetate and ethanol extracts of *Luffa operculata* against *C. albicans* was investigated by Jagessar *et al*., (2007). Alessandra *et al*., (2008) reported the antifungal activity of *M. charantia* seed essential oil. Hexane, dichloromethane, ethyl acetate and ethanol extracts of *M. charantia* were investigated against *C. albicans* by Jagessar *et al.*, (2008), where it was found that prominent activity was shown by ethanol extracts. Hadizadeh *et al*., (2009) worked on ethanol extracts of *Colocynthis* and other plants against *Alternaria alternata*, *F. oxysporum*, *F. solani* and *Rhizoctonia solani*. Petroleum ether and methanol extracts of leaf and fruit of *M. charantia* were investigated for antifungal activity against *C. albicans* and it was found that methanolic fruit extracts showed higher activity than leaf extracts (Mwambete, 2009). Belsem *et al*., (2009) investigated the anticandidal activity of Tunisian *Citrullus colocynthis*. Our results reported the significant antifungal activity of fruit extracts when compared to leaf, stem and root extracts which correlate with previous findings (Mwambete, 2009).

Our reports are the first account on comparision of antifungal activity of wild and cultivated variety extracts of different parts of *Coccinia indica*. Till date there have been no studies reported on antifungal activity of petroleum ether, chloroform, ethyl acetate, acetone, methanol and aqueous extracts of fruits and roots of *Coccinia indica*. Antifungal activity of *M. gypscum, M. canis, Aspergillus flavus, Cladosporium, Fusarium, Curvularia* and *Macrophomina* against *Coccinia indica* extracts has never been reported. These organisms were investigated for the first time in the present investigation. On review of literature, it was found that methanol and ethyl acetate extracts of stem and leaves have been tested for antifungal activity but petroleum ether, chloroform, acetone and aqueous extracts have been tested for these fungal strains for the first time.

CONCLUSIONS:

From the studies it was concluded that antifungal activity of *Coccinia indica* extracts against microorganisms indicates their medicinal value and supports the claim of the traditional healers that it has been used to relieve pneumonia, dysentery, cough and cold. Susceptibility of various microbes to the organic extracts of this plant suggests an immense scope for developing antimicrobial natural herbal agents.

Even though the present study on these extracts is an addition to the scientific literature, detailed investigations are needed to isolate bioactive principles from these extracts.

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Table - 2 : Antifungal activity of *Coccinia indica* leaf (Zone of inhibition in mm)

S.No	Fungi	Petroleum ether		Chloroform		Ethylacetate		Acetone		Methanol		Aqueous		Standard
		C	W	C	W	C	W	C	W	C	W	C	W	
1	*Candida albicans*	--	--	--	--	--	--	--	--	--	--	--	--	5
2	*Microsporum gypseum*	--	3	--	2	2	3	--	3	4	4	--	--	4
3	*M. canis*	1	2	1	2	2	1	--	4	2	3	--	--	3
4	*Aspergillus fumigatus*	2	1	2	1	3	1	3	1	2	1	--	--	3
5	*A. flavus*	3	1	2	--	--	3	2	2	3	3	1	--	5
6	*Cladosporium cladosporoides*	3	5	1	3	3	4	4	5	--	5	--	1	5
7	*Fusarium chlamydosporum*	2	4	2	4	2	3	2	5	4	5	--	1	4
8	*Curvularia lunata*	1	4	2	3	4	1	2	4	1	3	--	--	5
9	*Macrophomina phaseolina*	2	3	1	2	5	1	4	2	4	3	--	1	5
10	*Aspergillus niger*	4	7	4	3	2	3	3	5	4	2	2	--	4

C - Cultivated ; W - Wild

Table - 3 : Antifungal activity of *Coccinia indica* stem (Zone of inhibition in mm)

S.No	Fungi	Petroleum ether		Chloroform		Ethylacetate		Acetone		Methanol		Aqueous		Standard
		C	W	C	W	C	W	C	W	C	W	C	W	
1	*Candida albicans*	--	--	--	--	--	--	--	--	--	--	--	--	3
2	*Microsporum gypseum*	1	--	1	--	1	--	--	--	--	1	--	--	2
3	*M. canis*	--	--	--	--	1	--	--	--	1	--	--	--	2
4	*Aspergillus fumigatus*	--	1	--	1	--	1	1	--	1	--	--	--	3
5	*A. flavus*	1	--	1	--	1	--	--	1	1	--	--	--	2
6	*Cladosporium cladosporoides*	2	1	2	--	3	--	2	--	2	--	1	--	4
7	*Fusarium chlamydosporum*	2	1	1	--	2	1	1	--	3	2	2	--	5
8	*Curvularia lunata*	3	--	1	--	2	--	1	--	2	1	1	--	4
9	*Macrophomina phaseolina*	1	1	--	1	--	2	1	1	1	1	--	--	4
10	*Aspergillus niger*	1	--	--	1	2	--	1	1	2	1	1	--	4

C - Cultivated ; W - Wild

Table - 4 : Antifungal activity of *Coccinia indica* root (Zone of inhibition in mm)

S.No	Fungi	Petroleum ether		Chloroform		Ethylacetate		Acetone		Methanol		Aqueous		Standard
		C	W	C	W	C	W	C	W	C	W	C	W	
1	*Candida albicans*	--	1	--	--	--	--	--	--	--	--	--	--	3
2	*Microsporum gypseum*	--	2	--	--	--	--	--	1	--	2	--	--	2
3	*M. canis*	--	2	--	1	--	1	--	2	2	--	--	--	2
4	*Aspergillus fumigatus*	--	1	1	--	1	--	1	--	1	1	--	--	3
5	*A. flavus*	--	1	--	2	--	1	--	1	2	--	--	--	2
6	*Cladosporium cladosporoides*	1	1	2	1	2	--	3	2	4	3	--	--	5
7	*Fusarium chlamydosporum*	2	3	2	3	1	2	1	4	5	4	2	--	4
8	*Curvularia lunata*	2	2	3	2	1	2	1	2	4	3	--	1	3
9	*Macrophomina phaseolina*	3	4	2	1	4	3	2	3	4	2	1	--	4
10	*Aspergillus niger*	2	1	1	4	3	2	--	1	3	5	--	--	4

C - Cultivated ; W - Wild

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