

CHARACTERIZATION OF ANTIFUNGAL ACTIVITY OF THE VARIOUS ACTIVE CONSTITUENTS OF *COLEUS FORSKOHLII*

Ranjith Santhosh Kumar, D.S.* and B. Lakshman Kumar
Department of Biotechnology, Kongunadu Arts & Science College, Coimbatore.
*Email: ranjith.deva87@gmail.com

ABSTRACT

The aim of this study was to evaluate the anti-fungal effect of different solvent extracts of *Coleus forskohlii* which contains minor diterpenoids, deacetylforskohlin, 9-deoxyforskohlin, 1,9-deoxyforskohlin, 1,9-dideoxy-7-deacetylforskohlin, and four other diterpenoids, along with alkaloids and volatile oils. The solvent extracts included both polarized and non-polarized solvents like methanol, ethylacetate, benzene, chloroform, water, hexane, acetone and ethanol. As a result of this study, the antifungal effect was best observed with the methanolic extract against *Candida albicans* in comparison to the other extracts. This study also revealed that apart from forskohlin, which was confirmed by Thin Layer Chromatography, other compounds that were extracted with methanol are required to produce the antifungal effect and can be taken forward to pharmaceutical preparations.

Keywords: Antifungal, active constituents, *Coleus forskohlii*.

1. INTRODUCTION

Coleus forskohlii also known as *Plectranthus barbatus* (Lamiaceae) is one of the most significant potential medicinal crops of the future, as its pharmacopoeial properties have been discovered only recently. The tuberous roots have been identified as a rich drug for glaucoma, congestive, cardiomyopathy, asthma and certain cancers. Forskolin primary mode of action is to increase cyclic adenosine monophosphate (cAMP) and cAMP mediated functions, via activation of the enzyme adenylatecyclase (Metzger, *et al* 1981). Forskolin has been shown to increase cAMP formation in all eukaryotic cells except sperm, without hormonal activation of adenylatecyclase (Seamon, *et al* 1981). Forskolin's potentiation of cAMP in turn inhibits basophil, mast cell degranulation and histamine release (Marone, *et al* 1986); lowers blood pressure (Dubey, *et al* 1981), intraocular pressure inhibits platelet aggregation (Agarwal, 1982), promotes vasodilation (Wysham *et al* 1986), bronchodilation (Lichey, *et al* 1984), thyroid hormone secretion (Roger, 1987) and stimulates lipolysis in fat cells (Okuda, 1992).

Forskolin has been shown to enhance lipolysis, regulated by cAMP, also inhibits fat storage. Forskolin is responsible for virtually all pharmacological activities attributed to *Coleus forskohlii* and the extracts of this constituent have been used in nearly all existing studies though other plant constituents, such as volatile oils and other

diterpenoids and coleonols, contribute to the pharmacological activity and adsorption of forskolin.

Candida albicans causes Candidiasis which is an increasingly important disease that has a worldwide distribution due to the fact that it is a frequent opportunistic pathogen in patients. It is a fungus normally present on the skin and in mucous membrane such as vagina, mouth or rectum. The immune system keeps *Candida* proliferation under control, but when immune responses are weakened, *Candida* growth can precede unhindered. The uncontrolled growth of *Candida* is called *Candida* over growth. Solid media is generally employed for fungal culture, as the broths are not usually recommended except for fungal blood cultures were bi-phasic medium is used. The media commonly employed is Emmon's modification of Sabouraud dextrose agar. The media may be supplemented with antibiotics, such as gentamicin and chloramphenicol to minimize bacterial contamination and cyclohexamide to inhibit saprobic fungi (Jagdishchander, 1999).

The study of Senff *et al.*, (1990) showed the presence of *Candida albicans* in samples with scalp psoriasis or seborrheic dermatitis of the scalp (psoriasis capillitii). Yeasts were found in half of the tongue cultures and in 1/8th of the scalp scales. Further proof that *Candida* is one of the causes of psoriasis was seen in the study analyzed in more than 40,000 patients which showed that patients with atopic dermatitis and psoriasis had elevated levels of *Candida* in the intestines (Henseler *et al.*,

1997). The analysis showed that *Candida* colonization in the digestive tract can be one of the causes of psoriasis.

2. MATERIALS AND METHODS

2.1. Collection of raw drugs

The plants of *Coleus forskohlii* was collected randomly from Tamil Nadu Agricultural University (TNAU) Coimbatore, Coimbatore and Salem regions. The taxonomic identities of these plants were confirmed at Botanical survey of India and the voucher specimen numbers of the plants were preserved. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

2.2. Extraction procedure

3g of air-dried powder was extracted in water and all the solvents in Bio Sox apparatus and then kept on a rotary shaker at 190-220 rpm for 2h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min. The supernatant was collected. This procedure was repeated twice. The supernatant collected was pooled together and concentrated by evaporating in a vacuum drier to make the final volume one fourth of the original volume and stored at room temperature in airtight bottles.

2.3. Anti-fungal activity

2.3.1. Fungal Strains

In vitro antimicrobial activity was examined for aqueous and solvent extracts of *Coleus forskohlii*. The culture of *Candida albicans* was obtained from the PSG Institute of Medical Sciences and Research, Coimbatore (ATCC11778) and maintained at 4° C on nutrient agar slants.

The nutrient broth and nutrient agar were prepared as per the standard composition.

2.3.2. Method

Nutrient broth was prepared as per the standard composition and consisted of peptone 5 gm, NaCl 5 gm, Beef extract 3 gm, yeast extract 2 gm, pH 6.8 per 1 litre. Nutrient Agar- Peptone 5 gm, NaCl 5 gm, Beef extract 3 gm, Yeast extract 2 gm, Agar 20 gm, pH6.8 per 1 litre. Nutrient broth prepared for each culture and was sterilized in an autoclave at 1210C for 15 minutes. After sterilization the drug, different concentrations of the extracts were added. Further the cultures *Candida* sps. was inoculated in the tubes and subcultured weekly. Control sample was prepared along with culture in the absence of the drug. Only medium served as blank.

2.3.3. Media preparation and antifungal activity

The antimicrobial assay was performed by agar well diffusion method for all the extracts. The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1×10^8 cfu/ml) and poured into the Petri plate (Hi-media). For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (0.85 cm). Different concentrations of the test compound were introduced into the well. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of zone of inhibition. Controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done in triplicate and the mean values recorded.

3. RESULTS AND DISCUSSION

In the present study the different solvent extracts of *Coleus forskohlii* (methanol, ethyl acetate, benzene, chloroform, water, hexane, ethanol and acetone) at different concentrations 75µg and 150 µg was tested for its antimicrobial property against the growth of *Candida albicans*. The zone of inhibition observed after 72 hrs showed potent activity at 150µg of the methanol extract (20 mm) and benzene extract (13mm) and water (12mm), whereas with chloroform it was 6mm and other solvents like acetone, ethylacetate, ethanol, hexane did not reveal any zone of inhibition (Table 1 and Figure 1).

The anti microbial activity of plant oils and extracts has been recognized for many years and the significance of the microbial activity of oils and extracts of plants against *Candida albicans*, *Enterococcus faecalis* and *E. coli* has been reported. The oil of lemon grass, oregano and bay leaves were observed to inhibit the growth of most microorganisms (Naveens, 2008). The plant extracts, as methanolic extracts of *Peltophorum pterocarpum* and *Punica granatum* exhibited highest antifungal activity against *Candida albicans* (Duraipandyan et al., 2006).

The above results imply that the compounds from *Coleus forskohlii* that was extracted into methanol had the property to inhibit the growth of *Candida albicans* compared to the other solvents extracts. There was no antagonistic effect on *Candida albicans* by the *Coleus forskohlii* extracted in chloroform, petroleum ether and other solvents. However, maximum inhibition was observed with the reference drug clopidogrel, where as DMSO, used as vehicle control showed no inhibitory effect. The studies of screening results show a correlation with the antibiotic uses of the plant and the active

compounds present in extracts that may be responsible for the antimicrobial activity.

4. CONCLUSION

In the present study, methanolic *Coleus forskohlii* extracts exhibited high level of antifungal activity against *Candida albicans*. They have an excellent safety profile and are generally without toxicity or side effects at the recommended dosage. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with adequate efficacy can be used for the treatment and prevention of various ailments. The potential for developing therapeutic drugs from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against safely in humans. Plant-based drugs have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic drugs. Continued further exploration of plant-derived products is needed today.

Further research is necessary to determine the identity of the compounds from within these plants and also to determine their full spectrum of efficacy. However, the present study of *in vitro* evaluation of *Coleus forskohlii* forms a primary platform for further phytochemical and pharmacological studies.

Table 1

S.No	Forskolin extracts	Zone of inhibition in (mm)
1	Methanol	20
2	Ethyl acetate	-
3	Benzene	13
4	Chloroform	-
5	Water	12
6	Hexane	-

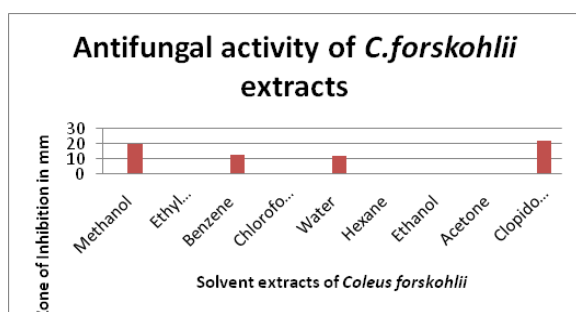


Figure 1

REFERENCES

Agarwal, K.C. and R.E. Jr. Parks, (1982). Synergistic inhibition of platelet aggregation by forskolin

plus PGE1 or 2-fluoroadenosine: effects of 2',5'-dideoxyadenosine and 5'-methylthioadenosine. *Biochem. Pharmacol.* **31**: 3713-3716

Buslau, M., I. Menzel and H. Holzmann, (1990). Fungal flora of human faeces in psoriasis and atopic dermatitis. *Mycoses* **33**: 90-94

Dubey, M.P., R.C. Srimal, S. Nityanand and B.N. Dhawan, (1981). Pharmacological studies on coleonol, a hypotensive diterpene from *Coleus forskohlii*. *J. Ethnopharmacol.* **3**: 1-13.

Marone, G., M. Columbo and M. Triggiani, (1986). Forskolin inhibits the release of histamine from human basophils and mast cells. *Agents Actions* **18**: 96-99.

Metzger, H. and E. Lindner, (1981). The positive inotropic-acting forskolin, a potent adenylate cyclase activator. *Arzneimittelforschung* **31**: 1248-1250.

Naveena, B.M., M. Muthukumar, A.R. Sen, Y. Babji and T.R.K. Murthy, (2006). Improvement of shelf-life of buffalo meat using lactic acid, clove oil and vitamin C during retail display. *Meat Sci.* **74**: 409-415.

Okuda, H., C. Morimoto and T. Tsujita, (1992). Relationship between cyclic AMP production and lipolysis induced by forskolin in rat fat cells. *J. Lipid Res.* **33**: 225-231

Roger, P.P., P. Servais and J.E. Dumont, (1987). Regulation of dog thyroid epithelial cell cycle by forskolin, and adenylate cyclase activator. *Exp. Cell Res.* **172**: 282-292.

Seamon, K.B., W. Padgett and J.W. Daly, (1981). Forskolin: unique diterpene activator of adenylate cyclase in membranes and intact cells. *Proc. Natl. Acad. Sci. USA* **78**: 3363-3367

Senff, H., C. Bothe, J. Busacker and D. Reinel, (1990). Studies on the yeast flora in patients suffering from psoriasis capillitii or seborrheic dermatitis of the scalp. *Mycoses* **33**: 29-32.

Srinivasan, S., M. Anitha, S. Mwangi and R.O. Heuckeroth, (2005). Enteric neuroblasts require the phosphatidylinositol 3-kinase/Akt/Forkhead pathway for GDNF-stimulated survival. *Mol. Cell. Neurosci.* **29**: 107-119

Veeramuthu Duraipandiyan, Muniyappan Ayyanar and Savarimuthu Ignachimuthu, (2006). *Complemen. Alter. Med.* 1-7.

Wysham, D.G., A.F. Brotherton and D.D. Heistad, (1986). Effects of forskolin on cerebral blood flow: implications for a role of adenylate cyclase. *Stroke* **17**: 1299-1303.