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ESSENTIAL OIL USED AS BROAD-SPECTRUM HERBAL THERAPY AGAINST SUPERFICIAL FUNGAL INFECTIONS

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ABSTRACT

The present study aimed to develop an effective chemotherapeutic agent from a renewable natural source that possesses potent antifungal properties, is biodegradable, thermostable, has a long shelf life, and is free from adverse side effects. The essential oil of *Artemisia maritima Linn.* was evaluated in vitro against human pathogenic fungi to determine its antifungal efficacy, thermal stability, storage durability, and safety on human skin. Its antifungal activity was also compared with that of several synthetic antifungal agents. Furthermore, the oil was formulated into an ointment and subjected to clinical evaluation. The results demonstrated that the essential oil exhibited strong antifungal activity, completely inhibiting the growth of tested dermatophytes at a concentration of 0.1 µl ml^{-1}. The oil remained stable and effective at temperatures up to 70°C and retained its activity after 48 months of storage. Compared with synthetic antifungal agents, the oil showed superior antifungal efficacy and produced no adverse effects on mammalian skin at concentrations up to 1%. These findings suggest that the *A. maritima* oil-based ointment is a safe, effective, thermostable, and long-lasting treatment for dermatophytosis. However, its commercial potential requires further validation through multicentre clinical trials. This version improves clarity, readability, and academic flow while preserving the original meaning and results.

KEYWORDS: Dermatophytes, Herbal Therapy, Thermostable, Antifungal, Dermatophytoses Storage Durability

Dermatophytosis, commonly referred to as tinea infection, is a fungal disease caused by dermatophytes belonging to the genera *Epidermophyton*, *Microsporum*, and *Trichophyton*. Among fungal infections, dermatophytosis are more prevalent than subcutaneous and systemic mycoses and continue to represent a major public health concern, particularly in tropical and subtropical regions. Despite the availability of numerous antifungal formulations, including ointments, lotions, paints, and powders, the effective treatment of these infections remains challenging. Most conventional antifungal products are derived from petrochemical sources, which are non-biodegradable and may be associated with adverse effects, residual toxicity, and environmental hazards. In addition, these formulations are often thermolabile and possess a limited shelf life, generally not exceeding 35 months. Naturally occurring antifungals described to date are mostly biodegradable (Beye 1978) and are devoid of side effects (Fawcett and Spencer, 1970) compared with commercially available antifungals. Recently, some products of higher plant origin have been shown to be effective source of chemotherapeutic agents without undesirable side effects with strong fungicidal activity (Dikshit *et al.*, 1985; Pandey *et al.*, 1996; Shahi 1997; Shahi *et al.*, 1999; Shahi *et al.*, 2000). These limitations encouraged the exploration of plant-derived products, particularly essential oils, as potential sources of effective antifungal agents. In the present study, the essential oil of *Artemisia maritima* was investigated for its antifungal activity against several dermatophytes, including *Epidermophyton floccosum*, *Microsporum canis*, *M. gypseum*, *Trichophyton mentagrophytes* and *T. rubrum*. The study also evaluated the thermal stability and storage durability of the oil to assess its suitability as a long-lasting antifungal agent. Furthermore, the oil was formulated into an ointment and subjected to clinical investigation to determine its therapeutic potential in the treatment of dermatophytosis. *A. maritima* is a small herbaceous plant belonging to the family Asteraceae. The genus is commonly known as worm wood comprises of about 400 species distributed throughout northern hemisphere. About 30 species of Artemisia documentedin in the flora of Western Himalaya (Marco and Barbera, 1990) As part of the programme on screening of odoriferous plants from natural habitat in the Western Himalaya region, Jammu and Kasmir, Himanchal Pradesh (Kangra, Kinnour,Kullu vally) of India (Weyerstahl *et al.*, 1988). *Artemisia maritima* is the medium size aromatic herb collected from Kinnor of Himanchal Pradesh, India. The plant grows up to 100cm in height.

MATERIALS AND METHODS

Maintenance of Human Pathogenic Fungi

The test pathogens, *Epidermophyton floccosum* (Hartz) Langeron et Mitochevitch, *Microsporum canis*

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Bodin, *M. gypseum* (Bodin) Guiart et Grigoraki, *Trichophyton mentagrophytes* (Robin) Blanchard, *T. rubrum* (Castellani) Sabouraud, were maintained on Sabouraud dextrose agar (SDA) medium. A seven-day old culture of test pathogens was used for antifungal study.

Isolation and Characterization of Essential Oil

The essential oil was extracted from the aerial part particularly leaves of *Artemisia maritima* by hydro distillation using Clevenger's apparatus (Clevenger, 1928). A light yellow colored oily layer was separated and dried with anhydrous sodium sulfate. The Physiochemical properties of the oil were determined by the technique described by Langenau (1948).

GC-MS analysis was carried out by GC-MS (QP2010) Shimadzu gas chromatograph- Mass spectrometer using helium as a carrier gas with a flow rate of 1.1 mL min¹. on split mode (1: 50) fitted with carbowax phase, BP-20 capillary column (length 30 X 0.25 mm with 0.25 mm film thickness). The temperature was programmed from 70ºC- 220ºC at 4ºC min¹ with 4 min hold at 70ºC and 5 min hold at 220ºC. Injector and interface temperatures were 250ºC for both. Ion source temperature was 200ºC. Samples were injected in 2mL quantities. The volatile oil was obtained by hydro distillation of *Artemisia maritima* aerial parts gave 0.5-0.7% yield (v/w) of the oil of *A. maritima.* The GC-MS analysis resulted in the identification of total 24 components (2 major and 22 minor). Among these1, 8-cineole is found in higest concentration (23.80%) Table-2

In Vitro Investigation

The minimum inhibitory concentrations (MIC) of the oil against test pathogens (dermatophytes) were determined following the poisoned food technique of Grover and Moore (1962) with slight modification (Shahi *et al.*, 1999). The nature of antifungal activity (fungistatic/fungicidal) of the oil at MIC was determined by the method of Garber and Houston (1959). The inhibited fungal discs (at MIC) were reinoculated upside down on plain SDA medium. Inoculated petri plate was incubated at 27 ^{0}C, observations were recorded on seventh day. Fungal growth on seventh day indicated fungistatic action of the oil while absence of fungal growth denoted fungicidal action

The minimum killing time of the oil was determined by mycelial disc killing technique of Shahi *et al.,* (1999). Two treatment sets were maintained one with pure oil (100 %) and other with the minimum fungicidal concentrations (MCC) of the oil. The treatment sets using MCC of the oil was prepared by dissolving the required quantity of the oil samples in 5 ml acetone and then added into 100 ml 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 7day old culture of the test pathogens were aseptically placed in the culture tubes of different treatment and control sets. These mycelial discs were taken out from the tube at different time intervals and washed immediately in the washing solution (containing acetone and sterilized distilled water in 1: 2 ratio) to remove the treatment solution. These washed mycelial discs were aseptically transferred upside down to the SDA medium (pH - 5.6) in petri plates. The same procedure was followed with the control sets. The inoculated petri plates were incubated at 27 ± 1^{0}C 1^{0}C and the observations recorded on seventh day and calculate the percentage of mycelial growth inhibition according to Shahi *et al.,* (1999). The effects of inoculum potentiality at MIC of the oil were determined by the method of Shahi *et al.,* (2000). Effect of the temperature, on antifungal activity of the oil was evaluated according to Shahi *et al.,* (2000). Five lots of oil were kept in small vials, each containing 5 ml of oil, these were exposed to 40, 60, 80 and 100^{0}C in incubator for an hour. Antifungal activity then tested at MIC by the poisoned food technique (Grover and Moore, 1962).

Expiry of toxicity of the oil was determined by storing the oil at room temperature (32 ± 4^{0}C) and antifungal activity were tested at MIC by the regular intervals 60 days up to 48 months following the poisoned food technique (Grover and Moore, 1962).

To comparative study of the oil with some synthetic antifungals (miconazole, ketaconazole, ciclopirox alamine) was carried out by comparing minimum inhibitory concentrations and minimum fungicidal concentrations. Various concentrations 0.01 to 10 ±l ml^{-1}, of synthetic antifungal substances were prepared from their stock solutions by incorporating into adequately cooled autoclaved 100 ml SDA medium. In controls, sterilized water (in place of the antifungal substances) was used in the medium in appropriate amount. The MIC was assayed following the poisoned food technique of Grover and Moore (1962). The nature of toxicity at MIC was determined by the method of Garber and Houston (1959). All experiments were repeated twice and each contained five replicates; data represent mean values. According to Wellman (1967), an ideal fungicide should maintain its antifungal efficacy under varying temperature conditions and remain stable during long-term storage.

RESULTS AND DISCUSSION

The volatile oil was obtained from aerial parts of *Artemisia maritima* by hydro distillation method gave 0.5-0.7% yield (v/w) of the oil. The physicochemical properties of the oil shown in table-1. The GC-MS analysis resulted in the identification of total 24 components (2 major and 22 minor). Among these1, 8-cineole is found in highest concentration (23.80%) Table-2

Table 1: Physicochemical properties of the oil of *Artemisia maritima* (Linn.)

SNo.	Properties Studied	Observations
1.	Plant height (cm.)	100 (approx.)
2.	Oil yield (%)	0.60
3.	Colour of the oil	Yellowish
4.	Specific gravity at 29.5^{0}C	0.8923
5.	Optical rotation	+4°25; 16°75
6.	Refractive index at 25^{0}C	1.4852
7.	Solubility	Acetone
8.	Ester value	71.0-175.0
9.	Carbonyle value	75.0
10.	Cineole	14.0-24.0

Table 2: Volatile constituents in *A. maritima* collected from western Himalaya, Himanchal Pradesh (kullu-vally)

S.No.	Constituents	%
1.	Thujone	o.64
2.	Dehydro 1,8-cineoleTerpinene	0.85
3.	1,8-Cineole	23.80
4.	1,8-Cineole terpinene	00 .93
5.	4,7,7-Trimethyle bicyclo(3.3.1) Hept-3-en-6-one	07.73
6.	t-Arbusculone	00.32
7.	Chrysanthenone	17.54
8.	t-Chrysanthenyl acetate	05.72
9.	Terpineol	02.99
10.	Dehydro sabina ketone	00.73
11.	Isopinocarveol	00.49
12.	Germacrene-D	04.54
13.	Piperitone	00.51
14.	Bicyclogermacrene	01.66
15.	5,8,10-Undecatriene-3-ol	00.93
16.	Davanone	00.74
17.	Caryophyllene oxide	00.88
18.	Davanone	01.07
19.	Artedouglasia oxide-C	01.11
20.	Filifolide	01.41
21.	Ethy cinnamate	00.78
22.	Artedouglasia oxide-A	01.71
23.	Artedouglasia oxide-D	00.43
24.	Spathulenol	00.75
25.	Artedouglasia oxide-B	00.54

In Vitro Test

The MIC of the oil of *A. maritima* were found to be 0.05 µl ml^{-1} for *E. floccosum* 0.05 µl ml^{-1} *M. canis,* 0.04 µl l ml^{-1} *M. gypseum,* 0.04 µl l ml^{-1} *T. interdigetale,* 0.04 µl l ml^{-1} *T. mentagrophytes,* 0.03 µl l ml^{-1} *Trichophyton rubrum.* The minimum fungicidal concentration (MCC) were found *E. floccosum* 0.06 µl l ml^{-1} *M. canis,* 0.05 µl l ml^{-1} *M. gypseum,* 0.05 µl l ml^{-1} *T. interdigetale,* 0.05 µl l ml^{-1} *T. mentagrophytes,* 0.04 µl l ml^{-1} *Trichophyton rubrum.* (Table 3). The oil inhibited heavy doses (30 mycelial discs, each of 5 mm in diameter) of fungal inoculum at 0.6 µl l ml^{-1} concentration. The activity of the oil did not expire even up to 48 month of storage and persisted up to 80^0C temperature. The pure oil (100 %) killed the fungi*, M. canis, T. mentagrophytes and T. rubrum* in just 5 sec while at its minimum fungicidal concentrations it required 80, 60 and 40 minutes respectively (Table 4).

The minimum inhibitory concentrations of the oil of *Artemisia maritima* against tested pathogens, *Epidermophyton floccosum, Microsporum canis, M. gypseum, Trichophyton interdigitale T. mentagrophytes, T. rubrum* were found to be 0.5, 0.4, 0.4, o.4, 0.4 and 0.3 µl ml^{-1} respectively

Table 3: Minimum inhibitory concentrations of the oil of *Artemisia maritima* against dominant superficial skin infective fungi (tinea corporis)

Test pathogens	Fungi Per cent inhibition (MGI %) at various concentrations (µl ml^{-1})					
	0.05	0.1	0.2	0.3	0.4	0.5
Epidermophyton floccosum	-	-	20	45	80	100^s
M. canis	-	30	45	78	100^s	100^c
M. gypseum	-	30	45	75	100^s	100^c
Tricophyton interdigitale	-	40	50	80	100^s	100^c
T. mentagrophytes	-	40	50	75	100^s	100^c
T. rubrum	10	50	57	100^s	100^c	100^c
^{c}Fungicidal ^{s}Fungistatic						
MGI, Mycelial growth inhibition						

Table 4: Minimum killing time of the oil of *Artemisia maritima* against test pathogens

Minimum killing time	Fungal Growth Inhibition (FGI) %					
	Mc			Tm		Tr
	PO	MFC	PO		MFC	PO	MFC
120min.	100	100	100		100	100	100
80"	100	**100**	100		100	100	100
70"	100	80	100		100	100	100
60"	100	76	100		**100**	100	100
50"	100	56	100		85	100	100
40"	100	35	100		48	100	**100**
30"	100	-	100		25	100	83
60 sec.	100	-	100		-	100	42
30"	100	-	100		-	100	28
20"	100	-	100		-	100	-
10"	100	-	100		-	100	-
5"	**100**	-	**100**		-	**100**	-
2"	95	-80			-	89	-
1"	87	-78			-	63	-

Mc = *Microsporum canis*, **Tm =** *Trichophyton mentagrophytes*, **Tr =** *T. rubrum,* **PO** = Pure oil; **MFC** = Minimum fungicidal concentration

Table 5: Comparative efficacy of the oil of *Artemisia maritima* with commercial antifungal drugs

Antifungals		Active Minimum inhibitory and fungicidal concentration (µl l ml^{-1}) ingredients					
		M. cannis		*T. mentagrophytes*		*T. rubrum*	
		MIC	MCC	MIC	MCC	MIC	MCC
Ointment	Essential oil	0.5	0.6	0.4	0.5	0.3	0.4
Dactrine	Miconazole	6.0	*	5.0	*	4.0	*
Nizral	Ketoconazole	6.0	*	5.0	*	4.0	*
Batrafine	Ciclopirox alamine	3.0	1.0	1.0	0.5	0.7	0.5

MIC = minimum inhibitory concentration, MCC = minimum fungicidal concentration, * = remained static

On comparison the MIC as well as MCC of the oil with those of synthetic antifungal drugs, the MIC of the oil was found to be many times more active than that of miconazole, ketoconazole and ciclopirox alamine against *M. cannis*, *T. mentagrophytes* and *T. rubrum*, with that of miconazole and ketaconazole respectively. However, these synthetic antifungals were remained fungistatic action except ciclopirox alamine (Table 5).

RESULTS AND DISCUSSION

The promising in vitro results obtained with the essential oil of *Artemisia maritima* against superficial dermatophytic fungi. The findings revealed that a 1% concentration of the oil produced no irritation or adverse reactions on human skin during a three-week observation period. The MIC of the oil of *A. maritima* were found to be 0.05 µl l ml^{-1} for *E. floccosum* 0.05 µl l ml^{-1} *M. canis,* 0.04 µl l ml^{-1} *M. gypseum,* 0.04 µl l ml^{-1} *T. interdigetale,* 0.04 µl l ml^{-1} *T. mentagrophytes,* 0.03 µl l ml^{-1} *Trichophyton rubrum.* The minimum fungicidal concentration (MCC) were found *E. floccosum* 0.06 µl l ml^{-1} *M. canis,* 0.05 µl l ml^{-1} *M. gypseum,* 0.05 µl l ml^{-1} *T. interdigetale,* 0.05 µl l ml^{-1} *T. mentagrophytes,* 0.04 µl l ml^{-1} *Trichophyton rubrum.* (Table 3). The oil inhibited heavy doses (30 mycelial discs, each of 5 mm in diameter) of fungal inoculum at 0.6 µl l ml^{-1} concentration. The activity of the oil did not expire even up to 48 month of storage and persisted up to 80^{0}C temperature. The pure oil (100 %) killed the fungi*, M. canis, T. mentagrophytes and T. rubrum* in just 5 sec while at its minimum fungicidal concentrations it required 80, 60 and 40 minutes respectively. The oil inhibited heavy doses (30 mycelial discs, each of 5 mm in diameter) of fungal inoculum at 0.6 µl l ml^{-1} concentration. The activity of the oil did not expire even up to 48 month of storage and persisted up to 80^{0}C temperature. The pure oil (100 %) killed the fungi*, M. canis, T. mentagrophytes and T. rubrum* in just 5 sec while at its minimum fungicidal concentrations it required 80, 60 and 40 minutes respectively (Table 4).

On comparison the MIC as well as MCC of the oil with those of synthetic antifungal drugs, the MIC of the oil was found to be many times more active than that of miconazole, ketoconazole and ciclopirox alamine against *M. cannis*, *T. mentagrophytes* and *T. rubrum*, with that of miconazole and ketaconazole respectively. However, these synthetic antifungals were remained fungistatic action except ciclopirox alamine Considering its potent antifungal activity, effectiveness against high fungal inoculum levels, extended shelf life, thermal stability, and absence of adverse skin effects, the essential oil of *Artemisia maritima* shows considerable promise as a broad-spectrum antifungal agent for the treatment of superficial dermatophytic fungi. Nevertheless, its commercial application should be supported by successful multicentre clinical trials to confirm its safety, efficacy and therapeutic value on a larger scale.

REFERENCES

Beye F., 1978. Insecticides from the vegetable kingdom. Plant Res. Dev., **7**: 13-31.

Clevenger J.F., 1928. Apparatus for the determination of volatile oil. J. Am. Pharm, Assoc., **17**: 346.

Dikshit A., Srivastava O.P. and Husain A., 1985. Effect of some essential oils on *Trichophyton mentagrophytes* in vitro and experimental ringworm. J. Antibac. and Antifun. Agent, **13**: 57-61.

Fawcett C.H. and Spencer D.M., 1970. Plant chemotherapy with natural products. Annu. Rev. Phytopathol., **8**: 403-418.

Garber R.H. and Houston B. R., 1959. An inhibitor of *Verticillium albo-atrum* in cotton seed. Phytopathology, **49**: 449-450.

Grover R.K. and Moore J.D., 1962. Toxicometric studies of fungicides against brown rot organism *Sclerotinia fructicola* and *S. laxa.* Phytopathology, **52**: 876-880.

Langenau E.E., 1948. The examination and analysis of essential oils, synthetic and isolates. In Guenther E (ed). The essential oil. Robert E Krieger Publishing Co. Hantington, New York.

Marco J.A. and Barbera O., 1990. In studies in Natural Product Chemistry, Atta-ur-Ralman (Ed.) Elesevier Science, Amsterdom, **7**: 201-264.

Pandey M.C., Sharma J. R. and Dikshit A., 1996. Antifungal evaluation of the essential oil of *Cymbopogon pendulus* (Nees ex Steud.) Wats var. Parman. Flavour and Fragrance Journal, **11**: 257-260.

Shahi S.K., 1997. Antimycotic studies of some plants (control of dermatophytoses in human beings). *D.Phil Thesis.* Allahabad University, Allahabad.

Shahi S.K, Shukla A.C., Bajaj A.K., Midgely G. and Dikshit A., 1999. Broad spectrum antimycotic drug for the control of fungal infection in human beings. Current Science, **74**: 836-839.

Shahi S.K, Shukla A.C., Bajaj A.K., Benerjee U., Remik D., Midgely G. and Dikshit A., 2000. Broad spectrum herbal therapy against superficial fungal infections. Skin Pharmacology and Skin physiology, **13**: 60-63.

Weyerstahl P., Marschall-Weyerstahl H., Schroder M. and Kaul V.K., 1988. Terpenes and terpene derivatives, XXIV. Isolation and stereochemistry of the four artedouglasia oxides. Liebbigs Ann. Chem., **1988(9)**: 917.

Wellman R.H., 1967. Commercial development of fungicides. in Plant apthology Problem and rogress. Haltoen et al (eds). Indian University Press, Allahabad. 1908-1958.