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In vitro Antimicrobial Activity of Essential Oil of *Pentanema indicum* var. *sivarajanianum* (L.) Ling. Against Dermatophytes*

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Abstract: The antimicrobial activity of essential oil of *Pentanema indicum* var. *sivarajanianum* (L.) Ling. was investigated. GC-MS analysis of the hydrodistilled oil resulted in the identification of 21 compounds constituting 99.4% of the total oil. The major constituents are hexadecane, α-humulene, myrecene and β-thujone comprising 51.6% of the oil. The oil strongly exhibited antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Micrococcus luteus*, *Micrococcus roseus*, *Candida albicans* and *Candida tropicalis*. The present study confirms that the essential oil possess significant antimicrobial properties *in vitro*.

Key words: *Pentanema indicum* var. *sivarajanianum*, essential oil, antimicrobial activity, GC-MS

Introduction

Many hundreds of plants worldwide are used in traditional medicines as treatment for bacterial infections. Particularly essential oils are known to possess antimicrobial activity (Hammer *et al*., 2002; Unlu *et al*., 2002; Alvaro *et al*., 2003; Tatjana *et al*., 2004) but the efficacy of such herbal medicines has seldom been rigorously tested in controlled clinical trails (Martin and Ernst, 2003). With the adverse reactions to the synthetic drugs and the emergence of resistant microorganisms are causing many problems for both the treatment of patients and infection controls. The resistant microorganisms are increasing and eradication with the current agents is not always successful (May *et al*., 2000). Efforts are being made to look for the products of natural origin, which have a great variety of bio-dynamic actions.

Pentanema indicum var. *sivarajanianum* (Asteraceae) is habituated in hill slopes and interior forest plains. It is known as 'Chiruchamanti' and the leaf extract has taken orally for several skin ailments by the aboriginal tribes of Eastern Ghats, which are common in tribes due to the lack of sanitation and hygienic food (Jeevan Ram *et al*., 2004). This is the first report on the biological activity and composition of the essential oil of *Pentanema indicum*. In the current study the essential oil derived from the aerial parts was screened against various pathological microorganisms *in vitro*. Our previous studies encouraged us to undertake the present investigations to assess the plant for any further beneficial effect of medicinal importance.

Materials and Methods

Extraction of the Essential Oil

The leaves of the plants were collected from the forests of Nallamalais, India during February 2002. The plant was identified with the help of regional flora (Pullaiah *et al*., 1997) and a voucher

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specimen (AJR-23816) has been deposited at the herbarium of Sri Krishnadevaraya University (SKU), Anantapur. The shade dried leaves were subjected to hydro-distillation for 3 h, using a Clevenger type apparatus. The oil was dried over anhydrous sodium sulphate and stored at 4°C until tested and analyzed.

GC-MS Analysis

The analysis was carried out on Shimadzu 17 A GC coupled with Shimadzu QP 5050A (Quadruple) Mass-Spectrometer equipped with EI and a fused silica column DB-5 (30 m x 0.25 mm i.d.) of 0.25 μm film thickness coated with polysilphenylene-siloxane. One microliter of the concentrated solvent fraction was injected and the GC oven temperature kept at 50°C for 5 min and programmed from 50°C-280°C for 40 min. Helium was used as carrier gas at a flow rate of 2 mL min^{-1} with a split ratio of 1:30 and ionization voltage of Mass spectral analysis was run by EI technique at 70 eV. The components were identified by comparing their relative retention indices with those of standard reference compounds and available literature data (Masada, 1989; Adams, 2001).

Antimicrobial Screening

The antimicrobial activity of the oil was assayed by using disc diffusion method (Bauer *et al*., 1966) and Minimum Inhibitory Concentrations (MICs) were determined by broth microdilution method (NCCLS, 1999). The assay was performed individually against *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 737), *Micrococcus luteus* (1541), *Micrococcus roseus* (MTCC 2522), *Candida albicans* (MTCC 183) and *Candida tropicalis* (MTCC 184). These were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. All bacteria were cultured overnight at 37°C in nutrient agar medium and yeasts were cultured overnight at 30°C in Sabouraud dextrose agar.

A suspension of the each microorganism (0.1 mL of 10^8 cells per mL) was seeded on to the respective media plates. Sterile Whatman No.1 filter paper discs (6 mm in diameter) were impregnated with 50 μL of the oil, are placed on the inoculated plates. These plates after staying at 40°C for 2 h, were incubated at 37°C for 24 h for the bacteria and 30°C for 48 h for the yeasts. The diameter of the inhibition zones around the discs were measured in millimeters. Standard antibiotics viz., ampilicin for bacteria and ketoconazole for yeasts were used as positive controls. Triplicates were carried out for each concentration.

The determination of Minimum Inhibitory Concentration was performed in nutrient broth for bacteria and Sabouraud dextrose broth for yeasts, supplemented with Tween-80 detergent. The hydrodistilled oil at starting concentration of 64 mg mL^{-1} was transferred into the first well and serial dilutions were performed ranging to 0.032 mg mL^{-1} were prepared in 96-well micro-titer plate, including one growth control (Nutrient broth + Tween-80) and one sterile control (Nutrient broth+ Tween-80+test oil). The plates were incubated at 37°C for 24 h for bacteria and 30°C for 48 h for the yeasts.

Results and Discussion

Dermatophytes (candidiosis, dermatomycosis, ringworm) are the most common forms of fungal infections found in most countries (Ribbon, 1988), cause diseases of the skin, nails and hair. The important reason being that a majority of the people of developing world still relies on indigenous

systems of medicine for the treatment of their common ailments (Ali and Azhar, 2000). Anecdotally, the leaf juice known as external remedy for bacterial and fungal infection in particular dermatophytes (Jeevan Ram *et al*., 2004).

Leaves of *Pentanema indicum* var. *sivarajanianum* on hydrodistillation yielded dark pungent yellowish green oil (1.2%v/w). GC analysis of freshly extracted distilled oil revealed the presence of 21 different components representing 99.4% of the total composition (Table 1). The oil consists non-terpenoidal alkanes (23.78%), sesquiterpene hydrocarbons (22.58%), alcohols (19.53%), monoterpene hydrocarbons (11.91%), oxygenated monoterpenes (7.86%) accomplished by relatively smaller amounts of esters (3.14%), monoterpene ketones (2.8%) and oxygenated sesquiterpenes (2.34%). The non-terpenoidal alkanes with hexadecane as the major constituent (21.85%) and accomplished by noticeable amount of tetradecane (1.93%). The sesquiterpene hydrocarbons represented by α-humulene (17.71%) as major component and small amounts of cubebene (4.87%). The alcohols with Pentanol (5.28%) and Hexanol (5.16%), monoterpene hydrocarbons with myrecene (6.58%), oxygenated monoterpenes with β-thujone (5.52%) comprises as major components.

The essential oil exhibited stronger antimicrobial activity against all the tested organisms. The growth of tested microorganisms ranged from 0.25 to 0.5 mg mL^{-1} (w/v) with the lowest MIC value against *Pseudomonas aeruginosa*, *Candida albicans* and *C. tropicalis* at 0.25 mg mL^{-1} (w/v). The Table 2 lists the zones (mm) and minimum inhibitory values (MIC) for the bacterial and fungal isolates studies. For *Staphylococcus aureus*, *Micrococcus roseus* and *M. luteus* the essential oil generally follows a concentration dependent antimicrobial activity.

The results show that the plant extract investigated demonstrate antimicrobial activity in their potential for helping to cure different skin diseases. The oil contains terpens, ketones, sesquiterpenes that have antimicrobial properties. Surprisingly the non-terpenoidal alkanes are present as major component which are very common in most of the Asteraceous members (Bohlmann, 1973). It is

Table 1: Chemical constituents of essential oil of *Pentanema indicum* var. *sivarajanianum*

RRI	Compound	Composition
685	2-Pentanone	1.86
743	3-Methylbutanol	1.16
777	1-Pentanol	5.28
795	2-Hexanone	0.94
833	Furfural	4.86
867	Hexanol	5.16
956	Camphene	1.93
993	Myrecene	6.58
1022	α-Terpinene	1.28
1046	β-Ocimene	2.12
1121	β-Thujone	5.52
1262	Linalyl acetate	1.16
1291	Anethole	2.34
1344	Cubebene	4.87
1384	Geranyl acetate	1.98
1400	Tetradecane	1.93
1461	α-Humulene	17.71
1562	Geranyl butyrate	5.46
1581	Caryophyllane oxide	2.34
1996	Hexadecane	21.85
2118	Phytol	3.07
	Total	99.4

RRI= Relative Retention Indices, calculated against n-alkanes

Table 2: Antimicrobial activity of essential oil of *Pentanema indicum* var. sivarajanianum

Microorganism	Essential oil		Antibiotic 30 μg disc^{-1} (mm)
	Disc diffusion (mm)	MIC (mg mL^{-1})	
Pseudomonas aeruginosa (MTCC 1688)	20	0.25	24
Staphylococcus aureus (MTCC 737)	19	0.5	24
Micrococcus luteus (MTCC 1541)	20	0.5	22
Micrococcus roseus (MTCC 2522)	18	0.5	22
Candida albicans (MTCC 183)	20	0.25	22
Candida tropicalis (MTCC 184)	18	0.25	22

evident that some minor compounds viz., camphene should also be taken into consideration for antimicrobial activity in combination. Camphene is known as decongestant, antiseptic (Bruneton, 1995) and is further known for its tropical use as a counter-irritant in fibrositis (Martindale, 1996). In light of the above activity that contribute to cure of different skin ailments it is important to use a battery of *in vitro* tests to evaluate the efficacy of this crude drug. The further studies aimed at the isolation and identification of novel antimicrobial substances with better therapeutic value.

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