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Inhibitory effect of *Tridax procumbens* against human skin pathogens

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ABSTRACT

Tridax procumbens is a common herb with significant medicinal properties traditionally used in the treatment of many skin diseases. The methanol extract of *T. procumbens* exhibited high antifungal activity against clinically important human skin pathogens such as *Microsporum fulvum*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichosporon beigelii* and *Candida albicans* with low MIC values. The fractionation of a methanol extract with dichloromethane yielded an oily viscous fluid with antifungal activity which was separated and characterized. The GC–MS analysis revealed the presence of 26 compounds. The major constituents were characterized as 9,12-octadecadienoic acid ethyl ester (18.04%), 5α-cholestane (12.42%), hexadecanoic acid ethyl ester (4.86%) and 9-octadecenoic acid ethyl ester (4.72%). This study demonstrated the efficacy of this herb against clinically important dermatophytes and also justified its traditional use.

1. Introduction

Plants are a good source of novel bioactive molecules with therapeutic potential. There is a plethora of pharmaceutically important molecules, but only a small percentage of plants have been explored for their phytochemical constituents (Hostettmann et al., 1998; Balandrin et al., 1985). *Tridax procumbens* is a common weed native to tropical America and distributed in tropical Africa, Australia and Asia. It is extensively used in the Indian *Ayurvedic* system of medicine for the treatment of diarrhoea, as an insect repellent, hair tonic and wound healer, i.e. the leaf juice is used to check haemorrhage from cuts and bruises (Srivastava et al., 1984; Udupa et al., 1991; Saraf et al., 1992; Bhat et al., 2007). It is a well known remedy for liver disorders and has been shown to have antidiabetic activity (Vilwanathan et al., 2005; Bhagwat et al., 2008). *T. procumbens* has demonstrated significant anti-inflammatory

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and antimicrobial activity (Nia et al., 2003; Mahato and Chaudhary, 2005). Interestingly some animal studies have shown that it may have potent immune-modulating property (Tiwari et al., 2004; Oladunmoye, 2006). *T. procumbens* contains alkaloids, carotenoids, saponins, flavonoids, flavones, glycosides and tannins from the leaves of this plant (Raju and Davidson, 1994; Yadawa and Saurabh, 1998; Ali et al., 2001; Jude et al., 2009). It also contains lipid constituents (Verma and Gupta, 1988) plus saturated and unsaturated fatty acids (Gadre and Gabhe, 1988). The presence of β-sitosterol-3-O-β-D-xylopyranoside in the flowers of *T. procumbens* was reported by Saxena and Albert (2005).

In tropical and subtropical countries, the infectious diseases that affect the skin and mucosal membranes are a severe problem. A number of these infections are most frequently caused by dermatophytes and yeasts (Hay, 2006). Due to an increase in the number of immune-suppressed patients in the last decade, there are more reports of systemic and superficial mycoses such as aspergillosis, candidiasis, and fungal infections (Gabardi et al., 2007; Nucci and Marr, 2005; Pfaller et al., 2006). Several reports have shown that the therapeutic potential of plant extracts against many diseases like skin and respiratory infection is due to their high antimicrobial activity against bacteria, yeasts and dermatophytes (Janssen et al., 1987; Rios et al., 1988; Griffin et al., 1999). The increasing recognition and importance of fungal infections in regard to resistance to antifungal drugs have stimulated the search for safe, natural therapeutic alternatives (Pina-Vaz et al., 2004). The use of indigenous folk medicines for the treatment of fungal infections may offer new effective remedies (Seneviratne et al., 2007; Li et al., 2008; Webster et al., 2008). The present study explored the bioactive constituents of a methanol extract of *T. procumbens* and its antifungal activity against dermatophytes.

2. Materials and methods

2.1. Plant material and extraction

T. procumbens was collected from Tezpur, Assam, India during September 2011. The plant was identified by Dr Jayshree Das, Pharmaceutical Technology Division, Defence Research Laboratory, Tezpur and a voucher specimen stored in their herbarium. The flowers were separated and only the aerial parts were dried in an oven at 50 °C for 72 h and powdered in a grinder. The powder (100 g) was extracted with 1000 ml of methanol for 48 h. The extract was concentrated to dryness using a vacuum rotary evaporator (Buchi Rotavapor) at 50 °C to remove all traces of methanol. The dried extract was then stored at 4 °C.

2.2. Fractionation of extract

The methanol extract was fractionated with dichloromethane. The dichloromethane soluble fraction was separated and yielded an oily, viscous fluid. This oily fraction was subjected to GC–MS analysis.

2.3. GC–MS analysis

The GC–MS analyses were performed in EI mode on a GCMS, Perkin Elmer, Turbomass gold, GC-Autosample xL (Perkin Elmer International, Boesch, Huenenberg, Switzerland) system with Elite-1 fused capillary column (composed of 100% dimethylpolysiloxane), 30 m × 0.25 mm × 0.25 μm, directly coupled to mass detector. The mass spectrometer was operated at 70 eV. Injection conditions were as follows: Column temperature 40–250 °C at a rate of 5 °C/1 min; carrier gas was He: 1 ml/min; sample injection volume 1 μl. The constituents of the essential oils were identified based on a comparison of mass spectra with those of data in the National Institute of Standards and Technology (NIST) libraries.

2.4. Antifungal activity

2.4.1. Microbial strains and culture conditions

The fungal strains used in this study included *Microsporum fulvum* (MTCC 8478), *Microsporum gypseum* (MTCC 8469), *Trichophyton mentagrophytes* (MTCC 8476), *Trichophyton rubrum* (MTCC 8477) and *Candida albicans* (MTCC 854) obtained from the School of Tropical Medicine, Kolkata. *Trichosporon beigelii* was isolated from a clinical sample by standard NCCLS (2002) method. All fungal strains were maintained on Sabouraud dextrose agar (SDA) medium (Himedia, Mumbai) at 28–30 °C for 10 days.

2.4.2. Preparation of spore suspension

The 10-day-old cultures were used for the preparation of inoculums. The spores were scraped with a sterile loop and macerated in sterile saline (0.85%) solution. The final spore suspension was adjusted to 10^5 CFU/ml.

2.4.3. Agar well diffusion method

An antifungal assay was carried out using the modified method of Kariba et al. (2001). The methanol extract (5 mg/ml) was reconstituted in dimethyl sulphoxide (DMSO) to assess the antifungal activity. The SDA media was inoculated with spore suspension (10^5 CFU/ml) of the test fungi. The test sample was placed in the 6 mm agar well. The plates were then incubated at 28 ± 1 °C. Griseofulvin was used as a standard and DMSO served as the control. The zone of inhibition around the well was determined as antifungal activity. Values are given as mean and SD of tests performed in triplicate.

2.4.4. Minimum inhibitory concentration (MIC)

The MIC was assessed according to the agar dilution method of Kariba et al. (2001) with modifications. The methanol extracts and fractions were dissolved in DMSO and concentrations ranging from 32 to 0.06 mg/ml were incorporated into SDA growth medium. The resulting SDA medium was inoculated with spore suspension (10^5 CFU/ml) of the test fungi. The plates were incubated at 28 ± 1 °C for 10 days. The minimal inhibitory concentration was recorded as the lowest concentration that produced no visible fungal growth. All the experiments were carried out in triplicate.