Chemical Composition and Biological Activities of the Essential Oil of *Plectranthus caninus* Roth

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ABSTRACT

The essential oil from the aerial parts of *Plectranthus caninus* Roth (Lamiaceae), obtained by hydro-distillation was analyzed by gas chromatography/mass spectrometry (GC/MS) and evaluated for antimicrobial, free radical scavenging and anti-inflammatory activities. Thirty-four compounds representing 91.02% of the total oil were identified. The major constituents of the oil were camphor (22.36%) and α-thujene (14.48%). The oil was tested against 21 bacterial and 4 fungal strains using disc diffusion method and found to be active against a broad spectrum of pathogens including Gram-positive and Gram-negative bacteria as well as some fungal strains. The minimum inhibitory concentrations (MICs) of the oil against the bacterial strains tested ranged from 10 to 400 µg/ml, and from 800 to 1000 µg/mL against the fungal strains employed. The *in vitro* antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging assay. The oil reduced DPPH in a concentration dependent manner with an EC_{50} value of 3.5 µl/ml. The *in vivo* anti-inflammatory activity was evaluated on the basis of inhibition of carrageenan-induced mouse hind paw oedema whereby doses of 200 and 300 mg/kg were found to inhibit significantly increase in paw volume during the late phase of inflammation. The study provides evidence for the broad-spectrum antimicrobial, significant antioxidant and anti-inflammatory effect of *Plectranthus caninus* essential oil, a possible explanation for the traditional use of the plant in the treatment of cold, teeth and gum disorders which may be related to microbial infections and inflammation.

Key words: Anti-inflammatory activity, antimicrobial activity, camphor, free radical scavenging activity, hydro-distillation.

INTRODUCTION

In recent years, an upsurge of interest in the use of natural substances as phytomedicines has resulted in a more thorough investigation of plant resources. Aromatic plants and their essential oils, used since antiquity in folk medicine and for the preservation of food, are known sources of natural secondary metabolites having biological activity such as antimicrobial, antioxidant and anti-inflammatory action among many others.[1] *Plectranthus* is a genus rich in essential oils. It contains about 300 species found in Tropical Africa, Asia and Australia with a diversity of ethnobotanical uses.[2, 3]

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DOI: 10.5530/pj.2011.26.1

Plectranthus caninus Roth (Lamiaceae) is a branched, succulent, and pubescent annual herb that attains a height of 15-40 cm. It grows in dry stony ground, rocky areas and road sides at an altitude between 1000-2600 m above sea level.[4] In Eastern Africa, it is used to treat teeth and gum disorders. [3] In northern part of Ethiopia *P. caninus* is known by its vernacular name "Endifdif" and it is traditionally used as anthelmintic and for the treatment of common cold. In Kenya, old men use its leaves as snuff, a habit that can become addictive.[3] Perusal of literature has revealed the isolation of phenolics[5] and diterpenes[6] from the leaves of *P. caninus*. The diuretic[5, 7], cytotoxic and antitumor promoting[5] activities of the plant are also reported.

As a continuation of our research on essential oil bearing plants of Ethiopia,[8-15] we report herein the chemical composition, antimicrobial, radical scavenging and anti-inflammatory properties of the essential oil distilled from the aerial parts of *P. caninus*.

MATERIALS AND METHODS

Plant material

The aerial part of *P. caninus* was collected in June 2007 from in and around the city of Addis Ababa, Ethiopia. The authenticity of the plant material was confirmed by Ato Melaku Wondaferash, the National Herbarium, Department of Biology, Addis Ababa University, where a voucher specimen was deposited.

Essential oil distillation

The fresh aerial part of *P. caninus* was extracted by hydrodistillation of 1 kg of the plant material using a Clevenger-type apparatus for 4 h. The oil obtained was stored in a sealed amber coloured vial in a refrigerator at -10°C until use.

Gas chromatography/Mass spectrometry analysis

Qualitative and quantitative GC/MS analyses were carried out on a Hewlett-Packard 5890 Series II Plus gas chromatograph interfaced to an HP 5989B mass spectrometer. Separation was done on a 25 m x 0.25 mm HP5-MS capillary column coated with 0.50 µm 5% phenyl 95% methylpolysiloxane. Temperature programming was set at 70-250°C, at a rate of 3°C/min. The carrier gas used was helium at a constant flow rate of 1.9 ml/min. Injector and interface temperature were adjusted to 250°C and 280°C, respectively. EI mass spectra were recorded at 70 eV ionization voltage (source temperature 250°C). Compounds were identified by mass spectral comparison with a commercial database (Wiley8 and NISTO5 mass spectral library) and the laboratory's own database. Spectral data were compared with linear retention indices published in the literature.[16- 18]

Bacterial strains

The oil was tested against the following Gram-negative bacterial strains: *Escherichia coli* K99, *E. coli* K88, *E. coli* 306, *E. coli* LT37, *E. coli* 872, *E. coli* ROW 7/12, *E. coli* 3:37C, *E. coli* CD/99/1, *Salmonella typhi* Ty2, *Shigella boydii* D13629, *S. dysentery* 1, *S. dysentery* 8, *S. flexneri* Type 6, *S. soneii* 1, *Vibrio cholerae* 1313, *V. cholerae* 293, *V. cholerae* 1315, *V. cholerae* 85. The Gram- positive bacterial strains used were *Bacillus pumilus* 82, and *B. subtilis* ATCC 6633 and *Staphylococcus aureus* ML 267.

All the bacterial strains were procured from the Department of Pharmaceutical Technology, Jadavpur University; Central Drug Laboratory, Kolkata and Institute of Microbial Technology, Chandigarh, India. The strains were first checked for purity on the basis of standard microbiological, cultural and biochemical tests and then used for sensitivity testing towards the essential oil.

Fungal strains

Antifungal activity testing was carried out on the following fungal pathogens: *Aspergillus niger* ATCC 6275, *Candida albicans* ATCC 10231, *Penicillium funiculosum* NCTC 287 and *P. notatum* ATCC 11625. All the fungal strains were procured in lyophilized state from the Institute of Microbial Technology (IMT), Chandigarh, India and preserved in the laboratory.

Antibacterial activity evaluation

The minimum inhibitory concentration (MIC) of the oil was determined by checkerboard technique using nutrient agar medium.[19-21] The zones of inhibition produced by the essential oil were determined and compared with that of pure ciprofloxacin by disc diffusion technique.[21, 22] Two sets of dilutions (100 µg/ml) whereby each of the oil dissolved in dimethyl sulphoxide (DMSO) and ciprofloxacin dissolved in sterile distilled water were prepared in sterile McCartney bottles. Sterile nutrient agar plates were prepared and incubated at 37°C for 24 h to check for any possible contamination. Three sterile filter paper discs (What man no. 1) of 6 mm diameter were soaked in the same dilution of the essential oil (each 6 mm disc was shown to absorb 25 µl of oil solution or reference drug stock solution in order to be saturated) and placed in an appropriate position on the plate, marked as quadrant at the back of the Petri dishes. The Petri dishes were incubated at 37°C for 24 h and the diameters of the zones of inhibition were measured in mm. A similar procedure was adopted for the pure ciprofloxacin and the corresponding zone diameters were compared.

Determination of mode of antibacterial action of the oil

The bacteria were allowed to grow in nutrient broth overnight, from which 2 ml were added to 4 ml of sterile nutrient broth and incubated for further 2 h at 37°C so that the culture attained a logarithmic phase of growth. The essential oil was then added at a higher concentration than the MIC value for that particular strain. The number of colony forming units (CFU/ml) were determined by the method described by Miles and Misra at an interval of 2 h up to 6 h and then after 18 h. [23]

Antifungal activity evaluation

The antifungal activity was first evaluated by estimation of MIC of the oil against the fungal pathogens followed