DOI:10.26524/KRJ301

Kong. Res. J. 6(2): 51-55, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

RESEARCH ARTICLE

EVALUATION OF ANTI-FUNGAL POTENTIAL OF *CINNAMOMUM ZEYLANICUM* BLUME (LAURACEAE) BARKS EXTRACT FOR ONYCHOMYCOSIS

Manivannan, S[1]. and S. Bhuvaneswari[2]

[1]Department of Zoology, Kongunadu Arts and Science College, Coimbatore- 641 029, Tamil Nadu, India.
[2]Department of Pharmaceutics, Periyar College of Pharmaceutical Science, Tiruchirappalli – 620021,Tamil Nadu, India.

ABSTRACT

Onychomycosis is a fungal infection of nails caused by dermatophytes, yeasts of nondermatophyte molds. Far more than being a simple cosmetic problem, infected nail serves as a chronic reservoir, which can give rise to repeated mycotic infections and represents about 30% of mycotic cutaneous infections. The prevalence rate of onychomycosis is determined by age, predisposing factor, social class, occupation, climate, living environment. To evaluation of Anti-fungal potential of *Cinnamomum Zeylanicum* Blume (Lauraceae) Barks Extract for Onychomycosis. The *C. Zeylanicum* barks crude extracts were obtained by soxhlet-solid-liquid extraction using ethanol and aqueous. The dermatophytes namely, *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton simii, Epidermophyton floccosum* and *Candida albicans* were recovered from rice farmers in Orathanadu, Thanjavur (Dt), Tamil Nadu. The antifungal activity, minimum inhibitory concentrations and minimum fungicidal concentrations against the onychomycosis of *C. zeylanicum* was determined by the disc diffusion methods in sabouraud dextrose agar using ketoconazole (2mg/disc) as a positive control. Phytochemical analysis revealed the presence of wide range of bioactive constituents like flavonoids, tannins, alkaloids, saponins, terpenes and steroids. The ethanol barks extract inhibited all test organisms at the minimum concentration of 25 mg/ml while fungicidal actions were observed at a concentration of 50 mg/ml for *T. rubrum,* 180 mg/ml for *T. mentagrophytes,* 160 mg/ml for *T. simii,* 120 mg/ml for *E. floccosum* and 60 mg/ml for *C. albicans.* The ability of the extracts to inhibit the growth of fungi likes dermatophytes and yeast is an indication of the antifungal potential of *C. zylanicum*, which makes the candidate for production of antifungal agents.

Keywords: *Cinnamomum zeylanicum*, phytochemical analysis, ethanol extract, antifungal, disc diffusion method, inhibition zone.

1. INTRODUCTION

Onychomycosis is an infection of the nail and may involve the nail bed, nail plate and matrix by fungi that include dermatophytes, nondermatohyte moulds and yeasts. Onychomycosis is the most common condition affecting the nails, accounting for 50% of all nail disorders and 33.3% of all mycotic infections of the skin [1, 2]. The toenails are affected in 80% of all cases of onychomycosis; dermatophyte infection, mostly due to *Trichophyton rubrum*, is the cause in over 90% of cases. 5–10% of these infections are caused by yeasts, especially *Candida albicans*. Onychomycosis occurs in 10% of the general population the incidence of onychomycosis has been increasing, owing to such factors as diabetes, immunosuppression, and increasing age. Population-based studies have found varied estimates of prevalence, ranging from less than 1 % to 8% percent in Europe and the United States and less than 1 % in Central Africa. Various workers have reported the incidence to vary from 0.5 to 5% in the general population in India [3].

Fungal nail infections are not life-threatening, yet they are associated with secondary bacterial infection, chronicity of disease, therapeutic failures and disfigurement like hyperkeratosis, discoloration of nail plate, and brittle nails [4]**.** The causative agents of the disease may vary depending upon geographic or temporal distribution. Even in developed countries, the importance of nail infections has been highlighted only in the last decade. In developing countries, socioeconomic constraints and other common prevalent health issues have led to a low awareness of onychomycosis by physicians and general population. Thus, even in the presence of good personal hygiene, it has continued to persist and spread.

Medicinal plants have been a part of modern life style of a man and these plants are a source of important therapeutic aid for alienating human ailments. With increasing realization of the health hazards and toxicity associated with the indiscriminate use synthetic drugs and antibiotics, interest, in the use of plants and plant based drugs

*Correspondence: MANIVANNAN, S., Department of Zoology, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: manivannanresearch@gmail.com

revived throughout the world. However, a large number of medicinal plants remain to be investigated, for their possible pharmacological value. Most of the pharmaceutical industry is highly dependent on wild population for the supply of raw materials for extraction of medicinally important compounds. The screening of natural products has been the source for new potential drugs is still largely unexplored and only a small percentage of them has been subjected to phytochemical investigation and the fractions submitted to pharmacological screening. Such screening of various natural organic compounds and identifying active agents is the need of the hour as due to successful prediction of lead molecule and discovery will pay of later in drug development. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties [5, 6].

Cinnamomum zeylanicum is a small, tropical, evergreen tree most noted for its bark, which provides the world with the commonly known spice, cinnamon. This species belongs to Lauraceae family, is native to Indonesia and cultivated in various regions of the world. Several biological properties of *C. zeylanicum* have been described such as antiseptic, analgesic, anti-spasmodic, astringent, insecticide and parasiticide properties [7]. Cinnamon is a spice tree contains several bioactive compounds that can be used against a wide range of microorganisms. Cinnamon bark crude extract has constantly been reported to have antifungal activity [8].

Antifungal susceptibility testing remains an area of intense interest. Susceptibility testing can be used for drug discovery and epidemiology. Number of reports is available showing efficacy of *C. zeylanicum* essential oils as antimicrobial agents [9]. The oil extracted from *C. zeylanicum* bark and leaves have been reported to possess fungicidal activity against fungi responsible for causing crown rot disease of banana. The major constituent possessing antifungal activity in *C. zeylanicum* bark and leaf oils were found to be cinnamaldehyde and eugenol, respectively. In addition other compounds having fungicidal property have also been reported to be present in bark and leaves [10]. Cinnamon barks represent important source of compounds like flavonoids, tannins, glycosides, saponines, alkaloids. The objectives of the current investigation to evaluate the anti-fungal activity of *C. zeylanicum* barks extracts is done in order to detect new sources anti-fungal agents.

2. MATERIALS AND METHODS

2.1. Collection of plant materials

The bark of *C. zylanicum* was collected from Spices Research Institute, Kerala in November 2018.

2.2. Preparation of plant extract

Freshly collected plant parts were shade-dried at room temperature for 10–15 days. Dried bark samples were separately crushed and ground into fine powder with mortar and pestle. Powdered plant materials were sequentially extracted with solvents in a Soxhlet apparatus for 8 h [11]. The solvents used for extraction included ethanol and aqueous. The respective extracts were filtered and dried under reduced pressure using rotary evaporator to yield solid/semisolid residues. The residues were lyophilized to get dry solid mass.

2.3. Preliminary phytochemical screening

The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of phenol and polyphenols, flavanoids, terpenoids, tannins, alkaloids and seponins, as described in literatures [12, 13].

2.4. Source of microorganism

Fifty samples were collected from rice farmers in Thanjavur, Tamil Nadu, with lesions suggestive of fungal infections. Dermatophytes and yeast were isolated and identified based on detailed study of their microscopic and macroscopic features [14].

2.5. Determination of anti-fungal activity

Sterilized discs (6 mm) prepared from Whatman No 1 filter paper were impregnated with different concentrations (10 mg, 20 mg, 40 mg, 80 mg) of ethanol extract dissolved in 2% Dimethyl Sulphoxide (DMSO). The disc of the ethanol and aqueous extracts were placed on Sabouraud dextrose agar (SDA) plates seeded with 0.1ml of 10^4 dilution of inoculum preparation [15]. The plates were prepared in triplicate, incubated at room temperature for 7 days and average diameter zone of inhibition recorded. Discs impregnated with 2% DMSO and 2 mg/disc ketoconazole served as negative and positive controls respectively.

2.5. Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC)

MIC:

Two hundred milligrams of the ethanol extract was dissolved in 2% DMSO and serially diluted two fold in sterile water. Different tubes containing different concentrations (25mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml) of the extracts were inoculated with 0.1ml of 10^4 dilution of the inoculum preparation (standardized suspension of the test organism) and incubated at room temperature for 7 days. These were done in triplicate and the broth medium containing no extract was used as control [16]. MIC was recorded as the tube with the lowest concentration of extract that failed to show any visible macroscopic growth.

MFC:

After determining the MIC, the inhibitory and two following higher concentrations as well as the positive controls were sub cultured on SDA plates in triplicate. After 7 Days of incubation at 30°C, the readings of MFCs were carried out based on growth controls and MFC was the lowest drug concentration that hindered visible growth of the subculture [17].

3. RESULTS AND DISCUSSION

The preliminary phytochemical analysis revealed that different active constituents present in different solvents such as phenol and polyphenols, flavanoids, terpenoids, tannins, alkaloids and seponins (Table.1).

Table: 1. Phytochemical analysis of ethanol and aqueous extracts of *C. zeylanicum* bark

S. No.	Phyto-constituents	RESULTS	
		Ethanol Extract	Aqueous Extract
1.	Phenol and Polyphenols	+	+
2.	Flavanoids	+	-
3.	Terpenoids	+	+
5.	Tannins	+	+
6.	Alkaloids	+	+
7.	Seponins	+	-

+ Presence; - Absence

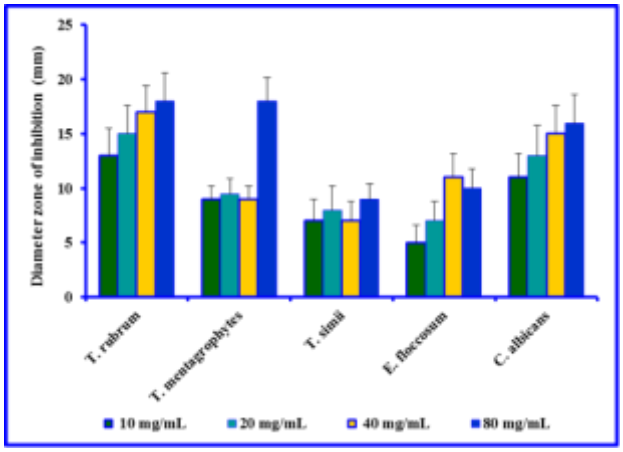

Fig. 1. Anti-fungal activity of ethanol extract of *C. zeylanicum barks*

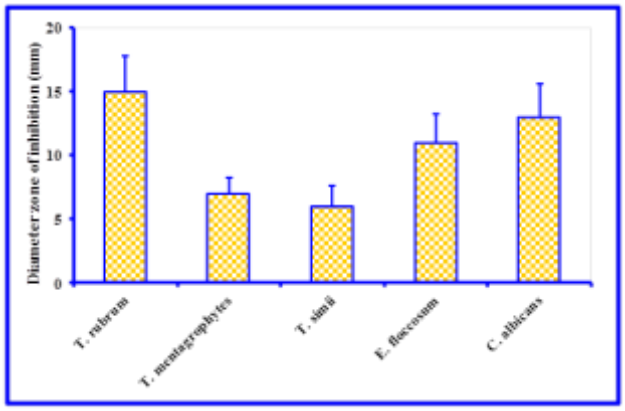

Fig. 2. Anti-fungal activity of aqueous extract of *C. zeylanicum barks*

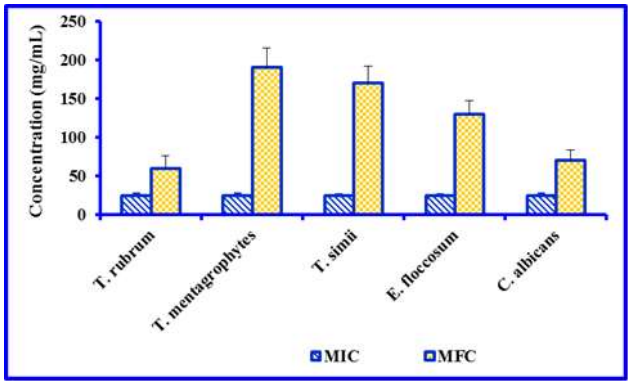

Fig. 3. Minimum inhibitory concentration and minimum fungicidal concentration of ethanol extract of *C. zeylanicum barks*

Abbreviations: MIC- Minimum inhibitory concentration MFC- Minimum fungicidal concentration

The macroscopic and microscopic features of the isolated fungi, a total of 5 species of dermatophytes namely, *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton simii, Epidermophyton floccosum* and *Candida albicans* were recovered from rice farmers in Orathanadu, Thanjavur (Dt), Tamil Nadu. The anti dermatophyte and anti yeast activities of crude ethanol barks extract of *C. zeylanicum* tested at four different concentrations with their zones of inhibition is represented (Fig:1). The crude ethanol barks extract of *C. zeylanicum* at a concentration of 10 mg/disc inhibited all test organisms. At that concentration, *T. rubrum* showed diameter inhibition zone of 14 mm, *T. mentagrophytes* (10 mm), *T. simii* (8 mm), *E. floccosum* (6 mm) and *Candida albicans* (12 mm). However, the highest diameter zone of inhibition (20 mm) was observed with *T. rubrum* at a concentration of 80 mg/disc. Based on the detailed study of the macroscopic and microscopic features of the isolated fungi, various species of dermatophytes and non dermatophytes . This result agrees with the work of other researchers who also recorded total inhibition of test dermatophytes by methanol extracts of *L. inermis* [18, 19]. From the results obtained, the diameter zones of inhibition recorded by the different dermatophytes increased as the concentration of the crude extract increases.

The aqueous barks extracts used in this work also showed inhibitory actions against the dermatophytes and *C. albicans*. Represented in Fig.2, is the MIC and MFC of the crude ethanol barks extract of *C. zeylanicum* against the dermatophytes and *C. albicans*. The ethanol barks extract inhibited all test organisms at the minimum concentration of 25 mg/ml while fungicidal actions were observed at a concentration of 50 mg/ml for *T. rubrum,* 180 mg/ml for *T. mentagrophytes,* 160 mg/ml for *T. simii,* 120 mg/ml for *E. floccosum* and 60 mg/ml for *C. albicans.* The results obtained in this work recorded increased bioactivity with ethanol extract than the aqueous extract which is contrary to the reports of other workers. As a general rule, plant extract is considered active against both fungi and bacteria when the zone of inhibition is greater than 6 mm [20]. The range of diameter zones of inhibition by methanol extract (6 mm - 20 mm) and aqueous extract (5 mm - 14 mm) of the leaf of *V. negundo* against the dermatophytes tested, confirms the anti dermatophyte activities of the extracts.

However, the data regarding the use of *C. zeylanicum* extracts as antifungal agents are scanty. Despite serious environmental implications associated with the excessive use of chemical fungicides still remain the first line of defense against fungal pathogens. Moreover, these fungicides when ingested by human beings and animals through food and water cause various ailments in the body. Search of natural fungicidal principles from the plant sources would definitely be a better alternative to these hazardous chemicals. Our study has indicated the anti-fungal potential of plant extracts, as the *C. zeylanicum* bark and leaf extracts displayed complete inhibitory effect on spore germination of aforesaid two dermatiaceous moulds [21].

The relative antifungal activity of *C. zeylanicum* extracts may not be easily correlated with any individual component but with a mixture of compounds present in these extracts. There are reports showing that alkaloids and flavonoids are the responsible compounds for the antifungal activities in higher plants. Moreover, secondary metabolites such as tannins and other compounds of phenolic nature are also classified as active antimicrobial compounds. Phenols, the aromatic compounds with hydroxyl groups are widespread in plant kingdom. They occur in all parts of plants. Phenols are said to offer resistance to diseases and pests in plants. Grains containing high amount of polyphenols are resistant to bird attack [22]. Interestingly, phytochemical screening of the current investigation has revealed that extracts from both the plant parts possess at least three to four of the following classes of secondary metabolites: phenols, flavonoids, terpenoids, tannins, alkaloids and saponins. Therefore, the presence of these phytochemicals could to some extent justify the observed antifungal activities in the current study.

4. CONCLUSION

The results of anti-fungal activities of crude ethanol and aqueous barks extracts of *C. zeylanicum* obtained in this work showed total fungicidal actions against the dermatophytes and yeast. It could be regarded as promising alternative antifungal preparation to be inserted in pharmaceutical formulations for used to treatment mycoses of different clinical severities, particularly, those caused by onychomycosis. The barks could be used in the production of antifungal drugs that will be effective and affordable to the developing countries to benefit from the emerging marks as the developing countries possess most biodiversity of medicinal plants.

ACKNOWLEDGEMENT

The present work has been carried out in the Department of Zoology, Kongunadu Arts and Science College (Autonomous), G.N. Mills post, Coimbatore, Tamil Nadu, India. We express their gratitude to the secretary and Principal for providing the laboratory facilities and encouragement.

REFERENCES

1. Ghannoum, M., and N. Isham, (2014). Fungal nail infections (Onychomycosis): A never-ending story?. *PLoS Pathog.* **10**(6): e1004105.
2. Moreno, G., and R. Arenas, (2010). Other fungi causing onychomycosis. *Clin. Dermatol.* **28**(2): 160-163.
3. Kaur, R., B. Kashyap and P. Bhalla, (2008). Onychomycosis-epidemiology, diagnosis and management. *Indian J. Med. Microbiol.* **26**(2): 108-116.
4. Scher, R.K., (1996). Onychomycosis: a significant medical disorder. *J. Am. Acad. Dermatol.* **35**(3): S2-5.
5. Dahanukar S.A., R.A. Kulkarni, N.N. Rege, (2000). Pharmacology of medicinal plants and natural products. *Indian J. Pharmacol.* **32**(4): S81-118.
6. Cowan, M.M., (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. **12**(4): 564-582.
7. Moreira, A.C.P., E.O. Lima, E.L. Souza, M.A.U. Dingenen and V.T. Trajano, (2010). Chemical composition and antifungal activity of *Hyptis suaveolens* (L.) poit leaves essential oil against *Aspergillus* species. *Braz. J. Microbiol.* **38**(1): 33-38.
8. He, Z.D, C.F. Qiao, Q.B. Han, C.L. Cheng, H.X. Xu, R.W. Jiang, *et al.,* (2005). Authentication and quantitative analysis on the chemical profile of cassia bark (cortex cinnamomi) by high-pressure liquid chromatography. *J. Agri. Food Chem.* **53**(7): 2424-2428.
9. Burt, S., (2004). Essential oils: their antibacterial properties and potential applications in foods- a review. *Int. J. Food Microbiol.* **94**(3): 223-253.
10. Delespaul, Q, V.G. Billerbeck, C.G. Roques, G. Michel, C. Marquier-Vinuales and J.M. Bessiere, (2000). The antifungal activity of essential oils as determined by different screening methods. J. Essential Oil Res. **12**(2): 256-266.
11. Pandey, A.K., (2007). Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed *Parthenium histerophorus* : An *in vitro* study. *Natl. Acad. Sci. Lett.* **30**(11-12): 383-386.
12. Bhuvaneswari, S., and S. Manivannan, (2014). Anti-diabetic and anti-inflammatory activity of *Caralluma adscendens* var. *adscendens*. *Int. J. Pharm. Bio. Sci.* **5**(1): 42-49.
13. Karthika, C., M. Rafi and S. Manivannan, (2016). Phytochemical analysis and evaluation of antimicrobial potential of *Senna alata* Linn. leaves extract. *Asian J. Pharm. Clin. Res.* **9**(2): 253-257.
14. Ekwealor, C.C, C.A. Oyeka and I. Okoli, (2012). *In vitro* anti dermatophyte activities of crude methanol and aqueous extract of *Lawsonia inermis*. *British Microbiol. Res. J.* **2**(2): 62-70.
15. Duraipandiyan V, M. Ayyanar, S. Ignacimuthu, (2006). Antimicrobial activity of some ehnomedicinal plants used by Paliyar tribe from TamilNadu, India. *BMC Comp. Alter. Med.* **6**:35-41.
16. Dash, D.K., and P.N. Murthy, (2011). Antimicrobial activity of few selected medicinal plants. *Inter. Res. J. Pharm.* **2**(1): 146-152.
17. Cheesbrough, M., (2006). District laboratory practice in tropical countries: 1st Ed. Cambridge UK: Cambridge University press.
18. Saadabi M.A.A., (2007). Evaluation of *Lawsonia inermis* Linn (*Sudanese Henna*) leaf extract as an antimicrobial agent. *Res. J. Biol. Sci.* **2**(4): 419-423.
19. Sharma, K.K, R. Saikia, J. Kotoky, J.C. Kalita and R. Devi, (2011). Antifungal activity of *Solanum melongena* L., *Lawsonia inermis* L and *Justicia gendarussa* B. against dermatophytes. *Inter. J. Pharm. Tech. Res.* **3**(3): 1635-1640.
20. Babu, P.D, R.S. Subhasree, (2009). Antimicrobial activities of *Lawsonia inermis* - a Review. *Acad. J. Plant Sci.* **2**(4): 231-232.
21. Mahomoodally, M.F, A. Gurib-Fakim and A.H, Subratty, (2005). Antimicrobial activities and phytochemical profiles of endemic medicinal plant of Mauritius. *Pharm. Biol.* **43**(3): 237-242.
22. Boussaada, O, J. Chriaa, R. Nabl, S. Ammar, D. Saidana, M.A. Mahjoub, *et al.,* (2008). Antimicrobial and antioxidant activities of methanol extracts of Evax pygmaea (Asteraceae) growing wild in Tunisia. *World J. Microbiol. Biotechnol.* **24**(8): 1289-1296.