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Antifungal activity of Jamarosa and Nagarmotha essential oils against *Microsporum gypseum* and *Trichophyton rubrum*

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ABSTRACT

In the present study, soil samples of 7 different public places in Varanasi, India were screened through hair baiting technique for the presence of dermatophytes. All the samples were found positive with dermatophytes including *Microsporum gypseum* and *Trichophyton rubrum*. Thirteen essential oils (EOs) were isolated from different plant parts through hydro-distillation and were screened against two dermatophytes *M. gypseum* and *T. rubrum* for their antifungal activity. At 250 ppm, Jamarosa and Nagarmotha EO showed strong antifungal activity and selected for determination of minimum inhibitory concentration (MIC) and minimum fungitoxic concentration (MFC) against *M. gypseum* and *T. rubrum.* The MIC of Jamarosa EO was found to be 100 ppm against both *M. gypseum* and *T. rubrum* and MIC of Nagarmotha EO was found at 250ppm against both test dermatophytes. The MFC of Jamarosa for *M. gypseum* and *T. rubrum* was found at 1000 and 500 ppm respectively. The MFC of Nagarmotha was not achieved up to 1000 ppm for test dermatophytes.

Keywords : Essential oils, Antifungal activity, Jamrosa, Nagarmotha.

Different species of *Microsporum, Trichophyton, Epidermophyton, Aspergillus, Candida, and Cryptococcus* cause various diseases in humans. Superficial fungal infections of the skin caused by the fungus are known as Dermatomycosis and the fungi that cause dermatomycosis is known as dermatophytes. The symptoms of dermal infection by dermatophytes include itching, swelling, blistering and scaling of skin (Ping-Hsien *et al*. 2007). Both healthy and immune-compromised persons are infected by dermatophytes.

Dermatophytes cause infections of the tissues which are rich in keratin such as skin, hair and nails, because they obtain nutrient from keratinized material (Midgley *et al.* 1994). For treatment of dermatomycosis, different modern antifungal drugs like amphotericin B, nystatin, ketoconazole, clotrimazole, griseofulvin, flucytosine etc. are used now a days. However, with increased use of antifungal drugs in treatment of fungal infection, the chance of resistance development in targeted organism also occur (Chee-Leok *et al*.1994, Zaias & Gerbert-Rebell 1996, Bennett *et al.* 2000; de Pauw BE, 2000). Several adverse effects on the body due to synthetic antidermatophytic drugs are also reported that include liver damage, redness, diarrhea, headache, abdominal (tummy) pain, indigestion etc. (Del *et al*. 1992, Torok *et al.* 1993, Lopez–Gomez *et al*. 1994, Gupta *et al.* 1998).

From ancient times plants and plant originated products are used in herbal medicines for the treatment of fungal infections (Mc Cutcheon *et al.* 1992). The most frequently used antifungal plants include black walnut (hull), barberry, cajeput, calendula, cedar, cinnamon (bark), cloves, geranium, garlic etc. Presently, essential oils; volatile and aromatic oily liquids extracted from different plant parts are gaining interest for the development of plant based antifungal agent in view of there well reported *in vitro* antimicrobial activity (Burt 2004, Prakash *et al.* 2015). Thus, essential oils can be used as green technology to treat antifungal infections.

Microsporum gypseum (E. Bodin) Guiart & Grigoraki and *Trichophyton rubrum* (Castell.) Sabour are well known soil borne dermatophytes usually infect the upper dead layers of the skin of mammals (Rippon *et*

al. 1988). Hence, in the present piece of work the essential oils from 13 different plants were screened for their antidermatophytic activity against *M. gypseum* and *T. rubrum* causing dermatomycosis to humid and moist region of the body. The essential oils of Jamarosa and Nagarmotha were subjected to detailed study against the test dermatophytes.

MATERIALS & METHODS

Chemicals and Equipments—High purity analytical-grade Sabouraud Dextrose Agar (SDA) media (mycological peptone 10 g; dextrose 40 g; agar 15g in 1 litre distilled water; pH 5.6 ±0.2), antibiotic Chloramphenicol and synthetic antifungal compound Cyclohexamide were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai India. The major equipments used were Clevenger`s hydro-distillation apparatus (Merck Specialties Pvt. Ltd., Mumbai), B.O.D. Incubator (Caltar NSW-152, New Delhi, India), Laminar Air Flow (Sonar, Associated Scientific Technologies, New Delhi, India) and compound microscope (Labomed, New York).

Collection of soil samples and baits to isolate keratinophilic fungi—The soil samples and baits were collected following Kumar *et al.* (2013). Twenty eight soil samples from seven different areas (four samples per site) near to and from Banaras Hindu University (BHU) campus, Varanasi, India were scrapped (approximately 400 gm) from the surface layer of each site up to a depth of 3-6 cm in sterilized plastic bags after removing superficial debris and other vegetative materials. The Samples were stored at 4 °C until use. The bait (horse hair) was collected from local horse farm of Varanasi, India. Before using as substrate, the baits were defatted by soaking them in diethyl ether (24 h) and later rinsing them 5-6 times with distilled water and subsequent air drying.

Keratinophilic fungi were isolated according to Vanbreuseghem (1952) by using sterilized horse hair as keratin bait. Sterile Petri dishes half filled with soil samples were used for hair baiting. Moisture in soil sample was maintained by utilizing double distilled water. Sterile horse hair was buried in the soil and each Petri dish was incubated at 27^{0}C and was continuously examined for fungal growth from the third day of incubation up to a period of 4-5 weeks. SDA medium supplemented with Chloramphenicol 50 mg/L and Cyclohexamide 500 mg/L was used for the culture of fungal isolates.

Isolated keratinophilic fungi were stained by lactophenol cotton blue and were observed under compound microscope for identification. Identification of keratiniphilic fungi was performed on the basis of microscopic characters with the help of monograph of Atlas of Clinical fungi (Hoog *et al*. 2000).

Plant materials used and extraction of essential oil—Different plant parts of 13 angiospermic taxa were collected from Botanical Garden, Banaras Hindu University, Varanasi, India and their identification was confirmed from Dubey (2004) and Duthie (1960). The voucher specimens were submitted to the Herbarium of Laboratory of Herbal Pesticides, Department of Botany, Banaras Hindu University, and Varanasi, India. The plant materials were thoroughly rinsed with double distilled water and 500 g of each plant part was subjected to hydro distillation in Clevenger's hydro-distillation apparatus (Prasad *et al.* 2009). The extracted fractions of plant parts exhibited two distinct layer; an upper oily layer and the lower aqueous layer. Both the layers were separated and essential oil was collected in clean glass vial. For excess moisture anhydrous sodium sulphate was added in the obtained essential oil and stored at 4^{o}C (Prakash *et al.* 2010).

Screening of isolated essential oils against test dermatophytes—The experiment was performed using poisoned food method for determining the inhibition of mycelial radial growth of the test fungus by the plant EOs. Each plant EO (0.25 µl/ml) dissolved in 0.5 ml acetone was taken in Petri plates (9 cm diameter) and 9.5 ml molten SDA was added to it so as to achieve a concentration of 250 ppm. SDA plates containing 0.5 ml acetone only along with 9.5 ml SDA, served as control. A 5 mm disc of test fungus (21 days old) was placed at the center of the prepared Petri plates and incubated in the dark at 27±2 °C Colony diameter of the test fungus in treatment and control sets were measured after 21 days and antifungal activity was calculated in terms of percent mycelial inhibition following Kumar *et al.* (2008). % mycelial inhibition = dc – dt/dc X 100 where, *dc* = average diameter of fungal colony in control set and *dt* = average diameter of fungal colony in treatment set.

From this experiment Jamarosa and Nagarmotha

essential oils showed the highest antifungal activity against both the test fungi and thus selected for detailed investigation.

Determination of minimum inhibitory concentration (MIC) of Jamarosa and Nagarmotha EO against test dermatophytes—Poisoned food method was used for determining the MIC of Jamarosa and Nagarmotha essential oil against test dermatophytes (Ramdas *et al*. 1998) to record their antifungal activity. EOs varying from 0.05 to 1.0 µl were taken separately in Petri dishes (90 mm diameter), mixed with 0.5 ml acetone and then added 9.5 ml SDA medium to obtain different concentrations from 50 to 1000 ppm. The control sets were kept EO free, parallel to the treatment sets. Five mm diameter disc of 21 days old culture of *M. gypseum* and *T. rubrum* were placed at the center of the poured Petri dishes of treatment and control sets. The dishes were incubated in BOD at 27±2 °C for 21 days. The minimum inhibitory concentration (MIC), the lowest concentration of EOs resulting in no visible growth was recorded.

Determination of minimum fungicidal concentration (MFC) of test essential oils—In previous test, discs which showed not any visible growth were sub-cultured on fresh SDA medium and incubated in BOD incubator at 27 °C for 21 days. MFC may be defined as lowest concentration of the essential oil that checks the growth of dermatophytes, resulting killing of at least 99.5% of original inoculum.

Statistical analysis—The experiments except mycobiota analysis were performed in triplicate and data are the mean ± standard error. The analysis of data was performed with the SPSS program version 16.0. and the statistical level of significance was fixed at $P<0.05$.

RESULTS

In the present study a total of 28 soil samples were collected from different parts of Varanasi region of India. By hair baiting technique, *Trichophyton rubrum, Paecilomyces lilacinus, Trichophyton ajelloi, Chrysosporium keratinophilum, Trichophyton erinacei*, *Trichophyton terrestre*, *Microsporum gypseum,* and few other non dermatophytes like *Aspergillus and Penicillium species were isolated from different samples*. However, from each site a particular species of dermatophyte was recorded. Table 1 shows the major dermatophytes isolated from soil samples of 7 different sites. Out of them, *M. gypseum* and *T. rubrum* are the most common human disease causing dermatophytes and hence selected for further detailed investigation.

The scientific name, common name and family of 13 different plants used for essential oil extraction are given in Table 2. The screening of isolated essential oils against test dermatophytes (*M. gypseum* and *T. rubrum*) at 250 ppm showed the superiority of Jamarosa and Nagarmotha essential oils over others as they caused 100% growth inhibition of both the test fungi (Table 2). The essential oils of *Citrus aurantium, Citrus limetta, Aegle marmelos*, *Olea europaea* and *Hedychium coronarium* were found not effective against these dermatophytes where as that of *Boswellia sacra, Geranium aculeolatum, Carum carvi, Ruta graveolens, Ocimum sanctum* and *Ocimum gratissimum* showed moderate activity. From this study Jamarosa and Nagarmotha essential oils were selected for further study as these oils caused 100% activity against both the test fungi at test concentration.

The test essential oils caused dose dependent decrease in mycelial growth on increasing concentration. The MIC of Jamarosa and Nagarmotha essential oils for complete inhibition of growth of test dermatophyte were found at 100 and 250 ppm respectively. The fungicidal concentration (MFC value) was recorded at 1000 and 500 ppm respectively for *M. gypseum* and *T. rubrum* for Jamarosa EO. For Nagarmotha EO the MFC value did not recorded up to 1000 ppm in both the test dermatophytes.

DISCUSSION

Soil of public places like park, garden, hospital, schools etc. are good source of different dermatophytes (Deshmukh 2004, Shadzi *et al.* 2002, Saxena *et al.* 2004, Zarei *et al.* 2008, Shrivastava *et al.* 2008). These fungi cause superficial infections of human and animals (Filipello *et al.* 1996, Spiewak *et al.* 2000). The ability of fungi to cause lysis of keratin has important ecological value (Filipello *et al.* 2000, Sharma *et al*. 2003, Zarrin *et al.* 2011). In the present study, the soil samples from 7 different public places were tested for the presence of any dermatophyte and all the samples were found positive suggesting the severity of occurrence of dermatophytes. The present research reports for the first

Table 1— Isolation of different dermatophytic fungi from hair baiting technique

S. N.	Major isolated keratinophilic fungus	Site of occurrence
1	*Microsporum gypseum*	Barber cabbage near to Hyderabad gate BHU
2	*Paecilomyces lilacinus*	Agricultural garden of BHU
3	*Trichophyton ajelloi*	Chhitturpur area of BHU
4	*Chrysosporium keratinophilum*	Vishavnath temple of BHU
5	*Trichophyton erinacei*	Botanical garden of BHU
6	*Trichophyton terrestre*	Dairy farm of BHU
7	*Trichophyton rubrum*	Sankatmochan mandir, Durgakund, Varanasi

Table 2— Screening of 14 plant essential oils against test dermatophytes at 250 ppm

S.N.	Botanical name	Common name	Family	Plant part used	% inhibition of M. gypseum growth	% inhibition of T. rubrum growth
1	*Aegle marmelos*	Bael	Rutaceae	Leaf	9.11±0.23	3.23±0.07
2	*Boswellia sacra*	Frankincense	Burseraceae	Resin	100.00±0.00	41.00±0.96
3	*Carum carvi*	Persian cumin	Apiaceae	Seed	56.90±1.21	9.91±0.06
4	*Citrus aurantium*	Bitter Orange	Rutaceae	Peel of fruit	11.33±0.09	0.00±0.00
5	*Citrus limetta*	Mosambi	Rutaceae	Peel of fruit	17.37±0.31	3.12±0.03
6	*Cymbopogon khasans*	Jamarosa	Poaceae	Leaf	100.00±0.00	100.00±0.00
7	*Cyperus scariosus*	Nagarmotha	Cyperaceae	Rhizome	100.00±0.00	100.00±0.00
8	*Geranium aculeolatum*	No common names	Geraniaceae	Leaf	100.00±0.00	52.81±1.11
9	*Hedychium coronarium*	White Ginger	Zingiberaceae	Rhizome	23.33±0.76	11.73±0.06
10	*Ocimum sanctum*	Tulsi	Lamiaceae	Aerial part	100.00±0.00	92.16±1.24
11	*Ocimum gratissimum*	Wild Basil	Lamiaceae	Aerial part	50.67±0.97	29.33±0.09
12	*Olea europaea*	Common Olive	Oleaceae	Fruit	0.00±0.00	0.00±0.00
13	*Ruta graveolens*	Rue	Rutaceae	Aerial part	49.91±1.21	53.41±0.98

Values are mean (n=3) ± standard error.

time the existence of keratinophilic fungi in and around the soil of BHU by hair baiting technique. The isolated fungi from different soil samples were found pathogenic to humans and animals. During the experimental work *Penicillium* was found most common contaminant in plates as saprophyte supporting earlier studies of Zarrin *et al*. (2011) and Shokohi (2005). So it is recommended to follow hygiene protocols to avoid the pathogenic fungi. Necessary treatment methods should be followed properly.

Dermatophytes cause a number of skin diseases in both human and animals. Antifungal agents must be used to treat such infections caused by dermatophytes. Due to the report of various harmful roles of synthetic antifungal drugs, there is a quick need of safe antifungal agent to cure dermatomycosis. Antimicrobial substances of plant origin are safer in nature and thus could be exploit for their formulation as antifungal agent (Martin & Ernst 2003). Various essential oils showed antifungal activity against many dermatophytes. Being natural in

origin, plant essential oils are good source for the development of antifungal agent for many infectious dermal diseases. In the present study, Jamarosa and Nagarmotha essential oils provided the highest antifungal activity against both *M. gypseum* and *T. rubrum* and hence may be useful as antifungal agents.

Cymbopogon khasans Sobti (Poaceae) commonly known as Jamarosa is used as a natural fragrance. The major component of this essential oil is reported as Z-citral and linalyl acetate (Mishra *et al.* 2012). The essential oil from this plant is reported to have antibacterial and insecticidal activity (Nayak *et al.* 2003), antifungal activity against food deteriorating fungi, antiaflatoxigenic and antioxidative activity (Mishra *et al*. 2012). *Cyperus scariosus* R.Br. (Cyperaceae) commonly known as Nagarmotha is a native of India. The main component of this essential oil is Cyperine. The oil is reported to have anti-inflammatory activity (Gupta *et al.* 1972). Both the essential oils are used in treatment of a number of diseases in ayurvedic system of medicine without any side effects (Prajapati *et al*. 2003).

Essential oils are used throughout the world for treatment of many diseases. Essential oils have no major side effects than synthetic drugs. Due to the side effects and increasing resistance of chemical drugs there is need for effective, safe, natural products, from plant essential oils and which may be used in making of a new antifungal agent, which may use plant essential oils to treat many diseases.

CONCLUSION

Conclusively, the soil samples in the public places of Varanasi region of India were found contaminated by *Microsporum gypseum and Trichophyton rubrum and other dermatophytes*. The screening of 13 plant essential oils against test dermatophytes revealed that essential oils from *Jamarosa* and *Nagarmotha* have potential antifungal activity and may be useful against various infections caused by dermatophytes.

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LITERATURE CITED

Bennett ML, Fleischer AB Jr, Loveless JW & Fledman SR 2000. Oral griseofulvin remains the treatment of choice for tinea capitis in children. *Pediatric Dermatology* **17** 304-309.

Burt S 2004. Essential oils: their antimicrobial properties and potential applications in food a review. *Inter. J. Food Microbiol.* **94** 223-253.

Chee-Leok G, Young Kwang T, Kamarudim BAM & Chew SS 1994. In vitro evaluation of griseofulvin, ketoconazole, and itraconazole against various dermatophytes in Singapore. *Inter. J. Dermatology* **33** 733-737.

de Pauw BE 2000. Is there a need for new antifungal agents? *Clinical Microbiol. Infection* **6** 23-28.

Del Aguila R, Gei FM, Robles M, Perera-Ramirez A & Male O 1992. Once-weekly oral doses of fluconazole 150 mg in the treatment of tinea pedis. *Clinical Experimental Dermatology* **17** 402–406.

de Hoog GS, Guarro J, Gene J, & Figueras MJ 2000. *Atlas of Clinical Fungi.* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, and Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain.

Deshmukh SK 2004. Isolation of dermatophytes and other keratinophilic fungi from the vicinity of salt pan soils of Mumbai, India. *Mycopathologia* **157** 265-267.

Dubey NK 2004. *Flora of BHU Campus,* Banaras Hindu University Press, Varanasi, India.

Duthie JF 1960. *Flora of Upper Gangetic Plain and of Adjacent Siwalic and Sub Himalayan Tract.* (Rep. Eds.) Botanical Survey of India, Calcutta, India.

Filipello MV, Preve L & Tullio V 1996. Fungi responsible for skin mycoses in Turin (Italy), *Mycoses* **39** 141-150.

Fillipello M V 2000. Keratinophilic fungi: Their role in nature and degradation of keratinic substrates. In: Kushawaha RKS & Guarri J (eds), *Biology of dermatophytes and other keratinophilic fungi.* Spain. Revista Iberoamericana de Micologia. Pp. 77-85.

Gupta AK, Del Rosso JQ, Lynde CW, Brown GH & Shear NH 1998. Hepatitis associated with terbinafine therapy: three case reports and a review of the literature. *Clinical Exp. Dermatology* **23** 64–67.

Gupta SK, Sharma RC, Aggarwal OP & Arora RB 1972. Anti-inflammatory activity of oil isolated from *Cyperus scariosus*. *Indian J. Exp. Biol.* **10** 41-42.

Lopez–Gomez S, Del Palacio A, Van Gutsem J, Soledad Cuetara M, Iglesias L & Rodriguez–Noriega A 1994. Itraconazole versus Griseofulvin in the treatment of tinea capitis: a double-blind randomised study in children. *Inter. J. Dermatology* **33** 743–747.

Martin KW & Ernst E 2003. Herbal medicines for treatment of fungal infection: A systematic review of controlled clinical trials. *Mycoses*. **47** 87-92.

McCutcheon AR, Ellis SM, Hancock REW & Tower GNH 1992. Antibiotic screening of medicinal plants of the British Columbian native peoples. *Journal Ethnopharmacology* **37** 213–223.

Midgley G, Moore MK, Cook JC & Phan QG 1994. "Mycology of nail disorders". *J. Am. Academy of Dermatology* **31** 68–74.

Nayak S, Debata BK, Srivastava VK & Sangwan NS 2003. Evaluation of agronomically useful somaclonal variants in Jamrosa (a hybrid Cymbopogon) and detection of genetic changes through RAPD. *Plant Sci.* **164** 1029-1035.

Ping-Hsien C, Chi-Wei Lee, Jia-Ying Chou, Murugan M, Bor-Jinn Shieh & Hueih-MinChen 2007. Anti-fungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. *Bioresource Technology* **98** 232-236.

Prajapati ND, Purohit SS, Sharma AK & Kumar T 2003. *A Handbook of Medicinal Plants.* Agrobios, India, Pp. 181.

Prakash B, Kedia A, Mishra PK & Dubey NK 2015. Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities-Potentials and challenges. *Food Control* **47** 381-391.

Prakash B, Shukla R, Singh P, Kumar A, Mishra PK & Dubey NK 2010. Efficacy of chemically characterized *Piper betle* L. essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity. *Inter. J. Food Microbiol.* **142** 114-119.

Prasad CS, Shukla R, Kumar A & Dubey NK 2009. In vitro and in vivo antifungal activity of essential oils of *Cymbopogon martini* and *Chenopodium ambrosioides* and their synergism against Dermatophytes. *Mycoses* **53** 123–129.

Mishra PK, Shukla R, Singh P, Prakash B, Kedia A & Dubey NK 2012. Antifungal, anti-aflatoxigenic, and antioxidant efficacy of Jamrosa essential oil for preservation of herbal raw materials. *Inter. Biodeterioration & Biodegradation* **74** 11–16.

Rippon, John Willard 1988. *Medical mycology: the pathogenic fungi and the pathogenic actinomycetes* (3rd ed.). Saunders, Philadelphia, pp. 797.

Saxena P, Kumar A & Shrivastava JN 2004. Diversity of keratinophilic mycoflora in the soil of Agra (India). *Folia Microbiol. (Praha)* **49** 430-434.

Shadzi S, Chadeganipour M & Alimoradi M 2002. *Mycoses* **45** 496-499.

Sharma R & Rajak RC 2003. Keratinophilic fungi: Nature's keratin degrading machines! *Resonance* **8** 28-40.

Shokohi T, Hedayati MT & Bakhshi H 2005. Isolation of fungi and aerobic actinomycetes from surface soil in Sari. *J. Kermanshah Uni. of Medical Sci.* **8** 25-32.

Shrivastava JN, Satsangi GP & Kumar A 2008. Incidence of keratinophilic fungi in waterlogged condition of paddy soil. *J. Environ. Biol.* **29** 125-126.

Spiewak R & Szostak W 2000. Zoophilic and geophilic dermato- phytoses among farmers and non-farmers in eastern Poland. *Annals of Agricultural and Environmental Medicine* **7** 125-129.

Torok I & Stehlich G 1993. Double-blind comparative examination of Ketoconazole 1% cream and Clotrimazole 2% ointment in superficial dermatomycosis. *Ther Hung* . **41** 60–63.

Vanbreuseghem R 1952. Biological technique for isolating dermatophytes from soil. *Annales de la Societe Belge de Medecine Tropicale* **32**(2) 173-178.

Zaias MD & Gerbert-Rebell MS 1996. Chronic dermatophytosis syndrome due to Trichophyton rubrum. *Inter. J. Dermatology* **35** 614–618.

Zarei MA & Zarrin M *2008.* Isolation of dermatophytes and related keratinophilic fungi from the two public parks in Ahvaz. *Jundishapur J. Microbiol.* **1** 20-23.

Zarrin MA & Haghgoo R *2011.* Survey of keratinophilic fungi from soils in Ahvaz, Iran. *Jundishapur J. Microbiol.* **4**(3) 191-194.