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In vitro Study of Antidermatophytic Activity of Mint (*Mentha Piperita*) Against *Trichophyton rubrum* and *Microsporum canis*

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Dermatophytes as the name suggest are the fungus that feed on skin. The chief source of their growth is keratin which is widely available in skin, nails and hairs. Here we have evaluated an *In vitro* study of antidermatophytic activity of Mint (*Mentha piperita*) against two dermatophytes i.e., *Trichophyton rubrum* and *Microsporum canis*. The data in the manuscript is very much helpful in curing dermatophytic infections as an application from Biotechnological point of view. Distribution of *Trichophyton rubrum* and *Microsporum canis* was found to be 19.23 and 32.69%, respectively. At variable temperature *Trichophyton rubrum* and *Microsporum canis* showed maximum growth at 37°C (0.23 and 0.19 g dry weight of mycelium, respectively). At variable pH *Trichophyton rubrum* showed maximum growth at pH 7.5 and 8.0 (0.32 g dry weight of mycelium) and *Microsporum canis* showed maximum growth at pH 7.5 (0.39 g dry weight of mycelium). *Mentha piperita* highest keratinase activity against *Microsporum canis* was found to be 2.99 unit mL^{-1} with 2.91 mg mL^{-1} extracellular release of protein and in *Trichophyton rubrum* it was found to be 2.99 unit mL^{-1} with 2.75 mg mL^{-1} extracellular release of protein.

Key words: Dermatophytes, *Mentha piperita*, *Trichophyton rubrum*, *Microsporum canis*, keratinase activity

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INTRODUCTION

Trichophyton, *Microsporum* and *Epidermophyton* are the three genera in which all the available dermatophytes fall (Bassiri-Jahromi, 2013). Cases of dermatophytosis have increased over the past few decades. These infections are often recalcitrant to therapy (Weitzman and Summerbell, 1995). Number of antifungal products is present in the markets to cure dermatophytosis (Al-Howiriny, 2002; Aderogba *et al*., 2006). But these drugs possess side effects on the proper healing of the skin from infection leading to skin wrinkling, pealing and distortion. Therefore there is a shift towards natural plant extracts for unfolding the folded medicinal importance of some of the medicinally important plants i.e., *Mentha piperita* (Mint) which have no side effects (Siramon *et al*., 2013).

Dermatophytic infections are very common in tropical and subtropical regions of the world which supports the favorable conditions where dermatophytes prevail on their chief source keratin which is present in nails, hairs and skin (Soares *et al*., 2012; Sharma *et al*., 2012). Keratinases are proteolytic enzymes in nature secreted by dermatophytes to feed on keratin which is the main constituent of structures that grow from the skin (Barchiesi *et al*., 2001).

The present retrospective work was aimed at isolation of dermatophytes using Baiting technique and its growth at different physiological conditions on Potato Dextrose Broth. Further we have evaluated an *In vitro* study of antidermatophytic activity of Mint (*Mentha piperita*) against two dermatophytes i.e., *Trichophyton rubrum* and *Microsporum canis* followed by screening of crude extract and essential oils of *Mentha piperita* against the fungus for determination of Minimum Inhibitory Concentration (MIC). The effect of plant extract on keratinase activity was also determined. The present data is very much helpful in curing dermatophytic infections as an application from Biotechnological point of view.

MATERIALS AND METHODS

Collection of soil samples: The present study was conducted for isolation of dermatophytic fungi from 52 different soil locations in Jaipur city of Rajasthan province (India). All the soil samples were collected in sealed polythene bags using sterile spatula at a depth of 5 cm below the ground surface. Soil was passed through a 2 mm sieve and stored at 10°C until isolation of dermatophyes.

Isolation and identification of keratinophilic fungi from soil: The experimental work was done by using the To Ka Va hair Baiting technique in which defatted human hairs were used as a growth medium for isolation of dermatophytes from the soil samples (Deshmukh, 2004). In this technique petri plates were sterilized and half filled with the soil samples. Short strand of sterilize defatted human hairs were spread over the surface of the soil. 10-12 mL of sterile water was added to the petri plates and incubated at room temperature (20-25°C). For identification of fungus species, internal and external morphological features were studied. Many different funguses was identified and isolated from the soil samples. Out of which *Trichophyton rubrum* and *Microsporum canis* were used for the present study.

Effect of variation of pH and temperature: For the study of growth and sporulation of dermatophytic fungi at different pH (Bhadauria and Sharma, 2001), temperature (Baxter and Illston, 1980) conditions and maintenance of fungal cultures Sabourauds Dextrose Agar/Broth Medium and Potato Dextrose Broth Medium was used with shaking for 15 days on rotation speed of 30 revolutions per minute. On sixteenth day of inoculation the mycelium was harvested for determining the growth and sporulation.

Inhibitory effect of crude extracts of plants on dermatophytes: Fresh leaves were collected and dried in shade. The dried leaves were ground to powder and suspended into petroleum ether and kept in refrigerator overnight for removing all the fatty substances. After overnight incubation, the supernatant was discarded and the residue was dried at room temperature. The residue was further divided into four parts and each part was suspended in methanol, ethanol, ethyl acetate and diethyl ether respectively in sterile 250 mL conical flasks and kept at 4°C overnight (Biswas *et al*., 2012). Each 100 g of powdered leaf material were soaked in 250 mL of methanol, ethanol, ethyl acetate and diethyl ether. After overnight incubation, the supernatant was filtered through whatman No.1 filter paper and the filtrate was dried to evaporate the organic solvent at room temperature. The sedimented extract was weighed and dissolved in 5% Dimethyl sulfoxide (DMSO). Filter diffusion method was used for determining the antidermatophyte effect of crude extracts of plants (Gould and Bowie, 1952).

Determination of minimum inhibitory concentration (MIC): The essential oils of *Mentha piperita* were screened against these fungus species to measure their Minimum Inhibitory Concentration (MIC). MIC was determined by incorporating various concentrations of plant extracts in SD broth. Twenty microliter of standard fungal inoculum was added to each tube and incubated at

room temperature for 21 days. Suitable controls were also included. SD broth with 20 μL of inoculum served as positive control. SD broth alone served as negative control (Nikkon *et al*., 2003). The tubes in duplicate for each agent were incubated at room temperature for 21 days. The MIC was regarded as the lowest concentration of the extract that did not show any viable growth after 21 days of incubation (compared with control) (Natarajan *et al*., 2003).

Keratinase activity: The effect of plant extracts on keratinase activity was determined by culturing fungal samples on Basal/Minimal Salt Medium (BSM/MSM) supplemented with human hairs and their total protein content, specific activity and percent inhibition of enzyme activity was measured (Fernandez-Torres *et al*., 2006).

RESULTS

Screening of 52 soil samples from different habitats of the Jaipur city, such as SMS Hospital, Gardens, Farmhouse, Colleges, Roadsides, Hostels, Malls, Airport, Colonies, Fort, Palaces etc. was carried out for the presence of dermatophytes. Many different funguses was identified and isolated from the soil samples. Out of which *Trichophyton rubrum* and *Microsporum canis* were used for the present study. They were identified on the bases of shape arrangement of spores and other structures through Scotch Tape Mount staining technique. Distribution of *Trichophyton rubrum* was found in 10 Petri plates (19.23%) and *Microsporum canis* was found in 17 petri plates (32.69%) out of 52 soil samples (Table 1). The color, texture, pigmentation on reverse surface of the colony and other characteristics were also recorded for fungal identification.

Environmental factors play an important role in the growth and sporulation of Keratinophilic fungi. Effect of variable temperature and pH on selected dermatophytic fungi was analyzed from weight of mycelium and spore count. These fungi were then subjected to growth at different physiological conditions such as temperature (5, 25, 30, 37, 45, 50 and 55°C) and pH (5, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5) on Potato Dextrose Broth (PDB). The growth index was then measured.

At variable temperature (Table 2) *T. rubrum* and *M. canis* showed maximum growth at 37°C (0.23 and 0.19 g dry weight of mycelium, respectively). At variable pH (Table 3) *T. rubrum* showed maximum growth at pH 7.5 and 8.0 (0.32 g dry weight of mycelium) and *M. canis* showed maximum growth at pH 7.5 (0.39 g dry weight of mycelium). The *in-vitro* antifungal activity of organic solvent extracts of leaves of *Mentha piperita* i.e., methanol, ethanol, ethyl acetate and diethyl ether (Table 4) was investigated against two selected dermatophytes i.e., *Trichophyton rubrum* and *Microsporum canis*.

The ethanolic extract of leaves of *Mentha piperita* exhibited the strongest activity against all the tested dermatophytes. For *Trichophyton rubrum* it showed inhibition zone of 24 mm and activity index of 0.75. *Microsporum canis* exhibited inhibition zone of 13 mm and activity index of 0.52 against ethanolic extract. El-Ghorab *et al*. (2003) demonstrated that the ethanolic leaf extract of *Mentha piperita* is known to have a very low toxicity.

One might conclude that the *Mentha piperita* would probably produce less side effects toxicity compared with conventional chemotherapeutic agent. Moreover essential oils of *Mentha piperita* were also screened against these fungus species to measure their minimum inhibitory concentration (Table 5). The minimum inhibitory concentration of essential oils of *Mentha piperita* against *Trichophyton rubrum* is 70% and against *Microsporum canis* is 90%. This suggested that at this value the growth of fungus was totally retarded.

In vitro degradation of keratin (human hair) was found to be associated with the release of extracellular keratinase into culture medium by the fungal species. Both the fungal strains (T*richophyton rubrum* and *Microsporum canis*) were able to produce keratinase at variable conditions but only in specific environmental conditions. Data obtained were analyzed and represented and percentage activity was determined.

It was found that *Trichophyton rubrum* and *Microsporum canis* grow best at temperature 37°C and pH 7.5 conditions. At this temperature and pH highest keratinase activity against *Microsporum canis* was found to be 2.99 unit mL^{-1} with 2.91 mg mL^{-1} extracellular release of protein (Table 6) and in T*richophyton rubrum* it was found to be 2.99 unit mL^{-1} with 2.75 mg mL^{-1} extracellular release of protein (Table 7).

DISCUSSION

Here, we have checked the growth pattern of two dermatophytes i.e., *Trichophyton rubrum* and *Microsporum canis* at variable temperature and pH, showing their excellent growth at pH 7.5 and 37°C (Table 2, 3). This holds the same favorable condition of human skin where the dermatopytic infections are common. A number of naturally derived therapeutic agents are in currently use against dermatophytes and one of them is of *Mentha piperita*. Traditionally *Mentha piperita* has been extensively used for the treatment of stomach ache, common cold, aromatherapy, antioxidants, etc (Zheng and Wang, 2001). The crude

Table 1: Abundance and external morphology of the mixed dermatophytic fungi from different soil samples of Jaipur

	External morphology			
Habitat	Abundance	Colour	Texture	Reverse pigmentation
Shantinagar (Durgapura)	+++	White	Granular	Wine Red
Gaushalla (Durgapura)	++	Cream, White, Grey	Powdery, Cottony	Olive Green, Light Brown
Shri Ram Garden (Shantinagar)	-	-	-	-
Barber Shop (Shantinagar)	+++	Cream, Grey	Powdery	Olive Green
Farm House (Bharat Petroleum Depot)	++	White	Cottony	Light Brown
Ram Bagh Circle	-	-	-	-
Narayan Singh Circle	-	-	-	-
Paach Batti	-	-	-	-
SMS Hospital	+++	White	Cottony	Golden Yellow & Light Brown
Central Park	++++	White	Cottony	White
Wilfred College (Mansarover)	-	-	-	-
Ajmeri Gate	++	White	Cottony	White
Maharaja College	-	-	-	-
Pratap Plaza	+++	White	Cottony	Golden Yellow
Vinodilalpura (Chaksu)	++++	White, Cream	Cottony, Powdery, Granular	Golden Yellow, Light Brown
Beelwa	++++	White	Granular	Pinkish, Wine Red
Gopalpura Bypass	+	White	Powdery	Golden Yellow, Light Brown
Ashok Vihar – Diggi Road (Sanganer)	+++	White, Cream	Cottony, Granular	Brown, Golden Yellow
Chokhi Dhani	-	-	-	-
EPIP Gate	-	-	-	-
Sanganer Airport	++++	White, Cream	Cottony, Powdery	Red, Golden Yellow, Brown
Chauti Chaupad	-	-	-	-
MGIAS	++	White	Cottony	White
Vatika	+	Cream, Grey	Powdery	Olive Green
Dhakad Colony (Sanganer)	++	White	Cottony	White
Govindpura (Sanganer)	+	Grey	Granular	Dark Brown
Barkat Nagar (Tonk Phatak)	+++	White, Cream	Cottony, Powdery	Maroon, Golden Yellow
Seel Ki Dungri (Chaksu)	+	White	Powdery	White
Pawan Vatika	+	Grey	Granular	Greenish
Ahemdabad	+++	White	Cottony, Granular	Wine Red, Golden Yellow, Pinkish
JECRC Garden	+	White	Granular	Golden Yellow
Sodala Thana	++	White	Granular, Cottony	Wine Red
Sector 30 (Mansarover)	-	-	-	-
Bus stand (Durgapura)	++	White	Cottony, Granular	Pinkish
Jhotwara	+++	White	Cottony, Granular	Golden Yellow, Dark Green
Ramganj	++	Cream	Cottony, Granular	Brownish, Pinkish
Seetla (Chaksu)	-	-	-	-
Lokesh Ka Baandh (Chaksu)	+++	White	Cottony , Granular	Golden Yellow, Wine Red
Jawahar Circle	++++	White, Cream, Grey	Cottony,	Pinkish, Dark Green, Brownish, Wine Red, Golden Yellow
Jal Mahal	++++	Cream	Cottony, Powdery	Golden Yellow, Light Brown
Shyam Nagar (Sodala)	++	White	Cottony	Golden Yellow
Shastri Nagar	+	White	Cottony	Golden Yellow
Chandpaul	++	White	Granular	Wine Red
Boli (Sawai Madhopur)	+	White	Cottony	Light Brown
Babaria (Sanganer)	-	-	-	-
Maharani Farm (Durgapura)	-	-	-	-
Malviya Nagar	+	White	Granular	Dark Green
JECRC Boys Hostel	+++	Black, White	Granular, Powdery	Black, Golden Yellow
Mahatama Gandhi Medical College	++	Grey	Granular	Greenish
Murlipura	-	-	-	-
Chandipura (Bhankrota)	-	-	-	-
Sukhpuria	+	White	Cottony	Golden Yellow

-: No growth, +: Poor growth, ++: Fair growth, +++: Good growth, ++++: Excellent growth

Table 2: Growth of *Trichophyton rubrum* and *Microsporum canis* at different temperature range

Temperature (°C)	Dry weight of *T. rubrum* mycelium (g)	*T. rubrum* sporulation	Dry weight of *M. canis* mycelium (gm)	*M. canis* sporulation
5.0	0.04	+	0.03	+
25.0	0.20	++	0.14	++
30.0	0.21	+++	0.16	+++
37.0	0.23	++++	0.19	++++
45.0	0.02	+	0.03	+
50.0	0.01	+	0.01	+
55.0	0.01	+	0.01	+

+: Poor growth, ++: Fair growth, +++: Good growth, ++++: Excellent growth

Table 3: Growth of *Trichophyton rubrum* and *Microsporum canis* at different pH range

pH Range	Dry weight of *T. rubrum* mycelium (gm)	*T. rubrum* sporulation	Dry weight of *M. canis* mycelium (gm)	*M. canis* sporulation
5.0	0.05	+	0.14	+
5.5	0.05	+	0.23	++
6.0	0.10	+	0.23	++
6.5	0.25	++	0.29	+++
7.0	0.30	+++	0.30	+++
7.5	0.32	++++	0.39	++++
8.0	0.32	++++	0.32	+++
8.5	0.19	++	0.29	+++
9.0	0.13	++	0.26	++
9.5	0.13	++	0.24	++

+: Poor growth, ++: Fair growth, +++: Good growth, ++++: Excellent growth

Table 4: Screening of different organic solvents extracts of *Mentha piperita* against *Trichophyton rubrum* and *Microsporum canis*

	Trichophyton rubrum		*Microsporum canis*	
Plant extract	Inhibition zone (mm)	Activity index	Inhibition zone (mm)	Activity index
Methanol	20	0.62	7	0.28
Ethanol	24	0.75	13	0.52
Ethyl acetate	22	0.68	10	0.40
Di ethyl ether	17	0.53	9	0.36

Activity Index (AI) = Inhibition zone of sample/ Inhibition zone of standard, Inhibition zone of standard Ketoconazole against *Trichophyton rubrum* = 32 mm, Inhibition zone of standard Ketoconazole against *Microsporum canis* = 25 mm

Table 5: Minimum inhibitory concentration of essential oils of *Mentha piperita* against *Trichophyton rubrum* and *Microsporum canis*

	Trichophyton rubrum		*Microsporum canis*	
Concentration of oil (%)	Inhibition zone (mm)	Activity index	Inhibition zone (mm)	Activity index
10	21	0.65	24	0.96
20	28	0.87	29	1.16
30	39	1.40	38	1.52
40	47	1.21	44	1.76
50	55	1.71	61	2.44
60	64	2.00	69	2.76
70	-	-	74	2.96
80	-	-	79	3.16
90	-	-	-	-
100	-	-	-	-

-: no growth of fungus

Table 6: Effect of different concentration of leaf extract of *Mentha piperita* on Keratinase activity of *Microsporum canis*

Plant extract concentration (%)	Keratinase activity (unit mL^{-1})	Protein (mg mL^{-1})	Specific activity (enzyme unit mg^{-1} protein)	Inhibition of enzyme activity (%)
100	-	-	-	-
90	-	-	-	-
80	2.36	0.58	4.06	21.08
70	2.50	0.96	2.60	16.40
60	2.61	1.11	2.35	12.72
50	2.76	1.99	1.38	7.70
25	2.82	2.75	1.02	5.69
0	2.99	2.91	1.02	0.00

Specific Activity (enzyme unit/mg protein) = Keratinase Activity (unit mL^{-1})/Protein (mg mL^{-1}), Inhibition of Enzyme Activity (%) = 100/Control concentration X concentration of enzyme activity-100

Table 7: Effect of different concentration of leaf extract of *Mentha piperita* on Keratinase activity of *Trichophyton rubrum*

Plant extract concentration (%)	Keratinase activity (unit mL^{-1})	Protein (mg mL^{-1})	Specific activity (enzyme unit mg^{-1} protein)	Inhibition of enzyme activity (%)
100	-	-	-	-
90	-	-	-	-
80	2.22	0.42	5.28	25.76
70	2.49	0.79	3.15	16.73
60	2.61	1.48	1.76	12.72
50	2.64	1.55	1.70	11.71
25	2.89	2.65	1.09	3.35
0	2.99	2.75	1.08	0.00

extracts and the essential oils of *Mentha piperita* had shown strong antifungal activity against the two isolated fungus from Jaipur city, their details are cited in the Table 4 and 5. The growth of *Trichophyton rubrum* was totally retarded at 70% concentration of plant extract, whereas in the case of *Microsporum canis* 90% concentration was found effective. The mint extracts has antifungal activity and suggested its therapeutic use in curing dermatophytosis.

CONCLUSION

The *Mentha piperita* extracts were found to be effective against *Trichophyton rubrum* and *Microsporum canis*. The results of the analysis of the essential oils of Mint were qualitative and quantitative. This study shows potential uses of extracts for antidermatophytic application. Use of plant extracts in the treatment regimen of various diseases are gaining importance as antimicrobial, antibacterial, antiviral and antifungal activities of many plants are reported. Antimicrobial properties of plant extracts are now recognized by several workers. Dose response studies are not needed because there are no side effects of natural products. Natural antifungals play an important role by restoring the barrier function of skin and allowing the skin to naturally replace itself. The present data is very much helpful in curing dermatophytic infections as an application from Biotechnological point of view, thus, this *In vitro* testing can help to determine the activities of new drugs and to find the right therapy.

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