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Antimicrobial Properties of Indian Medicinal Plants and Their Effect in Attenuating Fungal Virulence: A Herbal Approach

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Subject: Pharmacognosy

Abstract

Purpose: Extraction of bioactive agents from plant source to combat fungal and bacterial infections.
Results: The current study encompasses in vitro antimicrobial activities of methanol, hexane, chloroform, butanol, aqueous, ethanol, ethyl acetate and dimethylsulfoxide extracts of *Datura metel, Rosa indica* and *Citrus sinesis*. Phytochemical analysis of the plant extract exhibited the presence of tannins, alkaloids, terpenoids, flavonoids and saponins. These extracts were evaluated for antimicrobial activity using agar disc diffusion method against *Escherichia coli*, *Bacillus spp., Staphylococcus spp., Micrococcus spp.*, *Candida albicans, Aspergillus niger* and *Aspergillus flavus*. The methanol and ethanol extracts of *D. metel, R. indica* and ethyl acetate extract of *C. sinesis* were found to be highly potent against the bacterial strains. The chloroform, ethanol and dimethylsulfoxide extracts of *C. sinesis* and ethanol and chloroform extracts of *D. metel, R. indica* exhibited the most effective antifungal activity. The minimum inhibitory concentration (MIC) of plant extracts was determined using microdilution method. The MIC was found to be 0.45 to 1.25 mg/mL against bacterial strains and 0.4 to 2.5 mg/mL against fungal strains. It was also observed that these extracts drastically reduce the melanin content of the *A. niger* which plays a key role in its pathogenicity. The conidial cell wall and colony analysis of *A. niger* exhibited remarkable reduction in conidia and melanin deposition as seen microscopically and ergosterol content as estimated spectroscopically.
Conclusions: Melanin content in the cell wall is a key determinant of fungal pathogenicity making it an attractive target for antifungal drug discovery. Our results are the first report of melanin inhibitory activity of these extracts.

Keywords: *Antifungal, Antibacterial, Phytochemistry, Datura metel, Rosa indica, Citrus sinesis*

Introduction

Microbial infections and resistance to antimicrobial drugs pose a challenge to health community globally. Infectious diseases have contributed for 23.04 % mortality worldwide in 2002 (WHO, 2004). The mutagenic nature and rapid multiplication of microbial genome has resulted in antimicrobial resistance and thus reducing the efficacy of available antibiotics. These include multi-resistant *Mycobacterium tuberculosis* (Gillespie, 2002), penicillin-resistant *Streptococcus pneumonia* (Appelbaum, 1992), methicillin-resistant *Staphylococcus aureus* (Pasricha et al., 2013). Antibiotic resistance of wide range of pathogens coupled with high cost of antibiotics raises the concern in the present healthcare scenario. Thus, there is an urgent need is required to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. From time immemorial plants are used as alternative sources of treatment for various diseases. Over three quarters of world population relies mainly on plants and plant extracts for health care (Bhau, 2012). In India, drugs of herbal origin have been used in traditional system of medicine such as Ayurveda and Unani (Bhau, 2012). Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Presently, only a small percentage of plant biodiversity have been explored and further pharmacological screenings are required for its successful use in ethnomedicine. Geographic, climatic factors contribute to further diversity in bioactive constituents present in plants. Previous studies have established the antimicrobial activity of secondary metabolites present in plants such as alkaloids, flavonoids, tannins, terpenoids (Gayathri et al., 2011). Due to a rapid increase in the rate of antibiotic resistance in microorganisms and side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs In the current investigation, *D. metel (leaf)*, *R. indica (leaf and petal)* and *C. sinesis (peel)* were

screened for their potential antimicrobial activity against infectious microorganisms namely; *Bacillus species*, *Micrococcus species*, *Staphylococcus species*, *E. coli, A. niger*, *A. flavus* and *C. albicans*. Literature indicates that the leaves and seeds of *D. metel* have been widely used in herbal medicine as anesthetic, antispasmodic, bronchodilator and as hallucinogenic (Dabur et al., 2004a). The antibacterial efficacy of *Datura* has been reported against clinical bacterial isolates, *Streptococcus* b *haemolytic* (Akharaiya, 2011) and *S. aureus* (Venkanna et al., 2013).

Citrus essential oils besides being used as fragrance, have been reported to possess antibacterial activities against *Salmonella typhimurium* (Callaway et al., 2011) and *S. aureus* (Fisher and Phillips, 2006). In addition, antifungal activities against *Penicillium digitatum* (Caccioni et al., 1998) and *Penicillium italicum* (Caccioni et al., 1998) have been reported. Okwu and Emenike (2006) have demonstrated that the peel of *C. sinesis* contains extremely high alkaloid and phenol content which protects the seeds from microbial attack during fruiting.

Antibacterial properties of *R. indica* has also been explored against different pathogens, where methanolic and ethanolic extracts were tested against *Pseudomonas aeruginosa* (Kumar et al., 2012) and *S. aureus* (Khan and Tewari, 2011).

1. Materials and Methods

2.1 Collection of plant samples

The plant samples were collected from government nursery. The leaves, petals and peel were properly washed under running tap water and then rinsed in distilled water. Then the rinsed plant materials were air dried. The dried plants were grinded into powder using mortar and pestle.

2.2 Preparation of extracts

The extracts were prepared by fractional extraction procedure in which the powdered plant sample was extracted 4 to 5 times with warm methanol.
The methanol extract was filtered and evaporated.
The residue was used for further extraction with hexane (3 times) in a separatory funnel which was collected and concentrated. The aqueous layer left in the separatory funnel was extracted 3 times with chloroform. Finally, the aqueous layer obtained after this step was extracted 3 times with butanol which were collected and concentrated. 20% Ethanol, dimethylsulfoxide and ethyl acetate extracts were prepared separately.

2.3 Microbial strains

The bacterial strains tested were *Bacillus species*, *Micrococcus species*, *Staphylococcus species*, *E. coli* and fungal strains were namely, *A. niger*, *A. flavus* and *C. albicans*. Bacterial cultures were maintained on Luria Bertani agar slants. Fungal cultures were maintained on potato dextrose agar for *Aspergillus species* and yeast peptone dextrose for *C. albicans.* All cultures were kept at 4°C. The cultures were maintained as 15% glycerol stocks at -80° C for long time storage.

2.4 Preparation of inoculum

A loopful of the bacterial colony was inoculated into Luria Bertani media under laminar flow condition. This was incubated for 24 hours at 37^0C on shaker at 250 rpm. The slants of fungal cultures were prepared and incubated for 48 hours at 30^0C on shaker at 200 rpm. A loopful of *C. albicans* is inoculated into yeast potato dextrose media under laminar flow conditions and grown at 30^0C.

2.5 Preliminary phytochemical screening

The extracts were subjected to phytochemical screening for the presence of saponins, tannins, terpenoids, alkaloids and flavonoids. Phytochemical screening of the extracts was carried out according to the methods described earlier by Trease and Evans, 2002 and Geissman, 1963.

2.6 Agar disc diffusion method

The disc diffusion method was used for the test. Overnight *Candida* culture was mixed at a final concentration of $1x10^4$ cells to liquid YPD agar at 40^0C and poured into sterile petridishes. Sterile 6mm discs impregnated with 5 µl of the extract were placed on the agar petridishes. The plates were incubated for 48 hours at 30^0C. The zone of inhibition was measured in mm. For *Aspergillus*, $1x10^4$ conidial concentration was mixed in PDA. The plates were incubated for 5 days and the zone of inhibition was measured.
Overnight bacterial cultures were mixed at a final concentration of $1x10^6$ cells to LB agar at 40^0C and poured into sterile petridishes. Sterile 6mm discs impregnated with 5 µl of the extract were placed on the agar petridishes. The plates were incubated for 24 hrs at 37^0C. The zone of inhibition was measured in mm.

2.7 Minimum inhibitory concentration (MIC_{80})

MIC_{80} was conducted based on microdilution method with minor modification in a 96 well plate according to CLSI guidelines formerly known as NCCLS. The protocol has been described previously (V.Pooja et al., 2012) In case of bacterial species, the same protocol was followed and the final concentration was adjusted to 10^6cells/well and the 96 well plate was incubated for 24 hours at 37^0C. The plate was then read both visually and on an ELISA reader at 600 nm.

2.8 Estimation of ergosterol content of the cell wall of *A. niger*

The protocol of Young et al. (2003) was followed with minor modification. PDA were inoculated with freshly prepared conidial culture (10^6 cells/

ml) and grown at 28°C for 5 days. Cells were pelleted in a tabletop centrifuge and washed with sterile water. The pellets were weighed, and 3 ml of 25% alcoholic potassium hydroxide was added. The cells were incubated at 80°C for 1 h and allowed to cool to room temperature. 1 ml of sterile water and 3 ml of octane were added, and the cells were vortexed for 3 min. The octane layer was transferred into a separate glass tube and stored at -20°C for a maximum of 24 hr. For analysis 200 µl of the octane layer was diluted with 5 fold 100% ethanol (800 µl) and measured spectrophotometrically at both 281.5nm and 230 nm. The conversion from optical density to ergosterol content was calculated as follows:

Ergosterol %= $[(A_{281.5}/290 \times F)/\text{Weight of pellet}] - [(A_{230}/518 \times F)/\text{Weight of pellet}]$

F = Ethanol Dilution Factor

2.9 Inhibition of conidiation

The dose-dependent inhibition of conidiation in *A. niger* was performed according to the protocol described previously by us (V.Pooja et al., 2012).

2.10 Microscopic analysis of the *A. niger* colony and conidial morphology

Approximately 1×10^4 conidia were spread on potato dextrose agar plates in duplicate containing extracts of ethanol and chloroform. The concentration of the extract in each case was less than its MIC_{80} concentration. Fungal cultures were allowed to grow for 7 days at 30^0C and then examined visually for white colonies and microscopically for the presence of conidia using lacto phenol cotton blue staining and was recorded photographically.

2. Results

3.1 Preliminary phytochemical screening

In the present investigation, the antimicrobial activity of methanol, hexane, chloroform, butanol, aqueous and ethanol extracts of medicinal plants were analysed. These extracts were subjected to preliminary phytochemical screening represented in Table 1-4. The major phytochemicals were found in the ethanol extract of the medicinal plants.

Table 1: Phytochemical analysis of leaf extract of *Datura metel*

Test	Methanol	Hexane	Chloroform	Butanol	Aqueous	Ethanol
Tannin	+++	++	--	--	--	--
Saponin	--	--	--	--	--	++
Flavonoid	--	--	--	--	--	--
Alkaloids	--	--	+++	++	+	++
Terpenoid	--	--	+	--	--	--

Table 2: Phytochemical analysis of petal extract of *Rosa indica*

Test	Methanol	Hexane	chloroform	Butanol	Aqueous	Ethanol
Tannin	+++	--	--	--	+	+++
Saponin	+	--	--	--	--	+
Flavonoid	--	--	--	+	--	--
Alkaloids	+	+	--	--	--	+
Terpenoid	--	--	--	--	--	+++

Table 3: Phytochemical analysis of leaf extract of *Rosa indica*

Test	Methanol	Hexane	Chloroform	Butanol	Aqueous	Ethanol
Tannin	+	--	--	++	--	+++
Saponin	--	--	--	--	+	+
Flavonoid	--	+	+	--	--	--
Alkaloids	--	--	+	++	++	+++
Terpenoid	--	--	+	+	--	+++

Table 4: Phytochemical analysis of leaf extract of *Citrus sinesis*

Test	Methanol	Hexane	Chloroform	Butanol	Aqueous	Ethanol
Tannin	++	--	--	--	--	++
Saponin	++	++	--	--	+	++
Flavonoid	--	--	+	--	--	--
Alkaloids	+	--	+	+	--	+++
Terpenoid	+	--	--	--	--	+++

In table 1-4: +=indicates presence of phytochemical and --= indicates absence of phytochemicals; ++=shows moderate concentration; +++=shows high concentration.

3.2 Antifungal Assay

To further investigate the antifungal activity of different extracts of plant species, the zone of inhibition studies was performed by the disc diffusion method (Table 5). Significant reduction in the growth of *A. niger* was found in the ethanol extract of *C. sinesis*. *A. niger* was susceptible to chloroform and ethanol extracts of *D. metel* with zone of inhibition of 12mm and 14mm, respectively. Inhibitory effect of ethanol extract of *C. sinesis* and *R. indica* petal was observed on *A. flavus* with zone of inhibition of 20 mm.

Table 5: Antifungal activity of medicinal plants using disc diffusion assay, The results of zone of inhibition indicated the maximum potency of ethanol and chloroform extracts. The mean value of inhibition zone was measured in mm.

Plant	*Candida albicans*	*Aspergillus niger*	*Aspergillus flavus*
Datura metel			
Methanol	--	--	--
Hexane	--	--	--
Chloroform	9	12	--
Butanol	10	--	--
Aqueous	--	--	--
Ethanol	10	14	--
***Rosa indica* leaf**			
Methanol	12	--	8
Hexane	--	--	--
Chloroform	10	11	--
Butanol	11	--	10
Aqueous	--	--	--
Ethanol	15	--	--
***Rosa indica* petal**			
Methanol	11	--	16
Hexane	--	--	--
Chloroform	10	11	14
Butanol	11	--	18
Aqueous	--	--	--
Ethanol	--	--	20
Citrus sinesis			
Methanol	--	--	15
Hexane	--	--	--
Chloroform	10	12	--
Butanol	11	10	15
Aqueous	--	--	--
Ethanol	13	13	20
DMSO	--	--	10
Ethylacetate	--	--	16
Control			
Methanol	--	--	10
Hexane	--	--	--
Chloroform	6	--	10
Butanol	8	8	10
Aqueous	--	--	--
Ethanol	--	--	11
DMSO	--	--	8
Ethyl acetate	--	--	8

In Table 5: -- indicates the absence of zone of inhibition. The diameter of a disc was 5mm and the value mentioned for zone of inhibition was inclusive of diameter of the disc.

3.3 Antibacterial Assay

The bacterial growth inhibition as seen by zone of inhibition disc diffusion assay of different extract of the screened plants species is depicted in Table 6 and Figure 1-4. The results revealed that the selected medicinal plants exhibited antimicrobial activity of varying magnitudes. High activity was exhibited by ethanol, methanol and ethyl acetate extracts.

Table 6: Antibacterial activity of medicinal plants using disc diffusion assay, the results of zone of inhibition indicated the maximum potency of methanol and ethanol extracts. The mean value of inhibition zone was measured in mm.

Plant	*Bacillus spp.*	*Micrococcus spp.*	*E.coli*	*Staphylococcus spp.*
Datura metel				
Methanol	28.75	34.25	11	16.5
Hexane	--	--	--	--
Chloroform	9.5	18.5	--	--
Butanol	--	7.25	--	--
Aqueous	--	--	--	--
Ethanol	26	25.75	17.5	14.5
DMSO	--	--	--	--
Ethyl acetate	--	--	13.5	10.25
***Rosa indica* leaf**				
Methanol	23.5	24.5	9	24.5
Hexane	--	--	--	--
Chloroform	25.75	11.5	--	8.25
Butanol	12.5	13	--	12.5
Aqueous	--	--	11	7.5
Ethanol	20.25	18.75	13.75	15.25
DMSO	--	--	--	--
Ethyl acetate	--	--	12.25	11.75
***Rosa indica* petal**				
Methanol	10.5	30.5	8.75	9.25
Hexane	--	--	--	--
Chloroform	9	12.5	--	8.5
Butanol	13.5	9	--	16.5
Aqueous	--	--	13.75	--
Ethanol	22	21.25	17.75	14.25
DMSO	--	--	--	--
Ethyl acetate	--	--	13	12.75
Citrus sinesis				
Methanol	--	32.5	--	11
Hexane	--	--	--	--
Chloroform	21.25	7	--	--
Butanol	--	8	--	--
Aqueous	--	--	--	--
Ethanol	--	24.5	10	13
DMSO	--	--	--	--
Ethylacetate	25.5	36	14.25	18.25
Control				
Methanol	7.5	7	7	--
Hexane	--	--	--	--
Chloroform	8.75	7	--	--
Butanol	11.5	19	10	14
Aqueous	--	--	--	--
Ethanol	--	8.5	--	--
DMSO	--	11	--	--
Ethyl acetate	--	--	--	--

In Table 6: indicates the absence of zone of inhibition. The diameter of a disc was 5mm and the value mentioned for zone of inhibition was inclusive of diameter of the disc.

Among various extracts, the methanol and ethanol extract of *D. metel* exhibited maximum activity against *B. subtilis* with zone of inhibition of 28.75 mm and 26 mm, respectively. Other extracts that exhibited significant activity were methanol extract of *R. indica* leaf with zone of inhibition of 23.5 mm and ethylacetate extract of C. sinesis with zone of inhibition of 25.5 mm. In case of *Micrococcus spp,* ethyl acetate and methanol extract of *C. sinesis* exhibited significant activity with zone of inhibition of 36 mm and 32.5 mm, respectively. The ethanol extracts of all plant species were also potent against *Micrococcus spp.* Apart from this the ethanol extracts of *R. indica* petal and *D. metel* have shown significant antibacterial activity against *E. coli* with zone of inhibition of 17.75 mm and 17.5 mm, respectively. Zone of inhibition of 24.5 mm was observed against *S. aureus* for methanol extract of *R. indica* leaf.

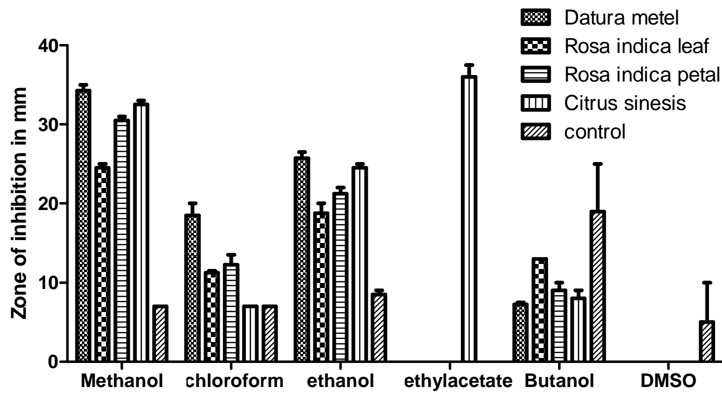

Figure 1: Comparison of antibacterial activity of extracts of plant species against *Micrococcus species*. The data are means and SEM from duplicates for each group along with two-way ANOVA (P<0.001) followed by Bonferroni Post test.

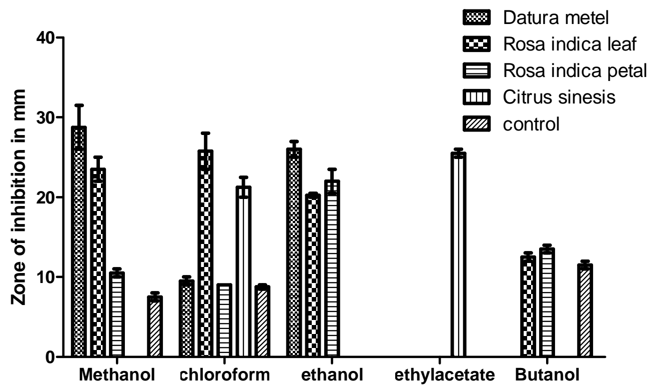

Figure 2: Comparison of antibacterial activity of extracts of plant species against *Bacillus species.* The data are means and SEM from duplicates for each group along with two-way ANOVA (P<0.001) followed by Bonferroni Post test.

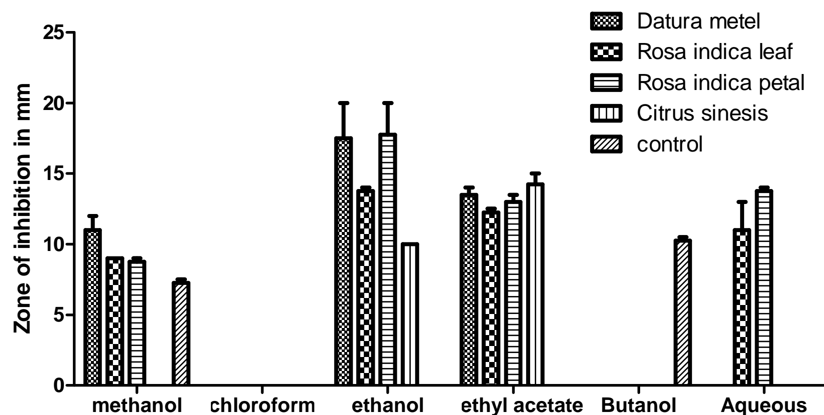

Figure 3: Comparison of antibacterial activity of extracts of plant species against *Escherichia coli*. The data are means and SEM from duplicates for each group along with two-way ANOVA (P<0.001) followed by Bonferroni Post test.

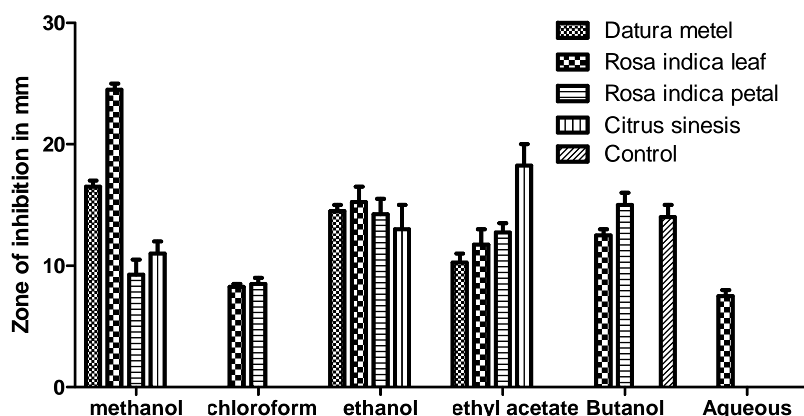

Figure 4: Comparison of antifungal activity of extracts of plant species against *Staphylococcus species*. The data are means and SEM from duplicates for each group along with two-way ANOVA (P<0.001) followed by Bonferroni Post test.

3.4 Minimum Inhibitory Concentration Assay

It was found that *C. albicans* was susceptible to chloroform extract of all the screened medicinal plant species, inhibitory effect was observed for chloroform extract of *D. metel* with MIC_{80} of 0.432 mg/ml. The ethanol extract of *D. metel* exhibited maximum inhibition in case of *A. niger* with MIC_{80} of 0.3125 mg/ml and chloroform extract of *C. sinesis* showed an MIC_{80} of 0.45 mg/ml. *A. flavus* was highly susceptible to methanol extract of *R. indica* petal with MIC_{80} of 0.212 mg/ml as represented in Table 7.

Table 7: Minimum Inhibitory Concentration of the extracts of medicinal plant species against *C. albicans*, *A. niger, A. flavus.* The MIC was determined by Micro Broth dilution assay in a 96 well plate according to the CLSI guideline.

Plant Extract	Minimum Inhibitory Concentration (MIC_{80} in mg/ml)		
	Candida albicans	*Aspergillus niger*	*Aspergillus flavus*
Datura metel			
Chloroform	0.36	0.56	--
Butanol	0.92	--	--
Ethanol	--	0.78	--
Rosa indica leaf			
Chloroform	0.427	--	--
Butanol	--	--	0.3
Methanol	--	--	0.35
Rosa indica petal			
Chloroform	0.935	0.35	--
Butanol	--	--	0.7
Ethanol	0.345	0.25	0.61
Methanol	--	--	0.212
Citrus sinesis			
Chloroform	0.4	0.875	--
Butanol	0.18	--	--
Ethanol	0.8	0.95	--
DMSO	--	0.212	0.65

In Table 7: -- indicates the absence of inhibition of growth of fungal species.

3.5 Inhibition of conidiation of *A. niger*

The presence of melanin plays a important role in reducing the pathogen susceptibility towards antifungal drugs and host defence mechanism (Wang and Casadevall, 1994). Aerosolized conidias are an essential prerequisite for rapid, pathogenicity of the fungal infection (Latge1999). In the current study, we further investigated the inhibitory effect of screened medicinal extracts on conidiation. Figure 5 shows the effect of a sub MIC_{80} concentration of the extracts on conidiation of *A. niger*. Cultures of *A. niger* were grown from germinated conidia on Potato dextrose agar in the presence of sub MIC concentration of each extract.

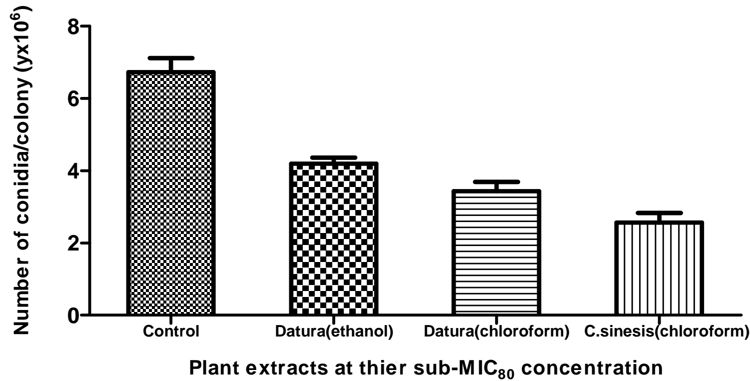

Figure 5: Effect of various extracts of medicinal plants on conidiation of *A. niger*. The inhibition of conidiation was determined using the agar block conidiation assay. The data are means and SEM from triplicates for each group along with one-way ANOVA (P<0.001) followed by Bonferroni Post test.

It was observed that the colonies grown at sub MIC_{80} concentration of 180 µg/ml of ethanolic extract of *R. indica* and 400 µg/ml of chloroform extract of *D. metel* were white as compared to the control culture which was grown in the absence of the extract. Microscopic examination of fungal colonies depicted white conidias with depleted levels of melanin in *A. niger* on treatment with ethanol and chloroform extract of *D. metel*. Substantial inhibition of conidiation in *A. niger* was also observed in the presence of chloroform extract of *C. sinesis* (Figure 6).

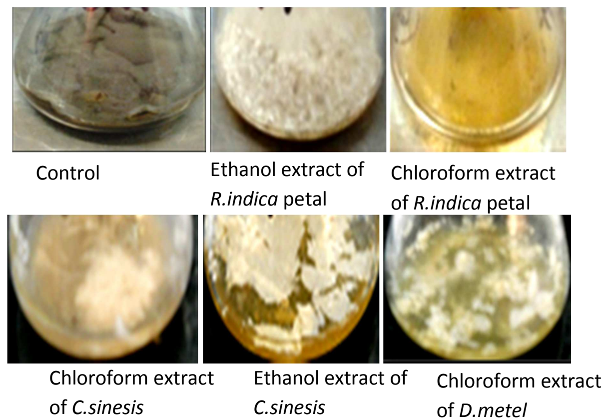

Figure 6: Liquid culture of *A.niger* (1 x 10^6 conidias) is grown in the presence of sub-MIC_{80} concentration of ethanol and chloroform extracts. When compared with *A.niger* colonies of the control, extreme reduction in the conidial growth can be seen after 4 day old culture.

Microscopic analysis of the conidias of *A. niger* stained with lactophenol cotton blue was also undertaken in the present study (Figure 7). Conidias from the treated colonies exhibited marked reduction in the melanin content. The cell surface morphology depicted smooth walls in the treated sample as compared to the control.

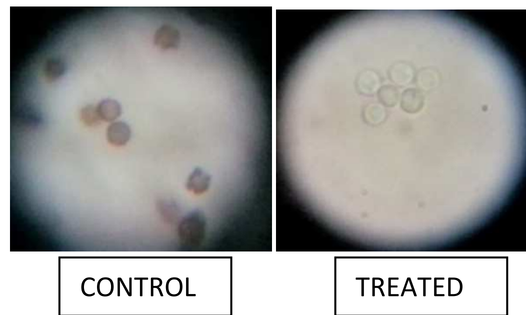

Figure 7: Reduced melanin in fungal conidia as seen under 40X magnification.

3.6 Estimation of ergosterol

The ergosterol content of the hyphal cell wall of *A. niger* was estimated spectrophotometrically and the percentage ergosterol content of the *A. niger* grown in the presence of the extracts was calculated. The control was taken as the *A. niger* grown in the absence of the any extract. The difference was given as % reduction in the ergosterol content of the test as compared to the control (Figure 8). It was found that there was 31.275 fold reduction in ergosterol content as compared to control.

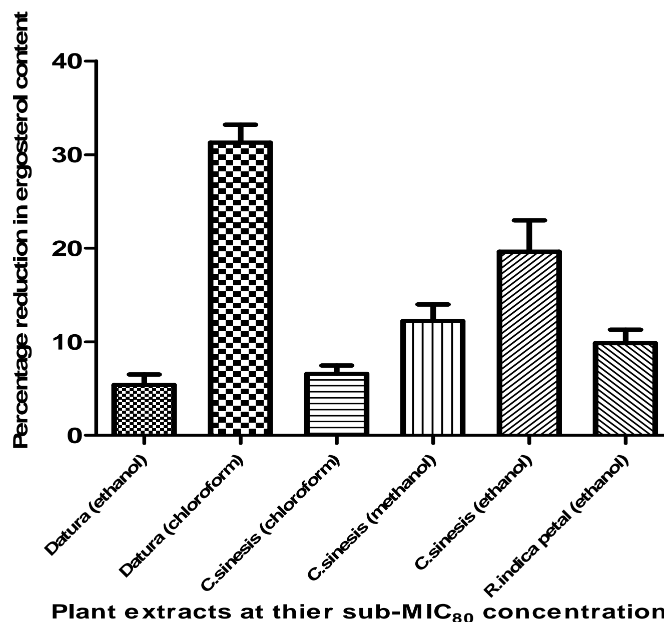

Figure 8: Percentage reduction in the ergosterol content of hyphal cell wall of *A.niger*. The ergosterol content of the hyphal cell wall was estimated spectrophotometrically and % ergosterol was calculated. The data are means and SEM from duplicates for each group along with one-way ANOVA (P=0.006) followed by Bonferroni Post test.

3. Discussion

Infectious diseases caused by bacteria, viruses and fungi involve complex interaction between the pathogen, host and environment. Indiscriminate use of antibiotics has led to the development of multidrug resistant pathogens (Chambers, 2001).

There are numerous reports on the development of resistance to the last line of antibiotic defence, which has led to the search for novel methods of therapy. Plant derived antimicrobials have been a source of novel therapeutics since ages. Plants are known to produce an enormous variety of small molecules having diverse structural space like terpenoids, glycosteroids, flavonoids and polyphenols. Inspite of the fact that plant derived antibacterials are less potent, plants fight infections successfully. Furthermore, the concept of synergism is combating infection makes them attractive alternatives to existing drugs.

Melanin is a biologically important pigment that is found throughout nature, often providing protection against ultraviolet radiation. Melanin production by fungi contributes to the virulence of pathogens of humans as well as those of food crops. Fungal melanin can influence the immune response of the host. Vast literature exists indicating strongly the interrelationship between virulence and melanin content. Melanin interferes with the normal function of phagocytic cells. Melanized *Fonsecaea pedrosoi* cells reduce the oxidative burst capacity of macrophages (Cunha et

al., 2010) and phagocytosis of the fungi (Cunha et al., 2005). Inhibition of phagocytosis has also been observed for melanized *C. neoformans* (Wang et al., 1995). In *Aspergillus fumigatus*, melanin inhibits apoptosis in macrophages that have phagocytosed melanized conidia (Volling et al., 2011). Melanin can modulate immune function in other ways. In experimental mouse infections, cryptococcal melanin alters cytokine levels in response to infection and activates the complement system (Rosas et al., 2002). Conversely, *A. fumigatus* melanin inhibits cytokine production in the host, possibly by blocking pathogen-associated molecular pattern (PAMP) recognition by the immune system (Chai et al., 2010).

In the present study, the extracts of medicinal plants exhibited novel effect of demelanization and remarkable reduction in conidiation at sub MIC_{80} concentration against *A. niger*. This result corroborated with our finding that chloroform and ethanol extracts reduced conidiation maximally. The ethanol extract of *D. metel* has exhibited maximum inhibition in case of *A. niger* with sub-MIC_{80} of 0.3125 mg/ml and chloroform extract of *C. sinesis* showed an sub-MIC_{80} of 0.45 mg/ml. *A. flavus* was highly susceptible to methanol extract of *R. indica* petal with sub- MIC_{80} of 0.212 mg/ml which is considerably low as compared to the previous studies performed on *D. metel* which has shown antifungal activity with sub MIC_{80} value of 1.250 mg/ml (Dabur et al., 2004b). The colony and hyphae cell wall analysis have shown drastic reduction in conidial and melanin formation as seen microscopically and spectrophotometrically. Our results are the first report of melanin inhibitory activity of these extracts. Thus, the extracts may exhibit unique ability for inhibition of pathogenic defense mechanism which will be further investigated.

Purification and structure elucidation of the active ingredient is underway to have better insights into the correlation between chemical structure and biological activity.

5. Conclusion

R. indica, *D. metel* and *C. sinesis* extracts possess a broad spectrum of activity against a panel of pathogens responsible for the most common opportunistic fungal and bacterial infections. The ability of these extracts to attenuate virulence by interfering with host defence mechanism opens opportunities to combat infections by rendering the pathogen more susceptible to the action of antimicrobial agents.

6. Acknowledgement

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