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In vitro anti-dermatophytic activity of essential oil extracted from *Artemisia japonica* Thunb

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Abstract
Dermatophytes are a group of filamentous fungi that can cause superficial mycoses in humans and other mammals. This is due to the ability of these dermatophytes to invade keratinized tissues including skin, hair, and nails. Riseofulvin (Grifulvin V, Gris-PEG), Terbinafine, Itraconazole (Onmel, Sporanox) and Fluconazole (Diflucan) are the major antifungal drugs which has been used for the treatment of dermatophytosis. To reduce the side effects and cost, natural compounds are mostly preferred. Many medicinal plants have been known for their occurrence anti-dermatophytic activity. However, the compositions of natural compounds are high in oils extracted from leaves. *Artemisia japonica* Thunb of *Asteraceae* family is widely distributed in India and the plant has been used as traditional medicine to treat various diseases. The present study aims to evaluate the *in-vitro* anti-dermatophytic activity of essential oil extracted from *Artemisia japonica* leaves. The essential oil (100 µL) has showed great potential to act against selected dermatophytes, *Trichophyton mentagrophytes* and *Microsporum canis.* The minimum inhibitory concentration (MIC) was found to be 1.534±0.201 mg/mL and 0.578±0.0311 mg/mL for *T. mentagrophytes* and *M. canis* respectively. The results of the present study revealed that the essential oil extracted from *Artemisia japonica* leaves may have potential use in the treatment of dermatophytosis.

Keywords: *Artemisia japonica,* essential oil, dermatophytes

Introduction
Dermatophytes are a group of pathogenic fungi that infect keratinous tissue through invading the hair, skin, and nails of a living host. This closely related group of organisms can be mainly categorized into three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton* [1]. The dermatophytic infections are epizootic in nature and can results in economic loss in the livestock industry. Hence, the diagnosis, control and the treatment is very important to reduce the spread of fungal infections in pets. Eventhough the antifungal drugs like Riseofulvin (Grifulvin V, Gris-PEG), Terbinafine, Itraconazole (Onmel, Sporanox) and Fluconazole (Diflucan) have been used for the treatment of dermatophyic infections, the negative impact of these chemical agents to human and the environment demands the need for natural biocontrol measures. Here comes the advantage of medicinal plants with diverse metabolites and its broad spectrum bioactivity. Interestingly, these bioactive metabolites are rich in essential oils. Essential oils (EO) are concentrated natural extracts derived from plants containing a mixture of diverse constituents and are widely used in traditional medicine from ancient times[2]. Due to its higher biological activities and therapeutic promises, the EO has also been widely recommended as an active ingredient in pharmaceutical, antiseptic, household, and cosmetic products [3]. In addition, the application of this naturally derived product for the treatment of pathological conditions has also encouraged the exploration novel products through a sustainable manner.

In this context in the present study, the plant *Artemisia japonica* Thunb of *Asteraceae* family has been used as the source for EO extraction. It is a nonaromatic perennial herb used as traditional medicine and are widely distributed in all over the India. Eventhough, there are studies on the composition of essential oil from *Artemisia japonica* and the antibacterial efficacy of the genus *Artemisia* has been reported, the anti-dermatophytic activity of the essential oil extracted from *Artemisia japonica* leaves is not studied yet. Hence, the anti-dermatophytic activity of essential oil from *Artemisia japonica* against *Trichophyton mentagrophytes* and *Microsporum canis* as observed in the current study is highly significant.

2. Materials and Methods

2.1 Plant selection and extraction of essential oil

The plant *Artemisia japonica* (KVASU/CM/003) collected from herbal garden attached to the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of veterinary and Animal Sciences, Mannuthy was used for the study. The fresh leaves were washed and shade dried and the essential oil was extracted using hydro distillation method. For this, the distillation flask of 500 mL contain water about 2/3 of its volume and 10 g of the dried leaves. The operation proceeded by heating the flask at 100 ^{0}C. The essential oil collected was then extracted using a separating funnel with chloroform.

2.2 *In vitro* anti-dermatophytic activity of essential oil

The dermatophytic fungi, *Trichophyton mentagrophytes* and *Microsporum canis* were purchased from MTCC, Institute of Microbial Technology (IMTECH), Chandigarh. Cultures were maintained in Sabouraud dextrose agar (SDA) (Himedia, Cat. No. M063). The antidermatophytic activity was screened by well diffusion method [4]. For this, *T. mentagrophytes* and *M. canis* were inoculated into sabouraud's dextrose broth and incubated at 37^0 C for 4 days. After incubation, the lawn culture of the suspensions was done on sabouraud's dextrose agar plates. Wells were punched on the agar plate using a sterile borer. The wells were filled with 100 µL of the essential oil at a concentration of 5mg/mL and 100 µL of dimethyl sulphoxide as negative control. The antifungal agent fluconazol disc (10mg) was also run simultaneously as a positive control. All the plates were incubated at 37 ^{0}C for 48 h and the zones of inhibition diameter was measured.

2.3 Determination of minimal inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) of the essential oil against the selected test organisms was also determined by the broth dilution method [5]. The inoculam was prepared by counting the microconidia using light microscopy. For this, 7 days old cultures grown on SDA agar plates was flooded with sterile normal saline (0.85%) and was gently swabbed with a cotton tip applicator to dislodge the conidia from the hyphal mat. The suspension was transferred to a sterile centrifuge tube and the volume was adjusted to 5 mL with sterile normal saline. The resulting suspension was counted on a haemocytometer and was diluted in RPMI 1640 (Himedia) with L-glutamine but without sodium bicarbonate and buffered at pH 7.0 with monosodium salt (MOPS) to the desired concentration.

Microdilution plates were set up in accordance with the NCCLS M27-A reference method with modification in the inoculum preparation. Here, the first well of the microtitre plate was filled with 200 µL of medium to serve as negative control. Then, 100 µL of inoculum was added to 2nd to 11th wells of the microtitre plate. Then, 100 µL of the essential oil (5 mg/mL) was added to second well and then serially diluted until the 11th well. Whereas, 12th well was filled with suspension (200 µL) without any antifungal agents and served as positive control. The plates were then incubated at 35 °C of the visible growth and inhibition was observed after 4 days of incubation. The assays were performed in duplicate to confirm the value of MIC of essential oil for tested pathogens. The MIC was defined as the point at which lowest concentration of an extract that inhibits the visible growth of organism and which was also confirmed spectrophotometrically at 600 nm.

3. Results

3.1. Anti-dermatophytic activity of essential oil against the tested pathogens

The antifungal susceptibility test of essential oil against the tested pathogens has showed good inhibitory activity against both the fungi *T. mentagrophytes* and *M. canis* with a zone of inhibition diameter of 5mm and 22mm respectively. However, the inhibitory activity was also compared with the antifungal agent Fluconazol run as the positive control and it showed an inhibition diameter of 20 mm *T. mentagrophytes* and 17 mm to *M. canis* (Figure 1). The greater inhibition of essential oil against *M. canis* than the antifungal agent further confirmed the efficacy of essential for the treatment dermatophytosis.

3.2. Minimal inhibitory concentration of essential oil

The MIC is the lowest concentration of the essential oil at which no visible growth of the organisms was observed. In the current study, the results showed that the essential oil was active antifungal agent against tested dermatophytes. The MIC values were found to be 0.578±0.0311 mg/mL for *M.canis* and 1.534±0.201 mg/mL for *T.mentagrophytes* (Figure 2).

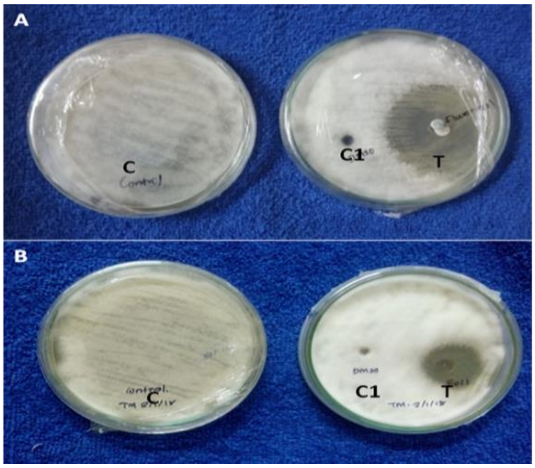

Fig 1: Antifungal activity of essential oil extracted from *Artemisia japonica* against *T.mentagrophytes* **(A)** zone of inhibition for Fluconazol; **(B)** zone of inhibition for essential oil along with respective control (c) and solvent control (C1).

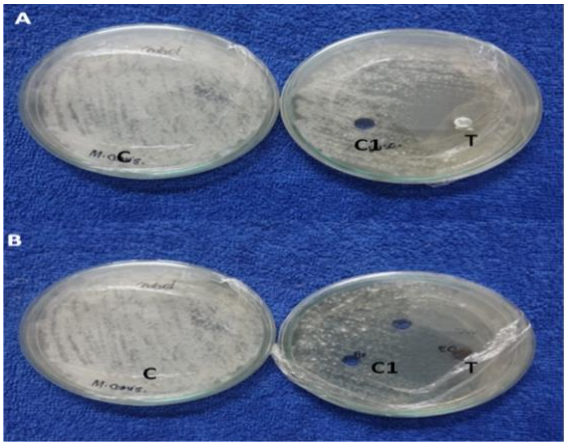

Fig 2: Antifungal activity of essential oil extracted from *Artemisia japonica* against *M. canis* **(A)** zone of inhibition for Fluconazol; **(B)** zone of inhibition for essential oil along with respective control (c) and solvent control (C1).

4. Discussion

Dermatophytosis commonly known as ringworm is a zoonotic disease, caused by fungi belongs to the genera *Micropsorum*, *Trichophyton*, and *Epidermophyton* [6]. The specific lesions by dermatophyte infections are localized in the keratinized surfaces like face, legs, and tail. The infected animals also demonstrate skin lesions with localized alopecia, erythema, and crust. As the humans are in contact with pet animals like cat or dog, they are easily susceptible to dermatophytosis. Dermatophyte infections in humans are majorly occurs through their contact with contaminated products or surfaces like soil, hair, or crust on the epidermal layer of infected animals [7]. The main causes of dermatophytosis in animals (especially in dogs and cats) are *Microsporum* spp. and *Trichophyton* spp. Hence the treatment fungi have played an important role in veterinary medicine. To reduce the side effects and cost of production, natural compounds are mostly preferred. The increased antimicrobial resistance (AMR) among the pathogenic microorganisms has also demand the need for alternative medicine with better efficacy and sustainability.

The current study thus aimed to evaluate the anti-dermatophytic activity of essential oil extracted from *Artemisia japonica* leaves. *Artemisia japonica* has previously been reported for their essential oil composition [8]. Cha *et al*., [9] has also described the chemical composition and antimicrobial activity of the essential oil of *Artemisia lavandulaefolia*. The antifungal susceptibility test of essential oil against *T. mentagrophytes* and *M. canis* revealed good inhibitory activity. Upon the comparison with the commercial antifungal agent Fluconazol, the oil showed higher inhibitory activity against *M. canis* than *T. mentagrophytes.* This was also supported by the previous reports on the antimicrobial and antioxidant activities of *Artemisia annua* and *Artemisia vulgaris* (L.) essential oil [10, 11]. The minimal inhibitory concentration of the oil was also determined and found to be 0.578±0.0311 mg/mL for *M. canis* and 1.534±0.201 mg/mL for *T. mentagrophytes*. The determination of minimal inhibitory concentration by broth dilution method is aimed to determine the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the pathogenic microorganisms [12].

Conclusions

As per the authors knowledge this is the first report on anti-dermatophytic activity of essential oil of *Artemisia japonica* against *T. mentagrophytes* and *M. canis*. Thus, the results obtained in the study are highly unique and can have significant application as antifungal agent for the treatment of dermatophytosis.

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