Print ISSN: 0976-2876 Online ISSN: 2250-0138

Available online at: http://www.ijsr.in

INDIAN JOURNAL OF SCIENTIFIC RESEARCH

DOI:10.32606/IJSR.V14.I1.00017

Received: 18-04-2023 Accepted: 23-07-2023 Publication: 31-08-2023

Indian J.Sci.Res. 14 (1): 111-116, 2023 Original Research

ECO-FRIENDLY POTENTIAL OF *Cymbopogon citratus* ESSENTIAL OIL AGAINST TINEA CAPITIS (SCALP RINGWORM)

PIYUSH MISHRA[a], ALKA KUSHWAHA[b1], SUNIL KUMAR SINGH[c] AND NITESH KUMAR SINGH[d]

[abcd]Department of Botany, D.A.V. College Civil Lines, Kanpur, Uttar Pradesh, India

ABSTRACT

Tinea capitis is a fungal infection of the scalp and hair shafts caused by dermatophytes, most commonly species of *Trichophyton* and *Microsporum*. It occurs mainly in children, although adults can also be affected. It is caused by mainly member of genera *Trichophyton* and *Microsporum*. Lemon grass *(Cymbopogon citrtus*) species possess pharmacological properties that are used for medical purposes worldwide. Essential oil of Lemon grass (*Cymbopogon citrates* DC.) leaves was extracted by steam distillation using Clevenger apparatus. During antifungal screening of *Cymbopogon citratus* essential oil, it exhibited the strong activity, completely inhibiting the mycelia growth (MICs) of common infecting fungi *viz. Trichophyton rubrum, T. mentagrophytes* at concentration 0.1µl/ml and *Micosporum canis* at 0.2µl/ml. The essential oil was found to be fungicidal (MFCs) at a concentration 0.2µl/ml against *Trichophyton rubrum* and *T. mentarophytes*, 0.3µl/ml against *Microsporum canis* due to its mainly high citral content. It inhibits fungal growth, and offers a natural alternative to conventional antifungal agents. The fungicidal activity of the oil was found no decrease in activity up to 36 months of storage. Moreover, it did not exhibit any adverse effect on mammalian skin up to 5% concentration. Environmentally, it is biodegradable, leaves minimal residues, and poses comparatively low ecological risks, making it a promising eco-friendly option for dermatophyte management and sustainable disease control. As such, the oil has a potential use as an effective herbal chemotherapeutic after undergoing successful clinical trials.

KEYWORDS: Dermatophytes, *Cymbopogon citrates*, Essential Oil, *Trichophyton*, *Microsporum*

Tinea capitis is caused by dermatophyte fungi that possess the ability to invade and utilize keratin, a structural protein found in hair and other keratinized tissues. It is commonly referred to as scalp ringworm**,** is a fungal infection that affects the scalp and hair shafts (Dei-Cas *et al.*, 2019). It is primarily caused by dermatophyte fungi belonging to the genera *Microsporum* and *Trichophyton* (Rippon, 1985). These fungi invade the outer root sheath of the hair follicle and may subsequently penetrate the hair shaft. Clinically, tinea capitis is classified into inflammatory and non-inflammatory forms. The non-inflammatory form typically presents with scaling and patchy hair loss without causing permanent scarring. In contrast, the inflammatory form may lead to the development of a kerion, a painful pus-filled lesion, and can result in scarring alopecia. (Souissi *et al.*, 2018) The infection predominantly affects children between the ages of 3 and 14 years, although individuals of any age may be affected. In some cases, the disease may also involve the eyebrows and eyelashes. Common causative species include *Trichophyton rubrum, T. mentagrophytes Trichophyton tonsurans* and *Microsporum canis* (Coxe, 1807) These are the most common paediatric dermatophytes infection worldwide (Rippon, 1985). The infection is transmitted through direct contact with infected humans (anthropophilic organisms), animals (zoophilic organisms), or contaminated soil (geophilic organisms). Indirect transmission can also occur through contact with contaminated objects such as hats, combs, hairbrushes, towels, and other personal items that act as fomites.

Tinea capitis is a common dermatological infection that occurs worldwide, with a higher prevalence in regions characterized by hot and humid climates, such as Africa, Southeast Asia, and Central America. Fungal infection in man and animal are common in tropical and sub -tropical countries due to prevailing moisture, over population, poor hygienic living conditions and temperature regimes (Weitzman and Summerbell, 1995, Ameen 2010, Jain *et al.*, 2014). The same fungus may also affect the nails or the hand (Kaushik *et al.*, 2015) The distribution of the disease between males and females may vary depending on the causative dermatophyte species. Infections caused by *Trichophyton* species generally affect boys and girls equally during childhood, whereas *Microsporum canis* infections are reported more frequently in boys. Tinea capitis predominantly affects children and is relatively

[1]**Corresponding author**

uncommon in adults, likely due to protective changes in the scalp that occur after puberty.

The infection typically begins as small red papules that gradually enlarge over time. As the disease progresses, it may spread to involve larger areas of the scalp or even the entire scalp. Characteristically, the central area of the lesion may appear relatively normal, while the margins are often erythematous, scaly, and inflamed. Common clinical manifestations of tinea capitis include scalp redness, itching, scaling, and varying degrees of hair loss (Souissi, 2018) The symptomatic presentation of ringworm infection of the scalp is quite different depending upon the causative organism. Commonly, the infection may look like severe dandruff that appears on various places on the scalp. Some infections cause patches of hair loss. The inflammatory type (kerion) is associated with pus discharge and might lead to permanent hair loss (Chodkiewicz *et al.*, 2018) Extension to the eyelashes and eyebrows is not uncommon. Cervical lymphadenopathy is often seen in patients with kerion.

The infections occur in both healthy and immune compromised persons. It is caused by a number of different fungi. The *Trichophyton mentagrophytes*, *T. rubrum* and *Microsporum canis* are commonly involved in such infection. (Chuang *et al.*, 2007, Shin and Lim., 2004) These pathogenic bio-agents are able to invades human keratinized tissues, causing lesions in the skin and other body parts (Hainer 2003, Ameen 2010). Infections are difficult to control effectively, and mostly synthetic antifulgals (azoles,allylamines and morpholine derivates) are used to treat these infections which are largely non-renewable petro-products that are non-biodegradable and cause adverse effects and residual toxicity.

Topical treatment is not recommended, as it is ineffective. Azole antifungal medications like itraconazole and fluconazole are also alternative treatment options. Allylamines are a great option orally, like terbinafine (Barlow, 1805). Antifungal shampoos can be part of the treatment plan and often help in preventing spread, but this is not the mainstay of treatment and will usually not cure tinea capitis.

Thus, in a meaningful search for new treatments with better and cheaper substitutes, plant resources are the natural choice. Plant derived essential oils are natural compounds that show potential effects against bacteria and fungi (Park *et al.*, 2007). Essential oils as antimicrobial agents have two main advantages: firstly, most essential oils are safer for consumers; secondly, there is low risk of the micro-organisms developing resistance to essential oils (Cardile *et al.*,2009). Thus, it can used to be an effective source of chemotherapeutic agents without undesirable side effects and with strong fungicidal activity (Dikshit *et al.*, 1985, Pandey *et al.*, 1996, Shahi *et al.*, 2000).

The objective of this work was to evaluate the antifungal efficacy of plants derived essential oils against pathogenic fungal strains that cause Tinea captis. Therefore, they can be used as a natural therapy to inhibit fungal pathogens causing infections (Prasad *et al.*, 2004). We hear report on the result of our investigation, the *Cymbopogon citratus* (DC) essential oil a member of Poaceae is very effective against Tinea captis causing fungi.

MATERIALS AND METHODS

Plant *Cymbopogon citratus* (lemon grass or oil grass) collected during the month of August from the botanical garden of D.A-V. College Kanpur UP India. The essential oil was extracted from the aerial parts (leaves) of *Cymbopogon citratus* by hydro distillation using a Clevenger apparatus (Clevenger, 1928). A clear light-yellow-colored oily layer was obtained on the top of the aqueous distillate, which was separated from the latter and dried over anhydrous sodium sulphate. The physicochemical properties of oil are given in (Table 1)

In *in vitro* studies, the minimum inhibitory concentrations (MICs) of the oil against test pathogens were determined following the poisoned food technique (Grover, 1962) with slight modification (Shahi *et al.*, 1999). The requisite quantity of the oil samples was mixed in acetone (2% of the required quantity of the medium) and then added in pre-sterilized sabouraud dextrose agar (SDA) medium, pH 5.6. In control sets, sterilized water (in place of the oil) and acetone were used in the medium in appropriate amounts. Mycelial discs of 5mm diameter, cut out from the periphery of 7-day old cultures, were aseptically inoculated upside-down on the agar surface of the medium. Inoculated Petri plates were incubated at 27 ± 1^0C and the observations were recorded on the seventh day given in (Table 2). Percentage of mycelial growth inhibition (MGI) was calculated according to the formula:

$$MGI= (dc - dt) \times 100/dc$$

Where, dc = fungal colony diameter in control sets.

dt = fungal colony diameter in treatment sets.

The minimum fungi static /fungicidal concentrations of the oil at minimum inhibitory concentrations (MICs) were ascertained by the method of Garber and Houston,1959. This was done by reinoculated the inhibited fungal discs at MICs on SDA medium.

Observations were recorded after 7 days of incubation. Fungal growth on the seventh day indicated a fungi static nature (Table 3), while the absence of fungal growth denoted fungicidal action of the oil (Table 4). The effect of inoculums density (increased progressively up to 30 discs in multiples of five, each of 5 mm diameter) of the test pathogens on MICs of the oil was determined following the procedure outlined by Dikshit and Dixit 1982. The effect of physical factors viz. temperature and expiry of toxicity during storage of the oil, was evaluated according to (Shahi *et al.*, 1999). Five lots of oil were kept in small vials, each containing 5 ml oil; these were exposed to different temperatures (30^0C, 50^0C and 70^0C) in an incubator for 1 h. Antifungal activity was then tested at MICs by the poisoned food technique (Grover and Moore 1962). Expiry of toxicity of the oil was determined by storing the oil at room temperature and testing antifungal activity at MICs at regular intervals of 60 days up to 24 months, following the poisoned food technique (Grover and Moore 1962).

The minimum killing time (MKT) of the oil was determined by the mycelial disc killing technique (MDKT) (Shahi *et al.*, 1999). Two treatment sets were maintained, one with pure oil (PO) and the other with the minimum fungicidal concentrations (MFCs) of the oil. The treatment set using MFCs of the oil was prepared by mixing the required quantity of the oil samples in acetone (5% of the total quantity of the treatment solution) and then adding this to the appropriate quantity of distilled water. Simultaneously, controls were maintained using sterilized water (in place of the oil) and acetone, adding into the distilled water in appropriate quantities.

Mycelial discs of 5 mm diameter, cut out from the periphery of 7-day-old cultures of the test pathogens, were aseptically placed in the culture tubes of different treatment and control sets. These mycelial discs were taken out of the tubes at different time intervals and washed immediately in the washing solution (containing acetone: sterilized distilled water, ratio 1:2) to remove the treatment solution. These washed mycelial discs were aseptically transferred upside-down to the SAD medium (pH 5.6) in the Petri plates. The same procedure was followed with the control sets. The inoculated Petri plates were incubated at 27 ± 1^0C and the observations recorded as an average value of five replicates on the seventh day. The percentage of fungal growth inhibition (FGI) was calculated by the formula of (Shahi *et al.*, 1999). All the experiments were repeated twice, each containing five replicates, and the data presented here are based on their mean values (table-5). To determined the maximum tolerable concentrations (MTCs) and long-term toxicity for irritant activity, if any, of the oil by their topical application on human skin, we followed the patch test method as described by (Shahi *et al.*, 1999*)*.

Table 1: Physicochemical properties of the oil of *Cymbopogon citratus*

Properties studied	Observations
Plant height (cm)	155–180
Oil yield (%)	0.4
Colour	Light yellow
Specific gravity at 29.5 ◦C	0.9050 -0.9475
Refractive index at 20 ◦C	1.4721-1.4861
Optical rotation	- 0.33 ◦
Acid value	6.99
Ester value	35.15
Citral (%)	68–80
Solubility	90% alcohol

Table 2: Minimum inhibitory concentration (MIC) of the oil of *Cymbopogon citratus* against dominant tinea captis causing fungi

Concentration (µl/ml)	Mycelial growth inhibition (MGI) (%)		
	Trichophyton rubrum	***Trichophyton mentagrophytes***	***Microsporum canis***
1.0	100	100	100
0.9	100	100	100
0.8	100	100	100
0.7	100	100	100
0.6	100	100	100
0.5	100	100	100
0.4	100	100	100
0.3	100	100	100
0.2	100	100	100
0.1	80	60	45

Table 3: Minimum fungistatic concentration and minimum fungicidal concentration (MFCs) and of the oil of *Cymbopogon citratus* against dominant tinea captis causing fungi

Concentration (µl/ml)	Mycelial growth inhibition (MGI) (%)		
	Trichophyton rubrum	***Trichophyton mentagrophytes***	***Microsporum canis***
1.0	100[c]	100[c]	100[c]
0.9	100[c]	100[c]	100[c]
0.8	100[c]	100[c]	100[c]
0.7	100[c]	100[c]	100[c]
0.6	100[c]	100[c]	100[c]
0.5	100[c]	100[c]	100[c]
0.4	100[c]	100[c]	100[c]
0.3	100[c]	100[c]	100[c]
0.2	100[c]	100[c]	100[s]
0.1	100[s]	100[s]	80

[c] Fungicidal, [s] Static

Table 4: Minimum killing time of the oil of *Cymbopogon citrtus* against test pathogens

Minimum killing time (MKT)	Mycelial growth inhibition (MGI) (%)					
	Trichophyton rubrum		***Trichophyton mentagrophytes***		***Microsporum canis***	
	PO	***MCCs***	**PO**	***MCCs***	**PO**	***MCCs***
120m	100	100	100	100	100	100
80m	100	100	100	100	100	100
70m	100	100	100	100	100	100
60m	100	100	100	100	100	100
50m	100	100	100	100	100	80
40m	100	88	100	63	100	45
30m	100	78	100	50	100	28
60s	100	-	100	-	100	-
30s	100	-	100	-	100	-
20s	100	-	100	-	100	-
10s	100	-	100	-	100	-
5s	93	-	89	-	76	-
1s	58	-	52	-	35	-

PO (pure oil), MCCs (minimum fungicidal concentration)

RESULTS AND DISCUSSION

The essential oil was extracted from the leaves of *Cymbopogon citratus* by hydro distillation using Clevenger apparatus. A clear light-yellow-colored oil on hydro distillation, yielded 0.4% essential oil in *Cymbopogon citratus*. The physicochemical properties of the oil are shown in (Table 1). The MIC of *Cymbopogon citratus* essential oil was found to be 0.1µl/ml against *Trichophyton rubrum* and *Trichophyton mentagrophytes, and* .2µl/ml against *Micrsporum canis* (Table 2) and fungicidal concentration of *Cymbopogon citratus* essential oil was found to be 0.2µl/ml against *Trichophyton rubrum* and *Trichophyton mentagrophytes, and* .3µl/ml against *Micrsporum canis* (Table 3). The oil also inhibited heavy doses of inocula which exhibited 100% mycelial growth at their respective fungicidal concentrations. The activity of the oil did not expire even up to 24 months storage and persisted up to 70^0C. The pure oil (100%) killed the fungi in just 10 seconds (s) while at its minimum fungicidal concentration it required 50 minutes (m) against *Trichophyton rubrum* and *Trichophyton mentagrophytes* and 60 minutes(m) against *Microsporus canis* respe (Table 4). The oil also exhibited a broad range of antifungal activity, inhibiting some other fungi, e.g. *Aspergillus flavus, A. fumigates, A.niger, Candida albicans, Malassezia, Epidermophyton floccosum, M. gypseum, M. nanum, T. violaceum* in range of 0.3- 1.2 µl/ml concentration. When tested for irritant activity and long-term toxicity on human skin and nails, the oil did not show any irritation or adverse effect at 5% concentration up to 3 weeks.

CONCLUSION

The essential oil of *Cymbopogon citratus* exhibiting strong toxicity against the test fungi causing Tinea captis. The oil also appears to possess wide range of antifungal activity. It inhibits fungal growth, and offers a natural alternative to conventional antifungal agents. The fungicidal activity of the oil was found no decrease in activity up to 36 months of storage. Moreover, it did not exhibit any adverse effect on mammalian skin up to 5% concentration. Environmentally, it is biodegradable, leaves minimal residues, and poses comparatively low ecological risks, making it a promising eco-friendly option for dermatophyte management and sustainable disease control. As such, the oil has a potential use as an effective herbal chemotherapeutic after undergoing successful clinical trials. The findings suggest that *C. citratus* may be used for the cure of tenia captis disease after suitable clinical trials.

ACKNOWLEDGEMENT

Author is grateful to Prof Arun Kumar Dixit Principle D.A.V. College Kanpur for proding facilities, inspiration, and Prof. Anupam Dixit, Ex-HOD Department of Botany, University of Allahabad UP India for his inspiration, valuable suggestions and critical review. I also thankful to BSI Allahabad UP for plant identification and its related information.

REFERENCES

Ameen M., 2010. Epidemiology of superficial fungal infections. Clin Dermatol, **28**: 197-201.

Argentina: a descriptive study. Int. J. Dermatol. 2019 Mar; **58(3)**: 288-295. (PubMed)

Barlow J., 1805. Observations on the Mode of Curing the Tinea Capitis. Med. Phys. J., **14(82)**:496. (PMC free article) (PubMed)

Cardile V., Russo A., Formisano C., Rigano D., Senatore F., Arnold N.A. and Piozzi F., 2009. Essential oils of Salvia bracteata and Salvia rubifolia from Lebanon: Chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. J. Ethanopharmacol, **26**: 265-272.

Chuang P.H., Lee C.W., Chou J.Y., Murugan M., Shieh B.J. and Shen H.M., 2007. Antifungal activity of crude extracts and essential oil of Moringa oleifera Lam. Bioresour Technol., **98**:232-236.

Clevenger J.F., 1928. J. Am. Pharm. Assoc., **17**: 346.

Coxe History of a Case of Tinea Capitis Cured by Vaccination. Med. Phys J. 1807 Oct; **18(104)**:340-341. (PMC free article) (PubMed)

Chodkiewicz H.M., Ranario J.S., Jahan-Tigh R. and MacFarlane D.F., 2018. Tinea Capitis Masquerading as Basal Cell Carcinoma. Skinmed., **16(4)**:269-271. (PubMed)

Dei-Cas I., Carrizo D., Giri M., Boyne G., Domínguez N., Novello V., Acuña K. and Dei-Cas P., 2018. Infectious skin disorders encountered in a pediatric emergency department of a tertiary care hospital in argentina: descriptive study. Int. J. Dermatology, **58(3)**: 288-295.

Dikshit A., Srivastava O.P. and Husain A.J., 1985. Antibact. Antifung. Agent. **13**: 57.

Dikshit A. and Dixit S.N., 1982. Indian Perfum., **26**: 216-227.

Garber R.H. and Houston B.R., 1959. Phytopathology, **49:** 449-450.

Grover R.K. and Moore J.D., 1962. Phytopathology, **52**: 876-880.

Hainer B.L., 2003. Dermatophyte infections. Am Fam Physician, **67**:101-108.

Jain N., Sharma M., Sharma M. and Saxena V.N., 2014. Spectrum of dermatophytoses in Jaipur, India. Afr. J. Microbiol Res., **8**: 237-43.

Kaushik N., Pujalte G.G. and Reese S.T., 2015. Superficial Fungal Infection. Primary care, **42(4)**: 501-516.

Pandey M.C., Sharma J.R. and Dikshit A., 1996. Flavour Fragr. J., **11**: 257-260.

Park M.J., Gwak K.S., Yang I., Choi W.S., Jo H.J., Chang J.W., Jeung E.B. and Choi I.G., 2007. Antifungal activities of the essential oils in *Syzygium aromaticum (L.)* Merr. Et Perry and *Leptospermum petersonii* Bailey and their constituents against various dermatophytes. J. Microbiol., **45**: 460-465.

Prasad N.R., Anandi C., Balasubramanian S. and Pugalendi K.V., 2004. Antidermatophytic activity of extracts from *Psoralea corylifolia* (Fabaceae) correlates with the presence of a flavonoid compound. J. Ethnopharmacol., **91**:21-24.

Shahi S.K., Shukla A.C., Bajaj A.K., Medgely G. and Dikshit A., 1999. Broad spectrum antimycotic

drug for the control of fungal infection in human beings. Curr. Sci., **74**: 836-839.

Shahi S.K., Shukla A.C., Bajaj A.K., Medgely G. and Dikshit A., 2000. Skin Pharm. Appl. Skin Phys., **13**: 60-64.

Shin S. and Lim S., 2004. Antifungal effects of herbal essential oils alone and in combination with ketoconazole against Trichophyton spp. J. Appl. Microbiol., **97**: 1289-1296.

Souissi A., Ben Lagha I., Toukabri N., Mama M. and Mokni M., 2018. Morse code-like hairs in tinea capitis disappear after successful treatment. Int. J. Dermatol., **57(12)**:e150-e151. (PubMed)

Rippon J.W., 1985. The Changing epidemiology and emerging patterns of dermatophytes species. Curr. Top. Med. Mycol., **1**:209-234.

Weitzman I. and Summerbell R., 1995. The dermatophytes. Clin Microbiol, **8**:240-49.