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Original Article

SCREENING OF *THYMUS VULGARIS* ESSENTIAL OIL AGAINST FUNGI CAUSING DERMATOPHYTOSIS IN HUMAN BEINGS

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ABSTRACT

Objective: The study was designed to determined antidermatophytic activity of *Thymus vulgaris* essential oil against fungi causing superficial skin infections in human and animal.

Methods: Minimum inhibitory concentration (MIC) of essential oil was screened against selected pathogenic fungi namely *Trichophyton rubrum* (MTCC 296), *T. mentagrophytes* (MTCC 7687), *Microsporum gypseum* (MTCC 4524), *M. fulvum*(MTCC2837), *T. soudanense* and *T. interdigitale* through semi solid agar antifungal susceptibility testing method(SAAS). Minimum fungicidal concentration (MFC) was also determined by modified semi solid agar antifungal susceptibility method. *T. soudanense* and *T. interdigitalis* were isolated through TO. KA. VA hair bating technique from animal habitats soil of Jaipur district.

Results: *T. vulgaris* essential oil exhibited excellent antideramtophytic activity against all selected dermatophytes. Minimum inhibitory concentration was ranged from 0.020±0.000µl/ml to 0.1±0.033 µl/ml. MFC were found little higher than MIC (0.02±0.000µl/ml to.2±0.000µl/ml). *M. gypseum* was found to be most susceptible fungus as compared to other test fungi.

Conclusion: *T. vulgaris* was found to be most effective fungicidal agent against human pathogenic fungi. Present findings provide for a rationale basis of a possible utilization of this oil in fields requiring safe and cheap compounds with antiseptic and preservative properties, such as cosmetic, pharmaceutical and food industries.

Keywords: *Thymus vulgaris*, Essential oil, Dermatophytes, *Trichophyton*, *Microsporum*

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INTRODUCTION

Infection caused by fungi in man and animal are more common in tropical and subtropical countries due to prevailing moisture, over population, poor hygienic living conditions and temperature regimes [1-5]. Skin infection due to dermatophytes has become a severe health problem affecting children, adolescent and adults. They produce keratinase which degrade the keratin and thus, invade the superficial skin tissue, nails and hair. These infections are generally cutaneous and restricted to the non-living, cornified layers of the skin. However, in constantly reoccurring conditions, the dermatophytes invade deeper tissues and simultaneously caused infections with other organisms. In general, the dermatophytes lack the ability to invade deeper tissues or organs of the host [6]. The dermatophytic infections are generally referred as ringworm infections due to their ring like appearance. Recently, there has been an increase in the incidence of fungal infections in developing countries. This may be the result of frequent use of antibiotics, environmental factors, resistant strains and various conditions, like organ transplantation, lymphomas, leukemia and human immunodeficiency virus [7]. However, clinical values of these agents have been limited due to having high toxicity and emergence of drug resistance in their antifungal activities [8]. These factors emphasize the urgent need for the development of new effective treatment alternatives.

The reduced adverse effects, decreased drug interactions, improved cure rate and cost effective medicine preparation from available topical agents are present day needs. In recent year there has been a gradual revival of interest in the use of medicinal plants and their products for the treatment dermatophytoses, and other fungal infections where a topical therapy is required. Spices and herbs are a major part of the daily food in India comprise the most important products used for flavoring foods and play a major role as the topical or systemic treatment of a wide range of diseases including infectious diseases. Beside their importance for general well-being, they are frequently used as traditional medicine [9]. In recent years, a lots of studies has been conducted on the antifungal effects of plant-derived essential oils on fungi causing superficial infections [10-14]. These finding promoted us to explore more spice essential oil for the treatment of dermatophytic infections.

Thymus vulgaris L. belonging to family Lamiaceae is a pleasant smelling perennial shrub, cultivating all over the world. Thyme is widely used for seasoning fish, poultry farms, vegetables, soups, for flavoring liqueurs, herbal tea preparations. *T. vulgaris* essential oil and extract has also been used in the treatment of gum diseases, rheumatism, sore throat, tonsillitis, and arthritis [15-16]. The essential oil of *T. vulgaris* is a known antiseptic, antiviral, antimicrobial agents [17-20]. Lots of work on antifungal activity of *T. vulgaris* essential oil against plant pathogenic, food born fungi, saprophytic fungi and *Candida albicans* has been reported [21-24] but very few reports are available on the management of human pathogenic fungi using *Thymus* oil despite its proven biological actions.

In present investigation a most important spice of European countries, *T. vulgaris* L. was explore against dermatophytic fungi. The high antidermatophytic activity suggests that thymus essential oil is a potential new antifungal agent for a natural product.

MATERIALS AND METHODS

Plant essential oil

Essential oil of *Thymus vulgaris* (red thyme) was procured from sigma Aldrich (São Paulo, SP, Brazil) company. Oil was clear light yellow colour with pleasant odor. Oil was stored in refrigerator.

Chemical and reagents

Sabouraud dextrose agar and Brain heart infusion agar media were purchased from Sigma Aldrich chemical company.

Microorganism for *in vitro* studies

T. vulgaris essential oil was evaluated for their antifungal properties against selected dermatophytes and keratinophilic fungi. For

antidermatophytic studies four dermatophytes were procured from the Imtech Chandigarh. These are *T. mentagrophytes* (MTCC 7687), *M. gypseum* (MTCC 4524) and *Microsporum fulvum* (MTCC2837), *T. rubrum* (MTCC 296). *Trichophyton soudanense* and *T. interdigitale* were isolated from soil sample collected from animal habitats of Jaipur district. These fungi were isolated through TO. KA. VA hair baiting technique of Vanbreuseghem [25]. Identification was based on macroscopic and microscopic examination of the culture isolates. The macroscopic examination of fungi was characterized by duration of growth, surface morphology and pigment production on the reverse. Strains were identified by their morphological and physiological characteristics according to the procedure described by Conant *et al.*,[26] and Forbes *et al.*, [27]. These selected fungi are maintained on Sabouraud's dextrose agar media and Potato dextrose agar media. Cultures were stored at 4 °C and sub cultured once in the month.

Determination of MIC and MFC by semisolid agar antifungal susceptibility method (SAAS)

Semisolid agar antifungal susceptibility testing method of Provine and Hadley [28] was applied for end point determination. For this method Brain heart agar medium was used.

Inoculum preparation

Sterile swab dipped into sterile tween 80 was used to pick the pure colony of yeast. This was then suspended in 3-4 ml of sterile normal saline and vortexed. The turbidity of the homogenous suspension was adjusted to ~0.5 McFarland standard. Similarly inoculum was prepared for filamentous fungi (3-7 d old slant at 37 °C on potato dextrose agar). By swabbing the pure colony (mixture of conidia and hyphal fragments) was suspended in 3-7 ml of sterile saline. The mixture was vortexed and heavy particles were allowed to settle. The homogenous suspension was adjusted to 0.5 McFarland standard.

Inoculation of drug containing tubes

The semisolid agar tubes containing known concentrations of test oils as well as oil-free controls, prepared in duplicate, were inoculated with one loopful (Himedia Flexiloop 4) of 0.5 McFarland adjusted culture by inserting the loop deep within the semisolid agar. The tubes were incubated at 37 °C for 72 h for dermatophytes. A loopful of the inoculum suspension was streaked onto Sabouraud dextrose agar to check for purity and viability.

End point determination

Endpoint determination was done according to the NCCLS/CLSI guidelines, M27-A and M38-A. Growth was compared to that of oil-free control and scored by visual inspection as follows: +4: growth same as control; +3: slight decrease in growth; +2: significant reduction in growth reduction 80% in yeast and 50% in filamentous);+1 slight growth or few visible hyphal fragments; 0: no growth.

MFC was recorded through slightly modification of SAAS method. After 72 hour observation of drug containing tubes for MIC, these tubes were further restored as same temperature conditions for 3-4 more days. Observation were recorded for MFC.

Statistical analysis

Each parameter was tested in triplicate. Conventional statistical methods were used to calculated means and standard deviations. Statistical analysis (T-test) was applied to the data to determine differences ($p<0.05$).

RESULTS

T. vulgaris an important spice is widely used in various ailments. Lots of work has been done on the chemical composition of *Thyme* oil [20,22,29-30]. Thymol is major constituents of oil ranging from 42.6-57.8% according to collection time and cultivation place. Other components are α-cymene, carvacrol, α-thujene, α-pinene, β myrcene, trans-ocimene,γ terpinene, limonene.

Preliminary screening of the *T. vulgaris* essential oil for antidermatophytic activity was carried out against six selected dermatophytic fungi using semi solid agar antifungal susceptibility method. Data incorporated in table 1 show MIC and MFC of *Thyme* essential oil.

Thyme oil exhibited excellent antifungal potential against all selected fungi. *Microsproum gypseum* was found most susceptible fungus (0.020±0.000µl/ml)followed by *T. mentagrophytes* (0.05±0.000 µl/ml), *T. interdigitale* (0.05±0.000 µl/ml) and *T. soudanense* (0.053±0.003µl/ml). *M. fulvum* (0.1±0.033 µl/ml) was less susceptible fungus as compared to other test fungi. MFC was same to MIC in case of *M. gypseum* (0.020±0.000 µl/ml) and *T. mentagrophytes* (0.05±0.003 µl/ml). MFC against *M. fulvum* was found to be 0.2±0.000 µl/ml.

Table 1: Antifungal activity of *Thymus vulgaris* essential oil against dermatophytic fungi

Activity fungi	MIC (µl/ml)	MFC (µl/ml)
Trichophyton mentagrophytes MTCC 7687	0.05±0.000	0.05±0.003
Microsporum fulvum MTCC2837	0.10±0.033	0.2±0.000
Trichophyton rubrum MTCC 296	0.026±0.002	0.05±0.003
Microsporum gypseum MTCC 4524	0.020±0.000	0.020±0.00
Trichophyton interdigitale	0.05±0.000	0.10±0.033
Trichophyton soudanense	0.053±0.003	0.133±0.033

MIC=minimum inhibitory concentration, MFC=minimum fungicidal concentration, Statistical analysis was carried out through T-test($p<0.05$).

DISCUSSION

T. vulgaris L. (Lamiaceae) is a well-known medicinal shrub that contains important phenolic compounds such as thymol and carvacrol and it has been used in many pharmaceutical applications. Data incorporated in present studies revealed strong antidermatophytic activity of *T. vulgaris* essential oil against all selected filamentous fungi namely *T. mentagrophytes, T. rubrum, T. interdigitale, T. soudanense, M. fulvum* and *M. gypseum.* MIC was ranged between 0.020±0.000 to 0.1±0.033µl/ml. These promising antifungal activity against all selected pathogenic fungi may be due to presence of high concentration of phenolic compounds. Similar results were also contributed by other researchers. Shigeharu Inouye *et al.* [31] studied the vapour activity of 72 essential oil on *T. mentagrophytes*. Oils containing phenol as the major constituent showed the most prominent vapor activity, with an MFD of 1.56 µg/ml air. They suggested that essential oils such thyme thymol, wild thyme exhibited potent vapor activity against *T. mentagrophytes* may be useful as room disinfectants when patients with tinea pedis may drop the living pathogens on the floor.

Soković *et al.*[29] studied the chemical composition of *T. vulgaris* essential oil and their antifungal activity against 17 micromycetal food poisoning, plant, animal and human pathogens. MIC and MLC were tested against five dermatophytic fungi namely *Microsporum canis, Epidermophyton floccosum, Trichophyton rubrum, T. mentagrophytes* and *T. tonsurans*. The essential oil of *T. vulgaris* showed very strong antifungal activity at low concentrations, 0.05-1.0 µl/ml. Pina-Vaz *et al.* [32] screened *Thymus vulgaris*, *T. zygis* sub species *zygis* and *T. mastichina* sub species *mastichina* essential oils and their major components (carvacrol, thymol, *p*-cymene and 1,8-cineole) against different strain and species of *Candida.* The essential

oils of *T. vulgaris* and *T. zygis* showed similar antifungal activity, which was greater than *T. mastichina*. MIC and MLC values were similar for all the compounds tested.

During present investigation, *M. gypseum* was found to be most susceptible fungus, where MIC and MFC were found similar. MIC of *T. vulgaris* against *T. rubrum* was also 0.026±0.002 µl/ml but MFC was slightly increased (0.05±0.003 µl/ml). MFC of essential oil was slightly increased as compared to MIC against all filamentous fungi. MFC were ranged from 0.020±0.00 µl/ml to 0.2±0.000 µl/ml. Adams *et al.*[33] described that the high antifungal activity of *Thyme* essential oil are due to presence of the high percentage of phenol components. It believed that phenol components may interfere with cell wall enzymes like chitin synthase/chitinase as well as with the α-and β-glucanases of the fungus. Consequently, the high content of phenol components may account for the high antifungal activity of oils [34]. Rota *et al.* [20] explained that presence of phenolic compounds thymol and carvacrol are responsible factors of excellent antifungal activities of *T. vulgaris* oil. The antifungal activity of the oil is mostly incorporated with the interaction of phenolic compounds, thymol, carvacrol, and p-cymene with cytoplasmic membrane ergostrol [22, 32]. It is believed that the hydroxyl group on these compounds interact on the cytoplasmic membrane and changes its permeability by affecting lipid ordering and stability of its bilayer. Therefore proton passive flux across the membrane increased, which disrupted the cytoplasmic membrane and leading to leakage of cellular contents [35-38]. Antifungal activity of the essential oil of *T. vulgaris* L. and Thymol on experimentally induced dermatomycoses was carried out by Sokovi *et al.* [29] and found excellent results.

CONCLUSION

This study is a preliminary evaluation of antimicrobial activity of the *Thymus* plant essential oil.

Present study concluded that the essential oil of *T. vulgaris* is highly active as fungitoxicants and could safely be used as natural preservatives to replace synthetic allopathic preparations in the prevention and cure of superficial skin infection. The plant presented broad spectra of activity, may help to discover new antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases. This versatile medicinal plant is the unique source of various types of chemical compounds, which are responsible for the various activities of the plant. Hence the extensive investigation is needed to exploit their therapeutic utility to combat diseases.

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AUTHOR'S CONTRIBUTION

Neetu Jain researched and wrote the article while Meenakshi Sharma provided guidance, critical review, and revision. All authors read and approved the final version of present manuscript.

CONFLICT OF INTERESTS

No conflict of interest

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