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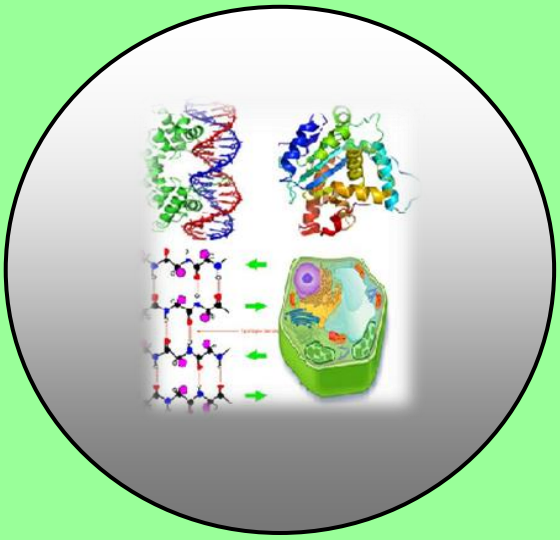

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RESEARCH PAPER

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Assessment of the Antifungal Activity of Five Traditionally Important Medicinal Plants from Bastar, Chhattisgarh

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ABSTRACT

Medicinal plants are the gifts of nature to cure several infectious diseases among human beings. In light of vast potentiality of medicinal plants as therapeutics an endeavour was made to explore the antifungal activity of five traditionally important medicinal plants viz., Amorphophallus campanulatus (Roxb.), Curcuma caesia (Roxb.), Dioscorea alata (L.), Urginea indica (Kunth.) and Zingiber capitatum (Roxb.) from Bastar region of Chhattisgarh. The root, stem and leaf of the plants were successively extracted with different solvents of varying polarity index. The extracts were evaluated for their antifungal efficacy against human pathogenic fungi viz., Aspergillus niger (MTCC 872) and Candida albicans (MTCC 183). The inhibition of both the fungal strains by the extracts of different plants revealed the presence of antifungal potentiality. The results revealed that the organic solvent extracts demonstrated more consistent antifungal activity as compared to aqueous extracts. The acetone root extract of U. indica showed significantly higher zone of inhibition against C. albicans whereas the acetone stem extract of A. campanulatus was found to be effective against A. niger as compared from other plant extracts. The highest activity index was recorded with root acetone extract of U. indica against C. albicans when compared with commercially available antifungal clotrimazole. The findings of the present study offer a scientific validation to the ethnobotanical use of these five important medicinal plants by the traditional community of Bastar in combating several fungal diseases.

Keywords: Antifungal activity, Activity index, Bastar, Medicinal plants and Phytochemical.

INTRODUCTION

Fungal infections have increased dramatically in humans over the past few decades. Aspergillosis is an opportunistic infection of humans related to the inhalation of *Aspergillus* conidia. The resistance of *Aspergillus* strain to some clinically used antifungal compound is a

major problem making to augment a search for compounds more effective and with minimum side effects (Canuto and Rodero, 2002; Curtis *et al*., 2005) *Candida albicans*, a nosocomial pathogen, has been documented to account for 50-70% cases of invasive candidiasis (Paula *et al*., 2006). The treatment of fungal diseases has lagged behind bacterial therapy and fewer antifungal than antibacterial are available for the amelioration of diseases (Fortes *et al*., 2008) However, the clinically used antifungal have various drawbacks in terms of side effects, efficacy, cost and their indiscriminate use which has led to the emergence of resistant fungal strains (Abad *et al*., 2007). Thus, in the current scenario of rapid spread of fungal infections and emergence of multidrug resistance the search for new antifungal compounds of plant origin is the need of the hour.

Medicinal Plants as herbal therapeutics are in great demand in the developed as well as developing countries owing to their wide medicinal and biological applications. They are used in traditional medicine for curing several ailments including bacterial and fungal diseases (Obafemi *et al*., 2006). The active principles of several herbal drugs include the secondary metabolites such as alkaloids, flavonoids, saponins, tannins, phenols, glycosides, resins, steroids and gums (Edeogal *et al*., 2005; Arokiyaraj *et al*., 2009; Kumar *et al*., 2010). These phytocompounds are more complex and specifically found in certain taxa but heterogeneity of secondary metabolites is found in the wild species (Balandrin *et al*., 1985). There is, therefore, urgent need to look for the search of efficacious medicinal plants with the aim of validating their ethnomedicinal importance and subsequently the isolation, purification and characterization of bioactive compounds which will be added to the potential list of drugs in future.

Chhattisgarh is identified as an 'Herbal State' due to its rich repository of medicinal and aromatic plants in many tribal districts including Bastar. The medicinal plants are distributed worldwide although they are most abundant in tropical regions (Calixto, 2000). The Bastar region is dominated by tribal who are traditionally dependant on plants for curing their ailments since long back. Despite of its abundance in medicinal flora the region is relatively less explored with reference to the antifungal properties of these plants. Thus, microbiologists, botanist, natural product chemists and ethnobotanists are in search of bioactive compounds from medicinal plants for curing several infectious diseases (Tanaka *et al*., 2006). So in the light of vast potentiality of medicinal plants as sources of antimicrobial drugs with special reference to antifungal compounds, an effort was made to assess five important medicinal plants from Bastar region for their antifungal activity against pathogenic fungi *viz*., *Aspergillus niger* and *Candida albicans*.

MATERIAL AND METHODS

Medicinal plants

Medicinal plants *viz*., *A. campanulatus (*Roxb.), *C. caesia (*Roxb.), *D. alata (*L.), *U. indica* (Kunth.) and *Z. capitatum* (Roxb.) were selected based on their traditional usage by the tribal community of Bastar district of Chhattisgarh in curing several ailments and its ethno-medicinal importance as herbal drug. Apparently healthy and diseased free plants were selected for the assessment of antifungal activity.

Sample collection and identification

The fresh and healthy root, stem and leaves of *A. campanulatus (*Roxb.), *C. caesia (*Roxb.), *D. alata (*L.), *U. indica* (Kunth.) and *Z. capitatum* (Roxb.) were collected from the fields near,

Shaheed Gundadhur College of Agriculture and Research Station, Jagdalpur (C.G.) after their authentication and identification at Department of Agronomy and Horticulture (SGCARS), Kumhrawand, Jagdalpur, Bastar district, Chhattisgarh, India. The plant samples were washed under running tap water to remove debris and were separated into root, stem and leaf and shade dried for about three weeks to attain a constant weight. The dried samples were mechanically grinded by using a pestle and mortar and finally powdered by laboratory blender (Remi) and stored in separate air tight bottles till use (Fig. 1).

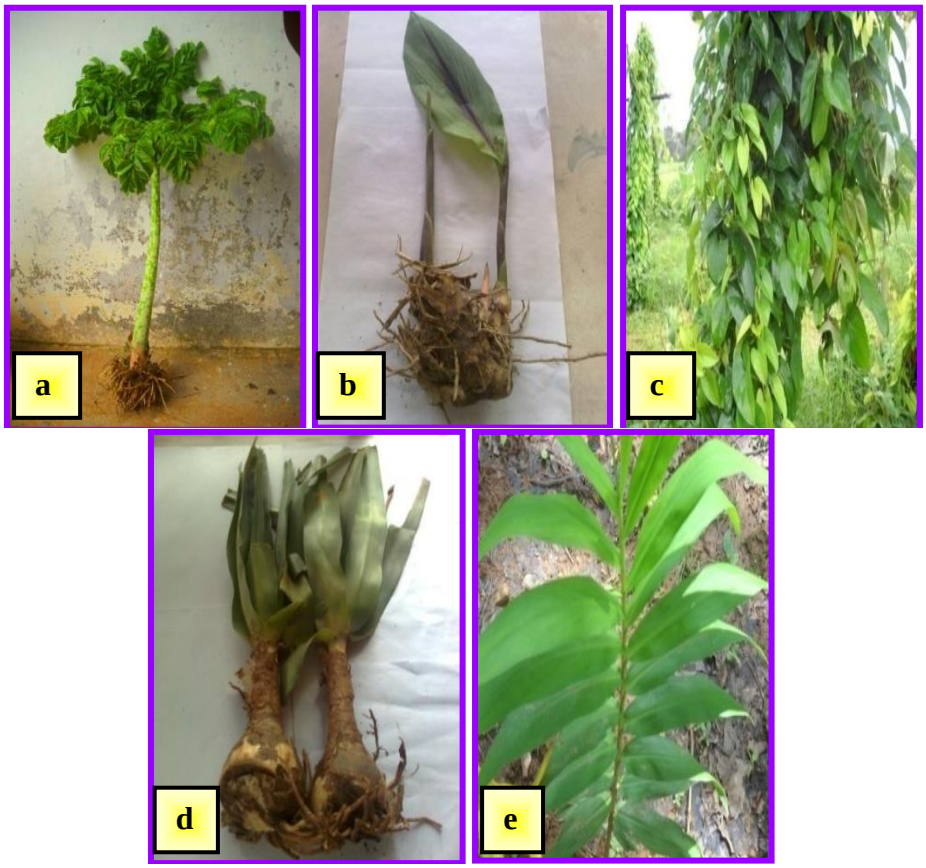

Fig. 1. Medicinal plant samples of (a) *Amorphophallus campanulatus (*Roxb.), (b) *Curcuma caesia (*Roxb.), (c) *Dioscorea alata (*L.), (d) *Urginea indica* (Kunth.), (e) *Zingiber capitatum* (Roxb.) collected from the field.

Preparation of plant extracts

15 g powdered material was extracted with 150 ml of different solvents according to their increasing polarity successively for 8-10 h in the soxhlet apparatus (Tempo) at a temperature not exceeding the boiling point of the respective solvents. After extraction excess solvent was removed by distillation and the concentrated extracts so obtained were further dried in incubator at 40^{o}C. The residual extracts after drying were dissolved in 50% dimethyl sulphoxide and stored in refrigerator at 4^{o}C in small and sterile glass tubes.

Fungal culture and growth conditions

The present study was carried out with the microbial strains procured from IMTECH, Chandigarh, India. The microbial strains used for antimicrobial assessment were *Aspergillus niger* (MTCC 872) and *Candida albicans* (MTCC 183).

The fungal strains were maintained on potato dextrose agar (PDA) slants at 28-30^{o}C, sub cultured regularly and stored at 4^{o}C for further use.

Preparation of fungal inoculum

The test fungal organisms were maintained on potato dextrose agar slants. One loop full of each fungal culture was inoculated in 25 ml potato dextrose broth and incubated at 28-30^{o}C in incubator. Stock inoculum suspensions were prepared from 7 day-old cultures grown on potato dextrose agar (Hi-Media) following National Committee for Clinical Laboratory Standards (NCCLS, 2002). Stock suspensions were adjusted spectrophotometrically to optical densities that ranged from 0.09-0.11 at 530 nm using a spectrophotometer which is equivalent to 0.9×10^4 to 4.7×10^4 cfu/ml.

Assessment of the Antifungal activity

The antifungal activity of the plant extract was evaluated by agar-well diffusion method (Gobdi and Irobi 1992). Potato dextrose agar was used to culture the fungal species. The spore suspension was spread on potato dextrose agar (Hi-media) plate using a sterile swab and air dried to remove the surface moisture. Wells were bored into the agar using a sterile 6 mm diameter cork borer. The crude extract was aseptically introduced into the well at a concentration of 2 mg/20µl, allowed to stand at room temperature for about 1 h as a period of pre-incubation diffusion to minimize the effect of variation in time between the application of different solutions and later the plates were incubated at 28-30^{o}C for 48 h. Controls were also set up in parallel and the effects were compared with clotrimazole as standard antifungal at a concentration of 10 µg/20µl. The plates were observed for the zone of inhibition after 48 h. The experiment was conducted in triplicates and the values are expressed as mean ± SE.

RESULTS AND DISCUSSION

Chhattisgarh is identified as an 'Herbal State' due to its rich diversity of medicinal plants in many tribal districts including Bastar. The present study highlights the vast potentiality of medicinal plants of Bastar for their antifungal efficacy against pathogenic fungi *viz*., *Aspergillus niger* and *Candida albicans*. Five traditionally important medicinal plants from Bastar region *viz*., *A. campanulatus (*Roxb.), *C. caesia (*Roxb.), *D. alata (*L.), *U. indica* (Kunth.) and *Zingiber capitatum* (Roxb.) were selected based on their ethnomedicinal use by the tribal community as the source of their food, medicine and cosmetics. The efficacy of traditionally used medicinal plant extracts on the pathogenic microorganisms has been well documented by the researcher's globally (Afifi and Abu-Irmaileh, 2000; Evans *et al*., 2002; Ates and Erdogrul, 2003; Pandey and Gupta, 2014). In the current investigation different parts of medicinal plants *viz.,* root, stem and leaf were assessed for their antifungal activity as the antimicrobial phytocompounds of plant origin may occur mostly in root, stem and leaf of the plants (Jalalpure *et al*., 2004; Siddiqui *et al*., 2009; Manivannan *et al*., 2010). The plant samples were successively extracted using four different solvents based on their polarity index as chloroform, acetone, methanol and aqueous with the soxhlet apparatus to ensure that a wide polarity range of compounds could be extracted (Pandey and Gupta, 2013).

Table 1. Antifungal activity of medicinal plants *A. campanulatus* (Roxb.), *C. caesia* (Roxb.), *D. alata* (L.), *U. indica* (Kunth.) and *Z. capitatum* (Roxb.) (Zone of inhibition in mm, Mean ± SE).

Medicinal Plants						
Fungal Species	***A. campanulatus* (Roxb.)**		***C. caesia* (Roxb.)**		***D. alata* (L.)**	
	A. niger	***C. albicans***	***A. niger***	***C. albicans***	***A. niger***	***C. albicans***
ROOT						
Chloroform	08.20±0.11	07.26±0.06	07.53±0.17	07.06±0.06	-	-
Acetone	10.93±0.06	08.66±0.06	09.26±0.13	07.80±0.00	08.33±0.24	08.46±0.06
Methanol	09.20±0.11	08.33±0.17	08.06±0.06	07.33±0.06	08.13±0.13	07.20±0.11
Aqueous	-	-	-	-	-	-
Clotrimazole	14.33±0.33	07.66±0.06	14.00±0.46	08.06±0.24	13.80±0.30	07.60±0.23
STEM						
Chloroform	10.26±0.17	07.13±0.06	09.06±0.06	07.00±0.00	-	-
Acetone	13.86±0.06	08.00±0.00	11.33±0.17	07.53±0.17	7.93±0.24	07.33±0.17
Methanol	10.93±0.06	07.60±0.11	09.40±0.11	07.20±0.11	7.60±0.11	07.00±0.00
Aqueous	-	-	-	-	-	-
Clotrimazole	14.06±0.06	07.80±0.11	13.86±0.17	07.73±0.35	14.04±0.43	08.33±0.37
LEAF						
Chloroform	-	-	-	-	-	-
Acetone	08.53±0.17	07.40±0.40	09.13±0.06	07.00±0.00	07.46±0.06	07.26±0.06
Methanol	08.06±0.06	07.20±0.11	-	06.60±0.11	07.00±0.00	06.66±0.13
Aqueous	-	-	-	-	-	-

Fungal Species	***U. indica* (Kunth.)**		***Z. capitatum* (Roxb.)**	
	A. niger	***C. albicans***	***A. niger***	***C. albicans***
ROOT				
Chloroform	-	-	-	07.33±0.17
Acetone	13.26±0.26	14.06±0.06	07.80±0.11	09.53±0.24
Methanol	09.80±0.20	11.13±0.13	07.26±0.06	08.33±0.13
Aqueous	-	-	-	-
Clotrimazole	14.00±0.46	07.66±0.06	13.86±0.48	08.26±0.17
STEM				
Chloroform	-	-	08.00±0.00	07.00±0.00
Acetone	11.06±0.06	11.53±0.26	12.46±0.24	09.26±0.26
Methanol	09.20±0.20	10.46±0.17	09.13±0.06	07.60±0.11
Aqueous	-	-	-	-
Clotrimazole	13.40±0.30	08.60±0.11	13.33±0.24	07.80±0.41
LEAF				
Chloroform	-	-	06.33±0.33	-
Acetone	08.20±0.20	09.20±0.11	07.13±0.06	-
Methanol	07.06±0.06	07.46±0.24	06.46±0.29	07.33±0.13
Aqueous	-	-	-	-
Clotrimazole	14.06±0.37	08.00±0.30	13.80±0.30	08.00±0.11

Clotrimazole	14.13±0.35	07.60±0.23	13.73±0.37	07.66±0.06	14.00±0.11	08.06±0.06

Standard antibiotic: Clotrimazole; (-) Not detected

Antifungal activity assessment of all the five tested plants revealed that the acetone extract of roots exhibited significant activity against *Candida albicans* with a maximum zone of inhibition (14.06±0.06 mm) in case of *U. indica (Kunth.)* followed by *Z. capitatum* (Roxb.) (09.53±0.24 mm), *A. companulatus* (Roxb.) (08.66±0.06 mm), *D. alata (L.)* (08.46±0.06 mm) and *C. caseia* (Roxb.) (07.80±0.00 mm) and as compared with that of standard antifungal with the zone of inhibition (07.66±0.06 mm).

Table 2. Activity index of five medicinal plant extracts of *A. campanulatus* (Roxb.), *C. caesia* (Roxb.), *D. alata* (L.), *U. indica* (Kunth.) and *Z. capitatum* (Roxb.) with respect to Clotrimazole.

Fungal Species	***A. campanulatus* (Roxb.) extract in different solvents**								
	Chloroform			Acetone			Methanol		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
A. niger	0.57	0.72	-	0.76	0.98	0.60	0.64	0.77	0.57
C. albicans	0.94	0.91	-	1.13	1.02	0.97	1.08	0.97	0.94
Fungal Species	***C.caesia* extract (Roxb.) in different solvents**								
	Chloroform			Acetone			Methanol		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
A. niger	0.53	0.65	-	0.66	0.81	0.66	0.57	0.67	-
C. albicans	0.87	0.90	-	0.96	0.97	0.91	0.90	0.93	0.86
Fungal Species	***D. alata* (L.) extract in different solvents**								
	Chloroform			Acetone			Methanol		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
A. niger	-	-	-	0.58	0.54	0.50	0.60	0.56	0.53
C. albicans	-	-	-	0.94	0.84	0.82	1.11	0.87	0.90
Fungal Species	***U. indica* (Kunth.) extract in different solvents**								
	Chloroform			Acetone			Methanol		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
A. niger	-	-	-	0.94	0.82	0.58	0.70	0.68	0.50
C. albicans	-	-	-	1.83	1.34	1.15	1.45	1.21	0.93
Fungal Species	***Z. capitatum* (Roxb.) extract in different solvents**								
	Chloroform			Acetone			Methanol		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
A. niger	-	0.60	0.45	0.56	0.93	0.51	0.52	0.68	0.46
C. albicans	0.88	0.89	-	1.15	1.18	-	1.00	0.97	0.91

However, the acetone extract of stem in case of *A. companulatus* (Roxb.) showed maximum inhibition (13.86±0.06 mm) for *Aspergillus niger*, followed by *U. indica* (Kunth.) (13.26±0.26 mm), *Z. capitatum* (Roxb.) (12.46±0.24 mm), *C. caseia* (Roxb.) (11.33±0.17 mm) and *D. alata* (Roxb.) (08.33±0.24 mm) as compared with standard antifungal with zone of inhibition (14.33±0.33 mm).

The above findings clearly indicates that acetone stem extracts of *A. companulatus* (Roxb.), *Z. capitatum* (Roxb.), *C. caseia* (Roxb.) and acetone root extracts of *U. indica (Kunth.)* and *D. alata* (L.) exhibited significant activity against *Aspergillus niger*. However, the acetone root extracts of all the five plants showed maximum inhibition for *Candida albicans* with respect to standard antifungal as clotrimazole. The antifungal activity of several plant extracts compared with standard antibiotics is well documented in literature (Ahmad *et al*., 2000; Rani and Murty, 2006).

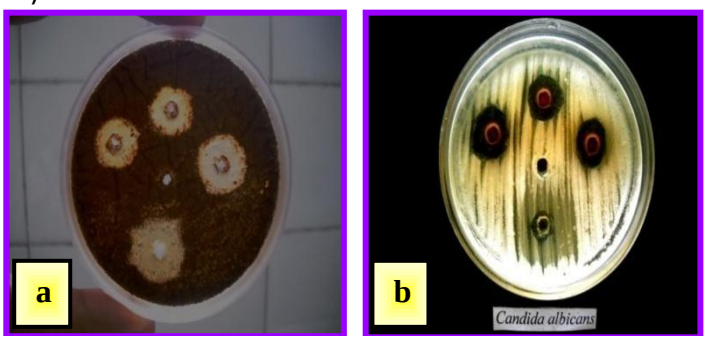

Fig. 2. Antifungal activity of (a) stem acetone extract of *A. campanulatus* (Roxb.) against *A. niger* and (b) root acetone extract of *U. indica* (Kunth.) against *C. albicans.*

The maximum zone of inhibition was recorded in case of acetone followed by methanol and chloroform extracts. Acetone as the choice of solvent for the extraction of phytochemicals has been used by several researchers (Mathkega *et al*., 2000; Lourens *et al*., 2004; Basri and Fan, 2005). However, no significant activity was observed in case of aqueous extract (Sharma *et al*., 2015). The findings clearly indicated that organic solvents were more suitable for the extraction of the active principles responsible for antimicrobial activity (Nair *et al*., 2005; Parekh *et al*., 2006; Mohanasundari *et al*., 2007; Das *et al*., 2010; Pandey and Gupta, 2014). However, the decline in activity of aqueous extract might be due to the excessive heating of the aqueous soluble active constituents during the extraction process which often affect bioactive phyto constituents of the extracts (Koduru *et al*., 2006; Aiyegoro *et al*., 2008; Ekwenye and Edeha, 2010). The phytochemical analysis of all the five medicinal plants showed the presence of alkaloids, flavonoids, tannins, saponins, polyphenolics, terpenoids, phytosterols, resins and glycosides which might be responsible for the potential antifungal activity (Santhi *et al*., 2006; Saxena and Sahu, 2012). The potential sensitivity of all the five medicinal plant extracts against two pathogenic fungi *viz*., *Aspergillus niger* and *Candida albicans* are presented (Table 1, Fig. 2). The activity index was calculated to express the relationship between zones of inhibition of the extracts with respect to that of standard antibiotics (Usman *et al*., 2009). Among the root, stem and leaf extracts of all the five plant extracts the highest activity index of (0.98) was observed with the acetone stem extract of *A. campanulatus* (Roxb.) against *Aspergillus niger* Whereas, for *Candida albicans* the highest activity index of (1.83) was recorded with the acetone root extract of *U. indica* (Kunth.). The antifungal activity was evaluated with respect to clotrimazole as the standard antifungal (Table 2). Higher activity index (>0.5) in the crude extract indicates the potential antifungal activity of the plants. The findings of the present study offer a scientific explanation to the ethnomedicinal use of the five medicinal plants of Bastar by the traditional healers in combating fungal diseases.

CONCLUSION

The results of the above investigation clearly depicts that all the five selected medicinal plant extracts exhibited antifungal activity. Although the spectrum of antifungal activity varies with different parts and solvent used for extraction. The present study would be meaningful to explore indigenous medicinal plants and scientifically validate the traditional usage of these five traditionally important medicinal plants by the tribal community of Bastar in curing several fungal infections**.** However, further purification of these crude extracts and the chemical characterization of bioactive principles using sophisticated analytical instruments such as UV-Visible spectroscopy, High Performance Liquid Chromatography, FT-IR, NMR and ESI-MS could be the future direction of this research.

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