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# Studies on the antifungal properties of some medicinal plants against *Filobasidiella neoformans* var. *bacilliospora*

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## Abstract

Anticryptococcal activity of six different solvent extracts namely aqueous, acetone, chloroform, di ethyl ether, ethanol and methanol extracts of six medicinal plants *viz Andrographis paniculata, Coleus forskohlii, Hibiscus esculentus, Pedalium murex, Solanum nigrum* and *Vetiveria zizanoides* were tested against *Filobasidiella neoformans var. bacilliospora* by agar well diffusion method. All the plant extracts showed antifungal activity and the chloroform extract of *Andrographis paniculata* was the best when compared to other extracts tested. Preliminary phytochemical analyses for screening of macromolecules in *Andrographis paniculata* was employed in chloroform extract and also for the identification active compounds.

**Key Words**: Anticryptococcal activity, *Andrographis paniculata,* medicinal plants, solvent extracts

## INTRODUCTION

The use of plants to treat ailments is time immemorial and many of the traditional medicines are still included as a part of habitual treatment of various maladies. Plants are rich source of bioactive compounds. Many secondary metabolites like alkaloids, phenolics, steroids and terpenoids have been characterized from plants which have much pharmacological importance and also the isolation and fractionation of the active constituents from the medicinal plants still remain a long and tedious process (Patra, 2012). Furthermore, they form the basis for the development of new antimicrobials. Traditionally used medicinal plants have recently attracted the attention of the biological scientific communities (Taylor *et al*., 2001).

*Cryptococcus neoformans* is known in both its asexual (anamorph) and sexual (teleomorph) states, for which the respective names *Cryptococcus neoformans* and *Filobasidiella neoformans* Kwon-Chung are used. The fungus can cause serious infections, especially in immuno- compromised patients. The main sites of infection are the lungs and the central nervous system, including cerebrospinal fluid (CSF). In the brain it causes meningitis, meningoencephalitis, and granuloma formation. Primary cutaneous infections are rare, and most skin infections are due to disseminated systemic infections. *Cryptococcus neoformans* var. *neoformans* is encountered in nearly all of the AIDS-related infections. The present article deals with the antimicrobial activity of six different plants against the human pathogen *Filobasidiella neoformans* Kwon-Chung.

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## MATERIALS AND METHODS

### Sample colection

Fresh, young and tender leaves of *Andrographis paniculata, Coleus forskohlii, Hibiscus esculentus, Pedalium murex* and *Solanum nigrum,* and roots of *Vetiveria zizanoides* were collected from the herbal garden of A.V.V.M Sri Pushpam College, Poondi, Thanjavur District were brought to the laboratory in separate polythene bags for further analysis.

### Extraction of Plant Materials

The freshly collected plant parts were thoroughly washed with tap water followed by sterile distilled water. The materials were dried in an oven at 50°C for 48 hrs followed by grinding in to fine powders in an electric blender (Lin and Lineback, 1990). The powders were used for extraction using different solvents such as aqueous, acetone, chloroform, diethyl ether, ethanol and methanols. 25 g of the powdered plant materials were dissolved separately in sufficient quantity of solvent to make 100ml of extract (25% w/v). The mixtures were kept undisturbed at room temperature for 24 hrs in a sterile flask covered with aluminum foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper. After filtration, the extract was evaporated in water bath until 25 ml extract was left in the container and immediately evaluated for antifungal activity through agar well diffusion method (Chen *et al*., 1987 : Barreto *et al*., 2002).

### Antifungal activity (Agar well diffusion method)

### Test organism

Pure culture of *Filobasidiella neoformans var. bacillispora* (MTCC No.1347) was obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. It was subcultured and

maintained in Malt Yeast Agar (Malt Extract 3.0 gm, Yeast Extract 3.0 gm, Peptone 5.0 gm, Glucose 10.0 gm, Agar 20.0 gm, Distilled water 1.0 L, pH 6.20).

**Antimicrobial Assay:**

The principle of agar well diffusion is similar to that of agar disk diffusion assay method (Das *et al.,* 2010). A standardized concentration of inoculum with fixed volume was spread evenly on the surface of the Potato Dextrose Agar plate. A hole (6 - 8 mm in diameter) was punched with a sterile cork borer aseptically in the middle. A fixed volume of plant extract was then introduced into the well and incubated at optimum temperature and duration depending upon the test microorganism (Mbata et al., 2006; Norrel and Messely, 1997).

**Qualitative determination of carbohydrates, proteins and amino acids (Harbone *et al.*, 1998)**

**Test for carbohydrates**

**Fehling's test:-**

**Fehling's Solution:**

**Solution A**: 34.6gms of pure copper sulphate were dissolved in distilled water and dilute to 500 ml.

**Solution B**: 173gms of sodium potassium tartrate and 30 gms of pure sodium hydroxide were dissolved in water and dilute to 500ml. Alternately, dissolved 121gms of pure sodium hydroxide and 93.1gms of pure tartaric acid in water, then the solution was diluted to 500ml (colourless). Equal volumes of solutions A and B were mixed immediately before use, and then used as the reagent.

1 ml of Fehling's solution A, was added to 1 ml of Fehling's solution B and a few drops of extract was also added and boiled for a few minutes. Formation of brownish red precipitate showed the presence of carbohydrate.

**Test for aminoacid and protein**

**Biuret test:-**

Equal volume of 5% sodium hydroxide solution (5 gm sodium hydroxide pellets dissolved in 100 ml distilled water) and 1% copper sulphate solution (1gm of copper sulphate was mixed in 100 ml distilled water) were added to 1 ml of extract. Formation of pink colour showed the presence of protein and amino acids.

**Qualitative determination of Secondary metabolites**

**Test for ascorbic acid**

2ml of 2% extract was added with 2ml of water, 0.1g of sodium bicarbonate and 20mg of ferrous sulphate. Then they were shaken and then kept undisturbed. A deep violet colour was developed, which disappeared when 5ml of 1M sulphuric acid was added that indicated the presence of ascorbic acid.

**Test for alkaloids**

**Mayer's test (Trease and Evans, 1978):-**

A drop of Mayer's reagent was added to a few ml of the filtrates by the side of the test tube. A creamy or white precipitate indicated the presence of alkaloids.

**Test for flavonoids (Mace, 1963)**

A portion of the aqueous extract was added to 5ml of the dilute ammonia solution followed by addition of concentrated sulphuric acid. Appearance of yellow coloration indicated the presence of flavonoids.

**Test for tannins (Mace, 1963)**

About 0.5mg of dried powered sample was boiled in 20ml of water in test tubes, and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green coloration.

**Test for phenol (Mace, 1963)**

**Ferric chloride Test:-**

The extract was diluted to 5ml with distilled water. To this a few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of phenolic compounds.

**Test for terpenoids (Mace, 1963)**

**Salkowski Test:-**

5ml of the extract was mixed with 2ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface showed the presence of terpenoids.

**Test for gums and mucilage**

About 10ml of each extract was separately added to 25ml of ethanol with constant stirring and kept for 2hrs. The swelling property of the precipitate showed the presence of gums and mucilage.

**Test for anthroquinones**

The extract was mixed with few drops of concentrated sulphuric acid. Red colour precipitation showed the presence of Anthroquinone.

**Test for cardiac glycosides**

About 1ml of glacial acetic acid was added to 2ml of extract and treated with ferric chloride and concentrated sulphuric acid. Appearance of greenish blue colour indicated the presence of cardiac glycosides.

**Test for saponins (Kokate, 1999)**

The extract was diluted with distilled water and made up to 20ml. The suspension was shaken in a graduated

cylinder for 15 min. 2 cm layer of foam indicated the presence of saponins.

**Test for sulfur**

2ml of 40% Sodium hydroxide and 10% lead acetate were added to 2ml of the extract solution, boiled for a minute and then cooled. Formation of a black precipitate indicated the presence of sulphur.

**Test for steroids**

2ml of chloroform, 3 drops of anhydrous aluminum chloride and 2 drops of concentrated sulphuric acid were added to 1ml of the ethanolic fruit extract. The obtained blue or green colour showed the presence of steroids.

**RESULTS**

**Culture characters of *Filobasidiella neoformans var. bacilliospora***

In the present investigation slant cultures of *Filobasidiella neoformans var. bacilliospora* was obtained from the institute of MTCC and regenerated on Malt extract 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 organism with lactophenol cotton blue stain showed the presence of encapsulated, round to oval shaped yeast cells (upto 6 µ in size), and it was confirmed that the culture was yeast-like fungus *Cryptococcus neoformans var. bacilliospora* (Anamorph) and *Filobasidiella neoformans var. bacilliospora* (Teleomorph).

**Effect of plant extracts**

All the six plants' extracts showed inhibitory effect against *Filobasidiella neoformans var. bacilliospora***.** The chloroform extract of the leaves of *Andrographis paniculata* was highly effective (22 mm) .

Table-1 Antifungal activities of some medicinal plants

| S.No. | Botanical Name | Family | Using Parts | Zone of inhibition(100%) in millimetre | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A | C | D | E | M | W |
| 1. | *Andrographis paniculata* | Acanthaceae | Leaf | 16 | 22 | 9 | 13 | - | 17 |
| 2. | *Coleus fovskohlii* | Lamiaceae | Leaf | - | 18 | - | - | 16 | 19 |
| 3. | *Hibiscus esculentus* | Malvaceae | Leaf | - | 18 | 11 | 15 | - | 17 |
| 4. | *Pedalium murex* | Pedaliaceae | Leaf | - | - | - | 15 | 19 | 19 |
| 5. | *Solanum nigrum* | Solanaceae | Leaf | - | - | - | 19 | - | 16 |
| 6. | *Vetiveria zizanoides* | Poaceae | Root | 13 | 12 | 14 | 15 | 17 | 16 |

A-Acetone, C-Chloroform, D-Diethyl ether, E-Ethanol, M-Methanol, W-Water.

Qualitative determination of phytochemicals

The chloroform extract of the leaves of *Andrographis paniculata* showed the presence of reducing sugars Ascorbic acid, Terpenoids and Anthroquinones.

Table-2 Phytochemical analysis of Chloroform leaf extract of *Andrographis paniculata*

| S.No. | Phytochemicals | Chloroform extract of Leaves |
|---|---|---|
| 1. | **Test for carbohydrates** | |
| (i) | Fehling's test | Positive |
| 2. | **Test for amino acid and protein** | |
| (ii) | Biuret test | Negative |
| 3. | **Preliminary qualitative analysis for secondary metabolites** | |
| (iii) | Test for ascorbic acid | Positivve |
| (iv) | Test for alkaloids | Negative |
| (v) | Test for flavonoids | Negative |
| (vi) | Test for tannins | Negative |
| (vii) | Test for phenol | Negative |

| | | |
|---|---|---|
| (viii) | Test for terpenoids | Positive |
| (ix) | Test for gums and mucilage | Negative |
| (x) | Test for anthroquinones | Positive |
| (xi) | Test for cardiac glycosides | Negative |
| (xii) | Test for saponins | Negative |
| (xiii) | Test for sulfur | Negative |
| (xiv) | Test for steroids | Negative |

## DISCUSSION

In spite of the existence of numerous antifungal and the therapeutic combinations, cryptococcosis is a part of the three most dangerous fungal infections in hospitable environment. In the traditional medicinal practice of treatment of various diseases mostly water was used as solvent. It was realized that the antifungal properties of the aqueous extracts were not complete and hence attempts were made with different organic solvents to elucidate the antifungal properties of the plants. Among the solvents such as ethanol, ethyl acetate, dichloromethane and hexane were found effective in fractionation and concentration of active ingredients (Yaye Yapi Guillaume et al., 2013). In the present investigation six solvents such as acetone, chloroform, diethyl ether, ethanol, methanol and water were used in different plants for its antifungal activity. Of which the chloroform extract of the leaves of *Andrographis paniculata* showed better result against *Filobasidiella neoformans* than the other plants and solvents (Table 1).

*Baccharis dracunculifolias* (hexane extract) was effective against *C. neoforman,*in inhibiting the growth of this fungus. The presence of alkaloids, saponins, flavonoids, anthraquinones and triterpenes or steroids was observed in hexane extract of *B. dracunculifolia. (*Maria Aparecida de Resende *et al*., 2007). But in the present study *A. paniculata* (chloroform extract) was more effective against the same organism than the other plants' extracts tested in inhibiting the growth of the fungus. The phytochemical constituents viz., reducing sugar, ascorbic acid, terpenoid, anthroquinones were present in the chloroform extract of *A. paniculata*. Hence, in line with the current trend of finding naturally occurring anticryptoccosis activity, this study was designed to evaluate anticryptococcosis activity of the extracts of *A. paniculata* on the *in vitro* growth of *Filobasidiella neoformans*, which causes illness in human.

In general, the antibacterial activity of the diterpenoids and flavones is probably due to the membrane disruption of terpenes, (Urzua et al., 1998; Cowan, 1999) and to their quilting the ability of flavones to form complex with extracellular soluble proteins to complex with bacterial cell walls and disrupt microbial membranes. But in the present investigation terpenoid present in the chloroform extract of *A. paniculata* is mainly responsible for its antifungal activity.

Phytochemical compositions of the medicinal plants are different from those of the frequently studied microbial sources, and therefore their mode of action might also differ (Fabricant and Fans worth, 2001). There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Prachayasittikul *et al*., 2008; Nogueira et al., 2008; Costa et al., 2008; Al-Bayati and Al-Mola, 2008; Chen *et al*., 2008; Pesewu *et al*., 2008; Turker and Usta, 2008).

The phytochemical screening of *Gymnema sylvestre* showed the presence of steroids or terpenoids and coumarins in the chloroform and steroids or terpenoids alone was found in its hexane extract. In case of *Andrographis paniculata* both the extracts showed the presence of steroids or terpenoids and coumarins (Shafi Thompson *et al*., 2011). In the present study the phytochemical screening of *A. paniculata* in chloroform extract alone showed the presence of reducing sugar, ascorbic acid, terpenoid, anthroquinones and absence of amino acid and proteins, alkaloids, flavonoids, tannins, phenol, gums and mucilage, cardiac glycosides, saponins, sulfur and steroids (Table 2). Hence terpenoids could cause the inhibition of the pathogen *Filobasidiella neoformans var. bacilliospora.*

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