Antimicrobial activity of different leaf extracts against some human pathogens

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Abstract
Human skin is prone to many types of infections from a range of microorganisms. Candidiasis and Cryptococcosis *infections* are common fungal infections. The currently antifungal therapies used such as amphotericin B, fluconazole, and itraconazole have certain limitations due to side effects and emergence of resistant strains. Naturally occurring plant metabolites are useful sources of new biocidal compounds. The ethanolic and diethyl ether leaf extracts from seven plant species (*Ocimum sanctum, Nerium oleander, Ricinus communis, Catharanthus roseus, Annona squamosal, Calotropis gigantean* and *Azadirachta indica*) were assayed for their antimicrobial activity against the opportunistic yeast *Candida albicans* and *Cryptococcus neoformans*. Ethanol and ether extract of *Ricinus communis*, *Catharanthus roseus* and *Calotropis gigantea* showed strongest efficiency against both fungal species. The ether extracts of *Ocimum sanctum*, *Ricinus communis* and *Nerium oleander* against *Candida albicans* were more active as compared to that of *Cryptococcus neoformans*. *Ricinus communis*, *Catharanthus roseus*, *Azadirachta indica* and *Calotropis gigantean* remained active 10 days after incubation.

Keywords: Ethanol, ether, plant extracts, antimicrobial effect, Candidiasis, Cryptococcosis

1. Introduction
The usefulness of ethano-botanical surveys in drug discovery has been reviewed by many researchers [1]. Naturally occurring plant metabolites are useful sources of new biocidal compounds and some cases have served as lead for new compounds that have been characterized [2]. However, search for new antimicrobial agent is a continuous exercise. *Candida albicans,* is a diploid fungus (a form of yeast) and a causal agent of opportunistic oral and genital infections in humans. Candidiasis is often observed in immune compromised individuals such as HIV positive patients. The clinical types of Candidiasis are intertriginous, paronychia and onychia, generalized cutaneous, vulvo-vaginal, oral thrush, pulmonary, endocardits, septicemia, meningitis and allergic [3].
Cryptococcosis is an important systemic mycosis. It is caused by yeast like fungus called *Cryptococcus neoformans*, a basidiomycete. *C. neoformans* usually grows as yeast, under certain conditions, both in nature and in the laboratory; *C. neoformans* can grow as a filamentous fungus. After inhalation of a massive dose of fungus, the organism lodges in the lungs, invades the bloodstream and finally reaches the central nervous system, where it causes characteristic lesions [4]. Those who have T -lymphocyte deficiency (especially the AIDS patients), Diabetes mellitus, Sarcoidosis or a Blood dyscrasia, Systemic Lupus Erythematous, Carcin bronchus, Tuberculosis are susceptible to *Cryptococcus* infection. The clinical types of cryptococcal disease are pulmonary, central nervous system, cutaneous, visceral [3].
Common antifungal antibiotics viz. Nystatin, Amphotericin B, Ketconazole, Fluconazole, Cotrimoxazole, etc. are used in treatment of Candidiasis and Cryptococcosis. But sometimes the use of these antibiotics carry drawbacks like hypersensitivity, allergic reactions and other side effects moreover rise in population, inadequate supply of drugs in certain parts of the world, prohibitive cost of treatment for common ailments, side effects of several allopathic drugs in current usage and development of resistance to currently used drugs for infectious diseases have led to an increased emphasis on the use of plant materials as a source of medicine for a wide variety of human ailments. In view of this scenario, the present study the antimicrobial activity of ethanolic and diethyl ether crude extract of some plant species have been investigated towards commonly found important pathogens *C. albicans* and C. *neoformans*. The study will be very much useful in the development of new fungicidal drugs against these human pathogenes on low cost basis without producing any side effects in patients.

2. Materials and Methods
Based up on the local availability and medicinal values leaves of seven plants were taken for the study.

2.1 Collection of Leaves Sample:
Healthy leaves of seven plants viz. *Ocimum sanctum, Nerium oleander, Ricinus communis, Catharanthus roseus, Annona squamosal, Calotropis gigantean* and *Azadirachta indica* were collected from botanical garden of Bharatiya Mahavidyalaya, Amravati (M.S.), India.

2.2 Preparation of Extracts:
The plants were freed from dirt by washing with running tap water followed by distilled water, cut into small pieces and dried under shade, pulverized and sieved through 1mm mesh, 100 mg of each powder material was weighed and kept in containers containing 250 ml each of ethanol and di ethyl ether. The mixers were kept overnight with occasional shaking. The materials of *Ocimum sanctum, Nerium oleander, Ricinus communis, Catharanthus roseus, Annona squamosa Calotropis gigantean* and *Azadirachta indica* were labelled as A1, B1, C1, D1, E1, F1 and G1 for ethanol extracts and A2, B2, C2, D2, E2, F2 and G2 for ether extract respectively. The mixtures were filtered through double layered muslin cloth and filter paper to remove debris. The ethanolic and ether filtrates of selected leaves were tested against the fungi *C.albicans* and *C. neoformans.*

2.3 Screening of fungicidal properties of plant extracts by DDM method:

The relative efficacy of some commonly used antifungal antibiotics was compared with plant extract discs by employing the Filter paper Disc Diffusion Method [5].

3. Result and Discussion

Both ethanolic and other forms of extracts of *Ocimum sanctum, Nerium oleander, Ricinus communis, Catharanthus roseus, Annona squamosal, Calotropis gigantean* and *Azadirachta indica* exhibited varying degree of antifungal activities against the test pathogenic fungi. On the general note, ethanolic extracts of the selected samples had higher antifungal activity compared to ether extracts (Table-1 and Table-2).

Ethanol extract of *Ricinus communis* and *Calotropis gigantean* showed best antifungal activity against *C. albicans* which shows DDM value of 18 mm and 20 mm respectively, followed by ether extract of *Nerium oleander* which shows DDM value of 17 mm and 15mm against *C. neoformans* respectively.The activity of ether extract generally declined after 5 days of incubation whereas, with ethanolic extracts antifungal activity was effective up to 7 days, while *Ricinus communis*, *Catharanthus roseus*, *Azadirachta indica* and *Calotropis gigantean* remained active 10 days after incubation.

Fungal infections are not related to personal hygiene. It can affect any person. Sharing bedding, towels and even bathing in an area where these fungi may reside, can lead to an infection. Skin fungi do not only infect the skin. It can also infect the hair and nails. It may lead to deformities of nails and loss of hair while on the skin it causes itching and rash. Fungal infections are most often seen in people with a weakened immune system, such as those who:

- Are infected with HIV
- Take high doses of corticosteroid medications
- Are on chemotherapy drugs for cancer

C. albicans and *C. neoformans* are *the* common yeasts to cause an infection inside and on the surface of the body and may prove life-threatening in immune compromised people. The currently antifungal therapies used such as amphotericin B, fluconazole, and itraconazole have certain limitations due to side effects and emergence of resistant strains. The result of present study indicates the potential of plant extracts tested as important sources of new antifungal agents.

Table 1: Antimicrobial activity of plant extracts against *Candida albicans* by DDM

Plant taken	Volume of extracts Incorporated in disc	Plant extracts			
		Diameter of zone of inhibition(in mm)			
		Ethanol		Di Ethyl Ether	
		Control	Extract	Control	Extract
Ocimum sanctum	50µl	---	13	---	15
Nerium oleander	50µl	--	11	--	17
Ricinus communis	50µl	---	18	---	12
Catharanthus roseus	50µl	----	17	----	11
Annona squamosa	50µl	----	15	----	--
Calotropis gigantean	50µl	----	16	----	10
Azadirachta indica	50µl	----	14	-----	10

All the values are mean of three replications

Table 2: Antimicrobial activity of plant extracts against *Cryptococcus neoformans* by DDM

Plant taken	Volume of extracts Incorporated in disc	Plant extracts			
		Diameter of zone of inhibition(in mm)			
		Ethanol		Di Ethyl Ether	
		Control	Extract	Control	Extract
Ocimum sanctum	50µl	---	--	---	--
Nerium oleander	50µl	----	--	----	15
Ricinus communis	50µl	----	17	----	--
Catharanthus roseus	50µl	----	17	----	11
Annona squamosa	50µl	----	---	----	--
Calotropis gigantean	50µl	---	15	---	11
Azadirachta indica	50µl	---	13	---	14

All the values are mean of three replications

4. Conclusion

This study open perspectives to find more effective drugs of plant origin in the treatment of some human pathogens like *C.albicans* and *C. neoformans.*

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6. Reference

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