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RESEARCH ARTICLE

CHARACTERIZATION, GROWTH AND CONTROL OF *Cryptococcus neoformans* Sanf. CAUSING CRYPTOCOCCOSIS

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ABSTRACT

Cryptococcosis is an acute, subacute or chronic fungal disease caused by an encapsulated yeast *Cryptococcus neoformans.* It is the second most common fungal infection after candidiasis in HIV infected individuals and it is consider as the potentially most serious infections. Commercial antibiotics especially chloramphenicol was more effective in controlling *C. neoformans.* A wide variety of plants can be screened which are potentially antimicrobial. Morphological and cultural characteristics of *C. neoformans* Sanf. were studied. Morphology of the organism was studied by direct observation under the microscope by using wet mount and negative staining techniques. The cultural characteristics of the organism were studied in Sabouraud's dextrose agar and broth under the influence of different carbon sources. The influence of some commercial antibiotics and plant extracts were assessed for their antimycotic activities. The identity of the fungus was confirmed by nigrosin staining and biochemical tests. Effect of some physico-chemical factors such as temperature, pH, salinity, sugar (dextrose) and peptone on the growth of *C. neoformans* was studied and the optimum requirements for its growth have been determined. Antibiotic activities of some commercially available 11 antibiotics were assayed against *C. neoformans* and chloramphenical alone inhibited the growth of the test organism. The presences of antimicrobial properties in 129 plant extracts were also tested against *C. neoformans.* Among the plant extracts tested *Coleus forskohlii* and *Pedalium murex* (leaf) was more effective than other plant extracts tested.

INTRODUCTION

Cryptococcosis is an acute, subacute or chronic fungal disease caused by encapsulated yeast belonging to the genus *Cryptococcus. C. neoformans* is significantly pathogenic to human beings and animals. A rapid increase of Cryptococcosis worldwide has followed the pandemic of AIDS. Cryptococcosis is the second most common fungal infection after cadidiasis in HIV-infected individuals and potentially the most serious infections. In India, *Cryptococcus neoformans* var. *gatti* has been reported from Northern India for the first time in 1993 followed by reports from other states also. It has been reported from Ferozepur district of Punjab, Chandigarh, Karnataka and Tamilnadu. Cryptococcosis is more prevalent among the male patients as compared to females. The disseminated form of disease is frequently found in the immune-compromised patients. The clinical features of cryptococcosis may be described depending upon the anatomical sites involved such as pulmonary cryptococcosis, CNS cryptococcosis or cryptococcal meningitis, visceral or systematic cryptococcosis, osseous cryptococcosis, cutaneous cryptococcosis. *Candida albicans* and *Cryptococcus neoformans* infections have been increased dramatically over the last few years, due to the explosion of AIDS and cancer epidemics as well as solid organ transplant recipients around the world (Basha *et al*., 2010; Jafari *et al*., 2011). Chariman and Sittambalam *et al.* (2012) reported a case of steroid-induced cryptococcal infection in a non-HIV-infected person.The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world due to their potent pharmacological activities, low toxicity and economic viability. India is one of the richest countries in biological diversity, with a variety of climatic conditions and different ecological habits. The world health organization (WHO) has approved the use of herbal medicines (Kamboj, 2000). Ayurvedic and Siddha (Hindu) physicians practicing thousand years ago (Lewis and Elwin Lewis, 1978). The objective of present study is microscopic and macroscopic identification of *C. neoformans,* growth studies and antifungal activity of plant extracts towards the understanding the control of pathogenic fungus and diseases.

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MATERIALS AND METHODS

Collection of culture

Pure culture of *Cryptococcus neoformans* sanf. was obtained from Institute of Microbial Technology (MTCC), Chandigarh, India.

Macroscopic examination

Culture medium

The culture was streaked on Sabouraud's dextrose agar medium for the cultural identification of organism.

Microscopic examination

The culture was examined microscopically by Wet mount preparation, Simple staining (Kannan, 1996), Nigrosin staining, LCB mounting (Sivamani, 1999) and Saline wet mount preparation.

Biochemical tests (Conventional identification method), (Aneja, 2001)

(Aneja, 2001) Carbohydrates utilization test, TSI test, Urease test, Phenol oxidase test, Nitrate reduction test were tested in *C. neoformans* for identification of biochemical characters.

Screening of suitable medium

Agar media

Six different types of media viz., Sabouraud's dextrose agar, Potato dextrose agar, Nutrient agar, Cornmeal agar, Malt extract agar, Rose Bengal agar, were prepared for the selection of suitable medium for the growth of *Cryptococcus neoformans*.

Sabouraud's dextrose agar (SDA) medium

The media were distributed separately in 90 mm diameter Petriplates and allowed to solidify. The *Cryptococcus neoformans* was inoculated and incubated at room temperature for 4 days. After incubation, the cultural characters and colony morphology were studied.

Different broth media

Six different types of media viz., Sabouraud's dextrose broth, Potato dextrose broth, Nutrient broth, Cornmeal broth, Malt extract broth, Rose Bengal broth were also prepared for the selection of suitable culture broth. The organism was inoculated in 100 ml conical flask containing the broth. The flasks were incubated at 30± 2°C. The growth rate was determined by UV-spectrophotometer 119 (Systronics, India) at 560 nm for 12 hrs intervals.

Influence of pH, temperature, salinity, sugar (dextrose) and peptone concentration of the growth of *C. neoformans*

pH

Sabouraud's dextrose broth was prepared and the pH was adjusted to 4, 5, 6, 7, 8 and 9 using HCl and 0.1 N NaOH respectively. The different pH adjusted broth was taken in test tubes and the organism was inoculated and incubated at 30± 2°C for 2-4 days. The growth rate (OD value) of the organism was measured.

Temperature

C. neoformans was inoculated in test tubes containing Saboraud's dextrose broth and were incubated at different temperature viz., 10, 22 and 30± 2°C upto 54 hours. The growth rate (OD value) was calculated for 24 hrs intervals.

Salinity

SD broth was prepared with different concentrations of salinity (40, 60, 80 and 100 ppt) by adding NaCl and measuring with Refractometer, and (Atago, Japan). The pathogen was inoculated an incubated at room temperature for 12 hrs intervals and the growth rate (OD value) of the organism was determined.

Sugar-g/l

Different concentration of sugar (dextrose) viz., 0, 2, 4, 6 and 8 g/ml of Sabouraud's dextrose broth were prepared. *C. neoformans* was inoculated and incubated at 30± 2°C and the growth rate (OD value) of the organisms was determined.

Peptone

Different concentration of peptone viz., 0, 0.5, 1.0, 1.5 and 2.0/100 ml of Sabouraud's dextrose broth were prepared. Culture of *C. neoformans* inoculated and incubated at 30± 2°C upto 54 hrs. Every 12 hrs intervals the growth rate (OD value) of the organism was determined.

Antimycotic activities

Commercial antibiotics

Sabouraud's dextrose agar medium was prepared and poured into sterile Petriplates. After solidification the organism was swabbed over the medium with the help of sterile cotton swabs. The commercial antibiotic discs of Amphotericin-B, Chloramphenicol, Clotrinazole, Cotrimoxazole, Flucanazole, Gentamycin, Griscofeclein, Itraconazole, Ketocotazole, Micanazole, and Roxithromycin were placed over the medium in the petriplates and incubated at room temperature for 2-4 days. The plates were then examined for the development of inhibition zone.

Plant extracts

Totally 129 plants were collected from in and around Karur and Thanjavur districts. Their plant extracts were taken and assayed for antimycotic activity.

Preparation of plant extracts (Damodaran and Venkatraman, 1993)

The collected plant parts were washed thoroughly in tap water and 0.1 percent mercuric chloride solution followed by sterile distilled water. 50 g of plant parts (Leaves, Bulbs, Rhizomes,

Seeds, and Roots) were weighed and ground in individually with 100 ml of sterile distilled water, by using mortar and pestle. The extract was filtered through a nylon cloth and centrifuged at 5,000 rpm for 10 minutes. The supernatant was taken and 25, 50 and 100 per cent concentrations of the extract were prepared. Extracts were assayed by disc diffusion method (Kirby-Bauer method, 1996). 5 mm diameter sterile discs were dipped in extracts and placed over the medium swabbed with the organism in the Petriplates. The plates were incubated at room temperature and observed and the measured the zone of inhibition (diameter).

RESULTS

Culture characters of *Cryptococcus neoformans*

In the present investigation lyophilized *Cryptococcus neoformans* Sanf. was obtained from the institute of Microbial technology, Chandigarh and regenerated on sabouraud's dextrose agar. After 3 days of incubation, white with tan cream colour colonies were developed with smooth pasty and yeast like appearance. Microscopic examination of the nigrosin staining revealed encapsulated, round to oval shaped yeast cells (upto 6 µ in size), so as to confirmed that the culture was yeast-like fungi.

Biochemical test

Twelve biochemical characters were tested for *C. neoformans* and recorded. The organism grew in seven carbon sources such as dextrose, glucose, lactose, maltose, melibiose, rhamnose, sucrose, xylose and TSI and grew at 37°C The organism produced urease and phenoloxidase and could not reduce nitrate to nitrite.

Table 1. Biochemical tests for *C. neoformans*

S.No.	Biochemical tests	Results
1.	Dextrose	Positive
2.	Glucose	Positive
3.	Lactose	Negative
4.	Maltose	Positive
5.	Melibiose	Negative
6.	Rhamnose	Positive
7.	Sucrose	Positive
8.	Xylose	Positive
9.	Triple sugar iron test	Positive
10.	Urea test	Positive
11.	Phenol oxidase test	Positive
12.	Nitrate reduction test	Negative

Screening of suitable medium (solid and liquid medium)

Among the different medium tested the pathogen grew very well in Sabouraud's dextrose agar (SDA) and broth. Cornmeal agar (CMA) and broth and PDA and broth showed good growth, but nutrient agar (NA) and and Rose Bengal agar (RBA) and broth recorded only moderate growth.

Influence of pH, temperature, salinity, sugar (dextrose), and peptone concentration on the growth of *C. neoformans*

Effect of pH

C. neoformans grew in all the pH tested. However, better growth was observed at pH 7.0 to 9.0.

Effect of temperature

Temperature influenced the growth of *C. neoformans* Among the three temperatures viz., 10, 22 and 30 ± 2°C Maximum growth rate was observed only at 30 ± 2°C.

Effect of salinity

Among the various salinity (40, 60, 80 and 100 ppt) tested the growth rate was high at 60 ppt of salinity concentration.

Effect of sugar (dextrose)

C. neoformans inoculated in Sabouraud's dextrose broth amended with different concentration of sugar (Dextrose – 0, 2, 4, 6 and 8g/100 ml) the maximum growth was observed in the broth containing 2g/100 ml concentration of sugar.

Effect of peptone

C. neoformans inoculated in Sabouraud's dextrose broth amended with different concentration of peptone (0, 0.5, 1.0, 1.5, 2.0 g/100 ml). The maximum growth was observed in 1.5g/100 ml after the incubation period.

Antimycotic activity

Effect of commercial antibiotics

The commercial antibiotics showed zone of inhibition on the growth when placed on SDA medium swabbed with *C. neoformans.* But observed in there was no zone of inhibition.

In vitro inhibitory effect of antimicrobial agents against *C. neoformans*

S.No.	Antibiotics	Zone of inhibition (mm)
1.	Amphotericin-B	16
2.	Chloramphenicol	32
3.	Clotrinazole	3
4.	Cotrimoxazole	15
5.	Flucanazole	9
6.	Gentamycin	18
7.	Griscofeclein	14
8.	Itraconazole	-
9.	Ketocotazole	3
10.	Micanazole	4
11.	Roxithromycin	19

Effect of plant extracts

Aqueous extract of 129 medicinally important plant parts (leaves, bulbs, rhizomes, seeds and roots) were individually tested. Among the 129 plants, 37 plants were showed inhibitory effect on *C. neoformans.* The most effective plants were *Pedalium murex* (19 mm), *Coleus fovskohlii* (19 mm), *Hibiscus esculentus* (17 mm), *Vetiveria zizanoides* (16 mm), *Solanum nigrum* (15 mm), and *Andrographis paniculata* (16 mm).

DISCUSSION

The diseases caused by fungi in human beings are common and the are called mycoses. They are classified as superficial mycoses, cutaneous mycoses subcutaneous mycoses and opportunistic mycoses.

Zone of inhibition of *Cryptococcus neoformans* to aqueous medicinal plant extracts

S.No.	Botanical Name	Family	Using Part	Zone of inhibition (mm) 100%
1.	*Abrus precatorius* Linn.	Fabaceae	Seed	-
2.	*Acalypha indica* Linn.	Euphorbiaceae	Leaf	9
3.	*Achyranthes aspera* Linn.	Amarantaceae	Leaf	10
4.	*Acorus calamus* Linn.	Araceae	Leaf	-
5.	*Adhotoda vasica* Nees.	Acanthaceae	Leaf	10
6.	*Aegle marmelos* Correa.	Rutaceae	Leaf	12
7.	*Aleurites molucanna* Wild.	Euphorbiaceae	Seed	8
8.	*Allium cepa* Linn.	Liliaceae	Bulb	14
9.	*Allium sativum* Linn.	Liliaceae	Bulb	15
10.	*Aloe vera* Linn.	Liliaceae	Leaf	-
11.	*Alpinia galanga* Sw.	Zingiberaceae	Leaf	-
12.	*Alternanthera sessilis* Br.	Amarantaceae	Leaf	5
13.	*Andrographis paniculata* Nees.	Acanthaceae	Leaf	17
14.	*Anisochilus carnosus* Wall.	Lamiaceae	Leaf	-
15.	*Annona squamosa* Linn.	Annonaceae	Leaf	8
16.	*Arachis hypogea* Linn.	Fabaceae	Leaf	-
17.	*Aristolochia indica* Linn.	Aristolochiaceae	Leaf	-
18.	*Artemisia vulgaris* Linn.	Asteraceae	Leaf	-
19.	*Artocarpus integrifolia* Linn.	Moraceae	Leaf	-
20.	*Azadirachta indica* Linn.	Meliaceae	Leaf	6
21.	*Barleria cuspidata* Heyne.	Acanthaceae	Leaf	-
22.	*Bassia longifolia* Linn.	Sapotaceae	Seed	-
23.	*Cajanus indicus* Spreng.	Fabaceae	Leaf	-
24.	*Calophyllum inophyllum* Linn.	Clusiaceae	Leaf	-
25.	*Capsicum frutescens* Linn.	Solanaceae	Leaf	-
26.	*Cardiospermum halicacabum* Linn.	Sapindaceae	Leaf	-
27.	*Carica papaya* Linn.	Caricaceae	Leaf	-
28.	*Carum bulboehastranum* Koch.	Apiaceae	Seed	-
29.	*Carum copticum* Benth.	Apiaceae	Leaf	-
30.	*Carum copticum* Benth.	Apiaceae	Leaf and Seed	12 -
31.	*Cassia auriculata* Linn.	Fabaceae	Leaf	-
32.	*Cassia obtusifolia* Linn.	Caesalpiniaceae	Leaf	8
33.	*Celosia cristata* Linn.	Amarantaceae	Leaf	-
34.	*Cinnamomum zeylonium* Fr.	Lauraceae	Bark	-
35.	*Cissus quadrangularis* Wall.	Vitaceae	Leaf	10
36.	*Citrus acidia* Roxb.	Rutaceae	Leaf	10
37.	*Coriandrum sativum* Linn.	Apiaceae	Leaf	-
38.	*Coriandrum sativum* Linn.	Apiaceae	Leaf and Seed	-
39.	*Crataeva religiosa* Forsk.	Capparidaceae	Rhizome	-
40.	*Cucumis sativus* Linn.	Cucurbitaceae	Fruit	-
41.	*Cuminum cyminum* Linn.	Apiaceae	Seed	-
42.	*Cymopsis psoralioides* Dc.	Fabaceae	Leaf	-
43.	*Cynodan dactylon* Pers.	Poaceae	Leaf	-
44.	*Cyperus rotundus* Linn.	Cyperaceae	Leaf	-
45.	*Delonix regia* Raf.	Caecalpinaceae	Leaf	-
46.	*Dolichos biflorus* Linn.	Caesalpinaceae	Seed	-
47.	*Eclipta alba* Linn.	Asteraceae	Leaf	-
48.	*Eugenia caryophyllata* Thumb.	Myrtaceae	Seed	-
49.	*Eugenia jambolana* Lam.	Myrtaceae	Flower	-
50.	*Ferula asafetida* Linn.	Apiaceae	Root	15
51.	*Ferula asafoetida Linn*	Moraceae	Leaf	-
52.	*Ficus religiosa* Linn.	Moraceae	Leaf	-
53.	*Gossypium arboretum* Linn.	Malvaceae	Leaf	-
54.	*Gossypium arboreum Linn*	Asteraceae	Leaf	-
55.	*Helianthus annus Linn*	Asclepiadaceae	Root	-
56.	*Hibiscus canabinus* Linn.	Malvaceae	Leaf	-
57.	*Hibiscus esculentus* Linn.	Malvaceae	Leaf	17
58.	*Hibiscus rosasinensis* Linn.	Malvaceae	Leaf	-
59.	*Hydrocotyle asiatica* Linn.	Apiaceae	Leaf	-
60.	*Ilicium verum* Linn.	Magnoliaceae	Flower	-
61.	*Indigofera tinctoria* Linn.	Fabaceae	Leaf	12
62.	*Jasminum sambac* Ait.	Oleaceae	Leaf	-
63.	*Jatropha curcas* Linn.	Euphorbiaceae	Leaf	-
64.	*Lablap purpureus* (L.) Sweet.	Fabaceae	Leaf	-
65.	*Lagenaria vulgaris* Serrnge.	Cucurbitceae	Leaf	-
66.	*Lavendula vera* Dc..	Cucurbitaceae	Leaf	7
67.	*Leucas aspera* Spreng.	Lamiaceae	Leaf	-
68.	*Luffa acutangula* Roxb.	Cucurbitaceae	Leaf	-
69.	*Magnifera indica* Linn.	Anacardiaceae	Leaf	-
70.	*Mentha piperita* Linn.	Lamiaceae	Leaf	-

Continue

71.	*Mimosa pudica* Linn.	Mimosaceae	Leaf	-
72.	*Moringa pterigosperma* Goerth.	Moringaceae	Leaf	8
73.	*Mukia scarbrella* Ann.	Cucurbitaceae	Leaf	10
74.	*Murraya koenigii* Spr.	Rutacaeae	Leaf	6
75.	*Musa paradisiace* Linn.	Musaceae	Flower, Stem	-
76.	*Musa paradisiaca* Houtt.	Myristioaceae	Seed	-
77.	*Nyctanthes arbor-tristis* Linn.	Oleaceae	Leaf	-
78.	*Ocimum basilicum* Linn.	Lamiaceae	Leaf	-
79.	*Ocimum gratissium* Linn.	Lamiaceae	Leaf	-
80.	*Ocimum sanctum* Linn.	Lamiaceae	Leaf	-
81.	*Ocimum var. thyrsiflorum,* Benth.	Lamiaceae	Leaf	11
82.	*Papaver somniferum* Linn.	Papaveraceae	Seed	-
83.	*Pedalium murex* Linn.	Pedaliaceae	Leaf	19
84.	*Phascelus radiatus* Linn.	Fabaceae	Leaf	-
85.	*Phaseolus radiatus* Linn	Fabaceae	Seed	-
86.	*Phyllathus distichus* Muell.	Euphorbiaceae	Leaf	-
87.	*Phyllathus niruri* Linn.	Euphorbiaceae	Leaf	15
88.	*Physalis minima* Linn.	Solanaceae	Leaf	-
89.	*Pimpinella hyneana* Wall.	Apiaceae	Seed	7
90.	*Piper betle* Linn.	Piperaceae	Leaf	-
91.	*Piper nigrum* Linn.	Piperaceae	Seed	-
92.	*Pisidium gaujava* Linn.	Myrtaceae	Leaf	-
93.	*Pisidium gujava* Linn.	Fabaceae	Leaf	-
94.	*Polyalthia longifolia* Benth.	Annonaceae	Leaf	-
95.	*Polygala telephoides* Wild.	Polygalaceae	Leaf	-
96.	*Portulaca quadrifida* Linn.	Portulacaceae	Leaf	10
97.	*Prunus amygdalus* Bail.	Rosaceae	Leaf and Seed	-
98.	*Prunus amygdalus* Basl.	Rosaceae	Seed	-
99.	*Psoralea corylifolia* Linn.	Fabaceae	Leaf	-
100.	*Punica granatum* Linn.	Punicaceae	Leaf	-
101.	*Querus incana* Roxb.	Fagaceae	Seed	-
102.	*Raphanus sativus* Linn.	Brassicaceae	Leaf	-
103.	*Ricinus communis* Linn.	Euphorbiaceae	Seed	-
104.	*Rubia cordifolia* Linn.	Rubiaceae	Seed	-
105.	*Sesamum indicum* Dc.	Pedaliaceae	Leaf	11
106.	*Sesbania grandiflora* Pers.	Fabaceae	Leaf	7
107.	*Sida caprinifolia* Linn.	Malvaceae	Leaf	-
108.	*Sida spinosa* Linn.	Malvaceae	Leaf	-
109.	*Solanum mélangena* Linn.	Solanaceae	Leaf	-
110.	*Solanum nigrum* Linn.	Solanaceae	Leaf	16
111.	*Solanum tarvum* Swantz.	Solanaceae	Leaf	11
112.	*Solanum torvum* Swantz.	Solanaceae	Leaf	-
113.	*Solanum tuberosum* Linn.	Solanaceae	Leaf	-
114.	*Solanum viarum* Dunal.	Solanaceae	Leaf	-
115.	*Sphaeranthus amaronthoides* burm. f.	Amarantaceae	Leaf	-
116.	*Tabernaemontana coronacia* Br.	Apocynaceae	Leaf	14
117.	*Tamarindus indicus* Linn.	Casalpiniaceae	Leaf	-
118.	*Tectona grandis* Linn.	Caesalpinaceae	Leaf	-
119.	*Terminalia chebula* Retz.	Combretaceae	Seed	-
120.	*Thespesia populnea* Corr.	Malvaceae	Leaf	-
121.	*Trigonella foenum-graceum* Linn.	Fabaceae	Leaf	11
122.	*Vetiveria zizanoides* Nash.	Poaceae	Root	16
123.	*Vetiveria zizanoides* Nash.	Poaceae	Leaf	-
124.	*Vitex negundo* Linn.	Euphorbiaceae	Leaf	-
125.	*Wedelia pauciflora* W.A.	Asteraceae	Leaf	-
126.	*Wrightia tinctoria* Br.	Apocynaceae	Leaf	14
127.	*Zinzifer officinalis* Zosa.	Zingiberaceae	Rhizome	-
128.	*Zingifer officinalis* Zosa.	Rhamnaceae	Leaf	-

(-) Indicates no zone formation

Zone of inhibition of *C. neoformans* to selected aqueous plant extracts in various concentrations

S.No.	Botanical name	Family	Using part	Zone of inhibition (mm)		
				25%	50%	100%
1.	*Pedalium murex*	Pedaliaceae	Leaf	9	11	19
2.	*Coleus fovskohlii*	Laminaceae	Leaf	9	15	19
3.	*Vetiveria zizanoides*	Poaceae	Leaf	8	12	16
4.	*Andrographis paniculata*	Acanthaceae	Leaf	6	8	17
5.	*Hibiscus esculentus*	Malvaceae	Leaf	8	10	`17
6.	*Solanum nigrum*	Solanaceae	Leaf	10	12	16

Cryptococcosis is caused by an encapsulated yeast belonging to the genus *Cryptococcus* and it can cause disease in apparently immuno competent, as well as immuno compromised hosts. Most susceptible to infection are patients with T-cell deficiencies (Kwon-Chung, 1992; Mitchel and Perfect, 1995). Immuno-compromised patients are more susceptible to mycotic diseases than the healthy people. However, it was emphasized that the type of fungus and the nature of the diseases are to be considered in the study of mycoses (Gopalakrishnan, 2003). Methamphetamine enhances *Cryptococcus neoformans* pulmonary infection and dissemination to the brain. Notably, C. neoformans modifies its capsular polysaccharide after METH exposure, highlighting the fungus's ability to adapt to environmental stimuli, a possible explanation for its pathogenesis. The findings may translate into new knowledge and development of therapeutic and public health strategies to deal with the devastating complications of METH abuse. (Patel, 2013). The identification of the isolate was confirmed by microscopic examination for the presence of yeast like cells. Direct observation of yeast in KOH preparation Winn and Westernfeld (1997) and Kozel and Cazin (1971) and McGinnis (1980) reported an Indian-ink determined the capsule negative stain of *C. neoformans*. In the present investigation also pathogen (*C. neoformans*) was confirmed by negative staining and methylene blue staining method. The biochemical characters *C. neoformans* was described by Phaff and Fell (1970). In the present study, *C. neoformans* grew in seven carbon sources did not reduced nitrate to nitrite.

Growth and morphology of fungi is controlled by various physico-chemical characteristics and composition of the culture media (Rai, 1989). Hence, the standardization of the media is essential to grow the organism for any kind of investigation. In the present investigation, *C. neoformans* was cultured in six different nutrient media and their broth. Of them, in the yeast like fungi grew in all the six media and their broth were slightly decreased compared to the others. Temperature, pH and salinity are the major factors found to affect the growth of any fungi. Maximum growth rate of *M. furfur* was at range of P^H 7-9. However the fungus was able to grow at all P^H levels (Moore, 1938; and Vijayakumar 2003). But, in the present investigation *C. neoformans* grew well at pH ranges from 6.0 to 8.0 indicated that the pH requirements may vary from medium to medium. In vitro study of growth rate of *C. neoformans* does not grow at 40°C or higher, but they grew at 37°C is normal (Fromtling *et al.* 1988). Roberts (1969) and Vijayakumar (2003) revealed that the growth rate of *Malassezia fu*rfur was well at 37°C on Malt extract agar or Sabouraud's dextrose agar medium. In the present study also revealed the pathogen (*C. neoformans*) grew well at 30± 2°C. Likewise the salinity also had its own varying impact on the growth of different species. Most of the pathogenic fungal species are well adapted to higher salinity concentration (Vasuki *et al.,* 2002; Gopalakrishnan, 2003; Vijayakumar, 2003). In the present investigation, the concentration of salinity was increased and the growth rate of pathogen was slightly increased. Vijayakumar (2003) used five different concentration of glucose and peptone (0, 1, 2, 3 and 4%) in sabourad's dextrose agar and noticed that the growth of *M. furfur* was observed at 12 hrs intervals. In glucose the concentration was increased at same time, growth rate was increase at 4 percent. In the present work it was found that *C. neoformans* grew well at 2.0g/10 ml in dextrose and 1g/100 ml in peptone. Weindling (1934), Florey *et al*. (1946), Dobos and Hirsch (1966) Bava *et al*. (1989) reported that the antibiotics, viz., amphotericin B, nystatin, ketoconazole and fluconozole were effective to control the growth of many fungal pathogen who also stated that many yeast like fungi were resistant many commercial antibiotics. In the present study the maximum and minimum effect to control the growth of *C. neoformans* was observed in chloramphenicol (32 mm), ketaconazole and clotrimazole (3 mm) are respectively. Out of 11 commercial antibiotics intraconazole had no effect on the growth *C. neoformans*. The antifungal effect of an aqueous extract of garlic was tested against 10 strains of *C. neoformans*. In the present study, 129 locally available medicinal plants extract examined for antimycotic activity against *C. neoformans.* Of which 37 plants were showed the inhibitory effect. *Pedalium murex* (19 mm) and *Coleus fovskohliii* (19 mm) were showed the maximum inhibition and minimum was in *Alternanthera sessilis* (5 mm).

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