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In vivo antifungal activity of *Acmella* essential oil on a dermatomycotic strain *Trichophyton mentagrophytes* (MTCC-7687)

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ABSTRACT

Trichophyton mentagrophytes is spotted as skin invasive dermatophyte. Further the strain is found to have endowed with innate drug avoidance mechanism. In topical countries, chronic dermatophytic infections are always in flash reports. In this tricky situation, ecofriendly, cost effective and safe medicinal principles from plants may be used reliable drugs against dermatopmycotic infections. Spilanthes acmella Murr. (Asteraceae) is commonly known as toothache plant is found luxuriously in our native land. This present pursuit aims at studying the antifungal efficacy of Acmella essential oil against Trichophyton (Trichophyton mentagrophytes, MTCC 7687) infections induced on a mouse model. Nude mice (Mus musculus) were topically inoculated with T. mentagrophytes spores by using "Spore suspension" method. After induction of the infection, the symptomatic mice groups were subjected to topical application of Acmella essential oil at a concentration of 1µl/ml (V/V, oil/emulsifier). Terbinafine (5mg/ml, W/V) was taken as standard drug. Four groups of mice were taken for the study. Two groups (3 mice/group) for positive and negative controls respectively, while the rest of the groups were studied for the test drug efficacy. There was 75% remedial effect of infected area by the activity of Acmella essential oil as observed on 11th day of experiment schedule as compared with Terbinafine which had absolute effect on 8th day. The results obtained from this in vivo study, it was suggestive about curing activity of Acmella essential oil against the induced Trichophyton infection in mouse models.

Key words: *Trichophyton mentagrophytes*, *Spilanthes acmella*, essential oil, *In vivo* study

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INTRODUCTION

Dermatomycoses are superficial fungal infections of the skin, hair and nails that affect more than 20-25% of the people worldwide. These infections can be caused by yeasts, dermatophytes and non-dermatophyte filamentous fungi (NDFF) and are considered a public health problem. Despite this, few studies have investigated the prevalence and antifungal susceptibility of causative agents of dermatomycoses in the developing world [1]. Many -a -number of antifungal agents are in use as topical pharmaceutical formulations. Topical delivery can be defined as application of drug formulation to the skin directly to treat cutaneous disorders or manifestations [2]. Increased resistance of pathogenic fungi to classical antifungal agents has led to sustained research efforts targeting alternative antifungal strategies [3]. The medicinal plants form a large group of economically important plants that provide the basic raw materials for pharmaceuticals [4]. Many essential oils produced from higher plants are reported to be with antifungal activity against dermatomycoses supported by *in vitro* studies [5]. There are reports regarding the wound healing property of herbal extracts which were experimented on animal models [6, 7]. However there is only limited data in

the literature on the antifungal activity of essential oils toward human fungal pathogens *in vivo* [8a]. The purpose of this *in vivo* study is to look at the potency of antifungal efficacy of Acmella (*Spilanthes acmella*, Murr. Asteraceae) essential oil against a strain of *Trichophyton mentagrophytes* (MTCC 7687) on mice models.

MATERIALS AND METHODS

Plant material: The flowering twigs of the plant locally named as Akarakara used in traditional medicament by the inhabitants was collected during its flowering period, from the sites of our native land belonging to Western part of Odisha, India. The collected plant was sent to Botanical Survey of India, Central National Herbarium (CNH), Botanical Garden, Howrah, India for authenticated identification.

Hydrodistillation of flowering heads: The flowering heads of the plant was subjected to hydro distillation by using Clevenger's apparatus [9] in the Department of Pharmacognosy, The Pharmaceutical College, Barpalli, Odisha, India.

Identification of hydrodistillate: The hydrodistillate was subjected to identification by using standard pharmacognostical protocol [10]. Further a GLC (Gas Liquid Chromatography) analysis was performed at the Sophosticated Analytical Instrumental Facilities (SAIF), Central Drug Research Institute (CDRI), Lucknow, India.
The dermatopmycotic strain used for the study: The dermatomycotic strain was procured from the institute of Microbial Type Culture Collection (MTCC), Chandigarh, India. The strain was subjected to revival on Sabouraud Agar media (HI-Media, Mumbai).

In vitro study: An *In vitro* experiment was carried out to screen for the sensitivity of the strain of *Trichophyton mentagrophytes* (MTCC 7687) towards the hydro distillate by following "Disc diffusion" method. Further, to determine the effective concentration of the herbal distillate for inhibiting the hyphal growth of the dermatomycotic strain, a "Slant dilution" method was followed [11].

In vivo study: The induction of infection on mice models were done by following the modified method of Sokovic *et al*., (2012) [12a]. For this purpose, conidia were obtained from 7 days old culture of *Trichophyton* mycelia and were suspended with sterilized distilled water. Male Swiss albino mice (*Mus musculus*, BALB/C (albino) family: Muridae subfamily: Murinae) taken as animal model were of 8 weeks old and of 30±2 gms (measured weights). The Mice were housed by Deshpande Lab, Bhopal, India. The mice were grouped into 3 groups (3mice/group). The group I (Induced with infection but not applied with drugs) were taken as control groups, while Group II was Induced with infection, applied with test drug i.e, Acmella essential oil, Group III, comprising a group was induced with infection and applied with standard drug, Terbinafine. The Mice were subjected to immuno supression by subcutaneous injection of 500 mg (w/v) of estradiol valerate and were observed for 3 days. Immuno suppression was obligatory to induce the dermatomycotic infection. For this purpose, flanks of mice were shaved with electric razor and the exposed area lightly abraded with a sterile scalpel blade. The conidia suspension (100µl) was applied on the shaved site as the inoculums and was gently rubbed with the flat part of a sterile blade. The developments of the lesions (if any) were visually examined at a time interval of 24 hrs. After a period of 7 days, the degree and intensity of mycelial growth of the inoculated spores on mice skin surfaces was inveigled in the form of scores. On seventh day onwards the lesions were scored as follows: 0 (absence of lesion), 1 (appearance of erythema at infected site or new hair growth on the bald exposed area), 2 (moderate erythema spreading over entire infected site), 3 (intense erythema with abrasions, swelling and scaling), 4 (severely erythematous lesion with crusting spreading over the entire exposed area). The average lesion scores were calculated for each group by dividing the sum of the lesion scored by the number of animals in the group.

The drug application period started after the development of lesions. The Group I was not subjected to any drug application. The Group II were applied with herbal distillate calculated from MFC (Minimum Fungicidal Concentration) value maintaining 50% dosage form with the diluent Sodium taurocholate salt in an aqueous base. The Group III was subjected to topical applications of Terbinafine, taken as standard antifungal drug maintaining the concentration 0.5 % (w/v). The visual examination was the method adopted to determine the percentage of therapeutic activity depicted by test drugs on the induced infection sites. The treatment scores were given to mice groups as follows: 0 (not cured), 1 (25% cured), 2 (50% cured), 3 (75% cured), 4 (100% cured).

RESULTS AND DISCUSSION

Plant material: The locally collected plant material from the native land sites was preliminarily identified as *Spilanthes acmella*. Further, the report from Botanical survey of India, Howarh validated the identification (Ref No. CNH/37/2011/Tech.II/499).

Hydrodistillation of flowering heads: The hydrodistillate could be recognized as essential oil fraction of flowering heads of the plant, *Spilanthes acmella.*

Identification of hydrodistillate: From the GLC analysis, the essential oil was identified as Acmella essential oil.

The dermatopmycotic strain (MTCC 7687) used for the study: The MTCC procured dermatomycotic strain used for the study was found to be revived on Sabouraud agar plates.

In vitro study: The disc diffusion method depicted the restriction of growth of mycelia of *Trichophyton metagrophytes*, when exposed to Acmella essential oil at a test concentration of 3µl/disc (3mm, disc size). From the slant dilution method the minimum fungicidal concentration (MFC) was determined as 1µl/ml (v/v).

In vivo study: From the *in vivo* study of the hydrodistillate (Acmella essential oil) against the induced dermatomycotic infection of *T.mentagrophytes*, the results are depicted in Table 1. Based on day wise observation on lesion development, it was found that the lesions were started to appear from day 7. All the untreated animal skin lesions observed to be severely erythematous lesion with crusting spreading over the entire exposed area on day eight up to fifteen days (Figure 1). The tested Acmella oil treated animal group showed moderate erythema lesions on the surface of mice after fifteen days of treatment (Figure 2). The standard drug Terbinafine treated animal group were found to be faded erythematous lesion at the site of induced trichophyton infection (Figure 3). The day wise Treatment Score were clearly observed in Figure 4. The treatment score for standard drug Terbinafine was found 4 (100% cure) on day eight. For tested Acmella oil the treatment score was 3 (75% cure) on day eleven. The Figure 4 depicts the result obtained from *in vivo* application of Acmella essential oil on induced dermatomycotic fungus, in the form of scores as induced lesion Scores and Treatment scores respectively. The treatment score for standard drug Terbinafine was found 4 (100% cure) on day eight. For tested Acmella oil the treatment score was 3 (75% cure) on day eleven. But the treatment score for untreated animal was 0 (not cured) always indicating the control. Table 1 noticeably showed the day wise average lesion score and treatment score for all untreated, treated and standard groups.

Table 1. Collective Average Lesion Score: Collective Treatment Score values obtained from three groups: Group I untreated; Group II treated; Group III Standard

SL No.	Average Lesion Score			Average Treatment Score		
	Untreated (Group I)	Treated (Group II)	Standard (Group III)	Untreated (Group I)	Treated (Group II)	Standard (Group III)
1	1.0	1.0	1.0	0.0	1.0	1.0
2	1.2	1.0	1.0	0.0	1.0	2.0
3	1.0	1.0	1.0	0.0	1.0	2.0
4	1.3	1.0	1.0	0.0	1.0	2.0
5	2.7	1.0	1.0	0.0	1.0	2.0
6	3.0	1.0	1.0	0.0	1.0	3.0
7	3.7	2.0	1.0	0.0	2.0	3.0
8	**4.0**	**2.0**	**1.0**	**0.0**	**2.0**	**4.0**
9	4.0	2.0	1.0	0.0	2.0	4.0
10	4.0	2.0	1.0	0.0	2.0	4.0
11	**4.0**	**2.0**	**1.0**	**0.0**	**3.0**	**4.0**
12	4.0	2.0	1.0	0.0	3.0	4.0
13	4.0	2.0	1.0	0.0	3.0	4.0
14	4.0	2.0	1.0	0.0	3.0	4.0
15	4.0	2.0	1.0	0.0	3.0	4.0

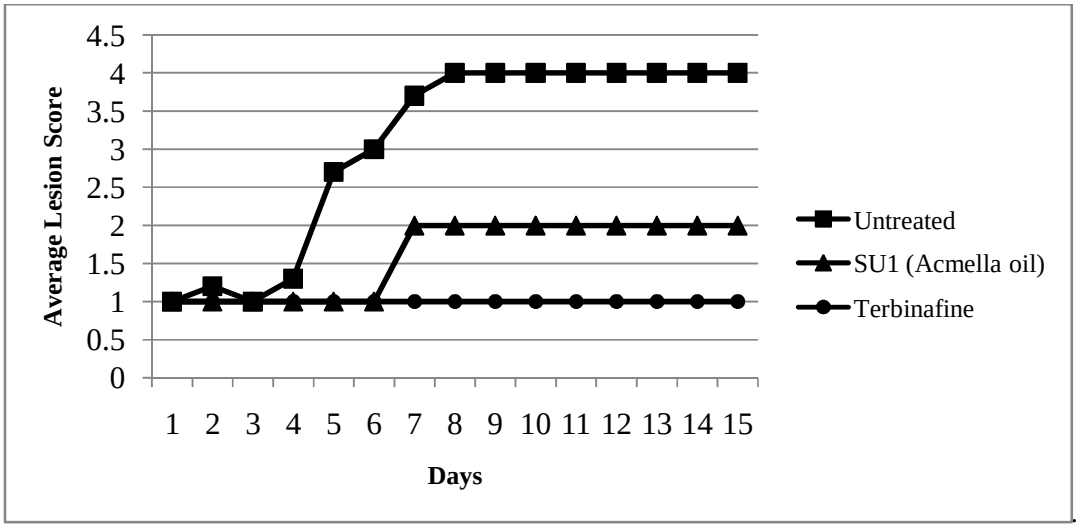
NB: Encircled numbers depict the average scores (%). Arrow indicates the 100% recovery of lesions by standard drug at day 8 and onwards; Scores written in box in percentages 70% and 100% for Acmella oil treated and Terbinafine treated lesions recovery respectively at day 11 onwards.

Figure 1- Control

Figure 2- Test

Figure 3- Standard

Graph 1: Average Lesion Score



Average lesion score: 0 (absence of lesion), 1 (appearance of erythema at infected site or new hair growth on the bald exposed area), 2 (moderate erythema spreading over entire infected site), 3 (intense erythema with abrasions, swelling and scaling), 4 (severely erythematous lesion with crusting spreading over the entire exposed area)

CONCLUSION

From this *in-vivo* study, it may be inferred that the test drug i.e. Acmella essential oil (1µl) had a comparable therapeutic activity like the referred drug Terbinafine against the test fungus, *T.mentagrophytes*, induced as infection in mice models in the form of lesions. Although Terbinafine had absolute inhibition at day 8, the Acmella oil, had 75% inhibitory effect at day 11. Accordingly, the test Acmella oil as a natural plant product could be considered as a better topically preferred drug in minimizing the side effects. Terbinafine is contraindicated in patients with a known hypersensitivity [13]. Hence, the Acmella oil and or the active constituent may be enlisted as a drug for topical preparations as excipient/drug active in Pharmaceutical industries, rendering the product cost effective. Normally herbal drugs are free of side effects[14]. Therefore this *in vivo* study had focused on remedial skill of a herbal hydrodistillate against a dermatomycotic infection induced on mice models.

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