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ESSENTIAL OIL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *LOBELIA PYRAMIDALIS* WALL

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ABSTRACT

The essential oil of *Lobelia pyramidalis* was analyzed by GC and GC-MS. A total of 21 constituents comprising 77.88 % of the total oil were identified. Perilla ketone constituted 25.61 % of the oil followed by camphorquinone (12.16 %), dibutyl phthalate (10.66 %) and allyl nonanoate (8.47 %). The antimicrobial activity of the oil was evaluated using the disc diffusion method and the microdilution technique. The results showed that the oil exhibited moderate antimicrobial activity.

Keywords: *Lobelia pyramidalis*, essential oil, perilla ketone, antimicrobial activity, minimal inhibitory concentration.

INTRODUCTION

Lobelia is a genus comprising over 200 species and 22 are found in India. The plants are herbs or shrubs and are often laticiferous (Bhattacharjee, 2008). *Lobelia* is reported to possess respiratory stimulant, antiasthmatic, antispasmodic, expectorant, and emetic properties. Traditionally, it has been used for bronchitic asthma, chronic bronchitis and, specifically, for spasmodic with secondary bronchitis. It is also used topically for myositis and rheumatic nodules (Ebadi, 2007). *Lobelia chinensis* L. has been used as a diuretic, an antidote, a hemostat, and as carcinostatic agents for stomach cancer in Chinese folk medicine. Various alkaloids, for example lobeline, lobelanine, and lobelanidine are constituents of this herb (Shibano et al., 2001). Thomson and Cutler reported that *Lobelia* was used for the treatment of asthma in the period 1805-1809. Thus, during the nineteenth century *Lobelia* was one of the most medicinally important plants and was used as a valuable remedy for asthma. *Lobelia inflata* (Indian tobacco), so-called because the native Americans (the Penobscot tribes) smoked the dried leaves as a substitute for tobacco, was used to produce the effect of alkaloids on the central nervous system (Felpin and Lebreton, 2004). Five alkaloids, two triterpenoids and sitosterol have been isolated from *Lobelia davidii* (Zhang et al., 1990). The whole plant of *Lobelia inflata* L. contains a variety of piperidine alkaloids such as lobeline, which has been used as a respiratory stimulant (Ishimaru et al., 1991). The hairy roots of *Lobelia sessilifolia* were shown to be rich in glycosylated polyacetylenes (Ishimaru et al., 1994). Acylated cyanidin 3-rutinoside-5,3'-diglucoside was also isolated from the purple-red flower of *Lobelia erinus* (Saito et al., 1995).

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Extracts of *Lobelia inflata* contain lobeline, which was found to show positive effects on the treatment of multidrug-resistant tumor cells (Ma and Wink, 2008), and the leaves contain *β*-amyrin palmitate, which possesses sedative and antidepressant properties. The rhizomes of *Lobelia chinensis* L. were reported to contain the polyfructosan lobelinin, and the plant is used in China to treat fever and asthma, and the roots are considered depurative and antirheumatic in Indo-China. This plant is one of the constituents of a tincture formulation used for the treatment of scars (Khare, 2007). Furthermore, lobeline can be modified to lobelane, which was shown to decrease methamphetamine self-administration in rats (Neugebauer et al., 2007).

Lobelia pyramidalis, locally known as Bhanguri, is an endangered herb that generally occurs at an altitude of 1500-3000 m. Its leaves and inflorescence are antispasmodic (Manandhar, 2002) and used for asthma, bronchitis, and fever (Kumari et al., 2011). This plant is also used to treat sciatica and back pain (Bajracharya, 1979). The available literature indicates a lack of work published on the chemical composition and biological activity of *Lobelia pyramidalis* Wall. Therefore, we focused our study on the essential oil composition and antimicrobial activity of *Lobelia pyramidalis* Wall.

MATERIALS AND METHODS

Plant material

The aerial parts of the plant were collected from Daman, Nepal, in the month of September, 2010. The plant identification was confirmed by the Botanical Survey of India (BSI), Dehradun. A voucher specimen (No.113408) was deposited in the Herbarium Section at BSI, Dehradun, India.

Extraction of essential oil

The fresh aerial parts of *Lobelia pyramidalis* (10 kg) were steam distilled and the distillate was saturated with NaCl and extracted with *n*-hexane. Anhydrous Na_2SO_4 was then added to dry the organic phase. This was separated using a separating funnel and the solvent was finally evaporated under reduced pressure. The oil yield was 0.2 % (w/w). The essential oil was stored under refrigeration for 3 days and then used immediately for the analysis and evaluation of antimicrobial activity.

GC and GC/MS

The oil was analyzed using a Nucon 5765 GC (30 m × 0.32 mm, FID) with split ratio 1:48, N_2 flow of 4 kg/cm^2. GC/MS analysis was performed using a thermoquest (Vienna, Austria) trace GC-2000 interfaced with Finnigen MAT Polaries-Q ion trap mass spectrometer fitted with RTX-5MS (Restek Corporation, Pennsylvania, USA) fused silica capillary column (30 × 0.25 mm, 0.25 µm film coating). The oven temperature was programmed from 60°–210°C at 3 °C/min using helium as carrier gas at 1 ml/min. The injector temperature was 210 °C; injection volume was 0.1 µL prepared in hexane, split ratio 1:40. Mass spectra were taken at 70 eV Electron Impact (EI) with mass scan range of *m/z* 40-450 amu with mass scan time of 4 s.

Microorganisms

The antimicrobial activity was conducted against one gram positive bacteria: *Staphylococcus aureus* (ATCC 25923) and three gram negative bacteria: *Klebsiella pneumoniae* (ATCC 22736), *Pseudomonas aeruginosa* (ATCC BAA-427), *Escherichia coli* (ATCC 9637), and six fungi *Candida albicans* (MTCC 183), *Cryptococcus neoformans* (ATCC 208821), *Sporothrix schenckii* (MTCC 1359), *Trichophyton mentagrophytes* (ATCC 9533), *Aspergillus fumigatus* (MTCC 343) and *Candida parapsilosis* (ATCC 22019). Required microorganisms were procured from the Institute of Microbial Technology, Chandigarh and National Chemical Laboratory, Pune, India.

Antimicrobial activity

The antimicrobial activity of essential oil from the aerial parts of *Lobelia pyramidalis* was tested using the paper disc diffu-

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sion method according to the slightly modified National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1997). A suspension (10^6 CFU/mL) of microbial strains was used. The filter paper discs (6 mm in diameter, Whatman filter paper 1) were individually impregnated with 10 µL of the essential oil aliquots (200 mg/mL) and subsequently placed onto the surface of inoculated Petri dishes containing Mueller Hinton Agar for bacterial strains and RPMI 1640 agar medium for fungal strains. The Petri dishes were kept at 4 °C for 2 h and were then incubated for 24 h at 37 °C for bacterial growth and for 48 h at 27 °C for fungal growth. The diameters of the inhibition zones were measured in millimeters, including the diameter of each disc. Controls were set up with equivalent quantities of DMSO (Qualigens), which was used as a solvent (20 %) when preparing the essential oil solution. All of the experiments were performed in triplicate and the results (millimeters of the inhibition zone) were expressed as mean values.

Determination of the minimal inhibitory concentration (MIC)

The broth microdilution technique was used to determine the MIC values (NCCLS, 1999). All of the experiments were performed in Mueller Hinton broth for the bacterial strains and RPMI 1640 medium for the fungal strains. Serial doubling dilutions of the essential oil were prepared in a 96-well microtiter plate ranging from 0.10 to 200 mg/mL. The prepared microtiter plates containing microorganisms and essential oil were then incubated at 37 °C for 24 h for bacterial growth and at 27 °C for 48 h for fungal growth. The growth of microorganisms was indicated by turbidity, which was visually observed. All of the experiments were performed in triplicate. Controls were set up with equivalent quantities of DMSO (Qualigens), which was used as a solvent (20 %) when preparing the standard solutions. Levofloxacin and clotrimazole were used as standards.

RESULTS AND DISCUSSION

Chemical composition of Lobelia pyramidalis essential oil

Steam distillation of the aerial parts of the plant yielded 0.2 % (w/w) of the essential oil. The oil sample was analyzed by GC and GC-MS and the constituents were identified on the basis of the retention index, library mass search database (NIST & WILEY) and Adams (Adams, 1995). A total of 21 constituents were identified, representing 77.88 % of the total oil constituents detected (Table 1), which included 54.33 % monoterpenes. The major constituents of the oil were perilla ketone (25.61 %), camphorquinone (12.16 %), dibutyl phthalate (10.66 %), isobornyl isobutanoate (2.94 %), isophytol (2.76 %) and 2,5,9-trimethyl decane (2.06 %). To the best of our knowledge, this is the first report to determine the constituents of the essential oil of this plant.

Antimicrobial activity

The essential oil of *Lobelia pyramidalis* was studied for its antimicrobial activity. *In vitro* antimicrobial studies were carried out against bacterial and fungal strains as previously described. The results obtained for the zones of growth inhibition (mm) and minimal inhibitory concentrations (MICs) in Mueller Hinton Agar for bacterial strains and RPMI 1640 medium for fungal strains are summarized in Table 2.

The results showed inhibition zones against all strains except *C. neoformans* and *K. pneumoniae.* The data obtained from the disc diffusion method indicated that the essential oil was most active against *S. aureus* among the bacterial strains and *T. mentagrophytes* among the fungal strains with largest inhibition zones of 18 mm and 20 mm, respectively.

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Table 1: Essential oil composition of *Lobelia pyramidalis* Wall

S.No.	Compound	K.I	Leaf Oil %	Mode of Identification
1	Mycrene	985	0.28	a,b
2	*α*-Phellandrene	999	.19	a,b
3	*δ*-Carene	1011	0.24	a,b
4	Limolene	1025	1.42	a,b
5	Acetophenone	1061	.64	a,b
6	Perilla Ketone	1243	25.61	a,b,
7	Camphorquinone	1290	12.16	a,b,
8	Allyl nonanoate	1378	8.47	a,b
9	*n*-Tetradecane	1400	1.17	a,b
10	*Iso*bornyl isobutanoate	1430	2.94	a,b
11	Vestitenone	1444	3.02	a,b
12	2,5,9-Trimethyl decane	1480	2.06	a,b
13	< (E)-o- > Methoxycinnamaldehyde	1525	1.24	a,b
14	< 1,10- > Decanediol	1545	.75	a,b,
15	Thujopsan-2- *β*-ol	1585	1.16	a,b
16	1,2-Benzenedicarboxylic acid, butyl-2-methylpropyl ester	1614	.88	a,b
17	Dibutyl phthalate	1624	10.66	a,b
18	1,2-Benzenedicarboxylic acid, butyl-2-ethylhexyl ester	1630	.98	a,b
19	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1636	1.025	a,b
20	Isophytol	1946	2.76	a,b
21	*n*-Tetracosane	2400	.23	a,b
	Total		**77.88**	

Table 2: Antimicrobial activity of the essential oil of *Lobelia pyramidalis* Wall

Microorganisms	Essential oil		Standards	
	IZ (mm)	MIC[a] (mg/ml)	MIC[b](µg/ml)	
			LVF	CL
Staphylococcus aureus (MTCC 3160)	18	3.12	.32	NT
Klebsiella pneumoniae (ATCC 22736)	NA	NT	4.0	NT
Pseudomonas aeruginosa (ATCC BAA-427)	14	12.50	.30	NT
Escherichia coli (ATCC 9637)	14	12.50	.20	NT
Candida albicans (MTCC 227)	16	6.25	NT	2
Cryptococcus neoformans (ATCC 208821)	NA	NT	NT	2
Sporothrix schenckii (MTCC 1359)	13	6.25	NT	32
Trichophyton mentagrophytes (ATCC 9533)	20	3.12	NT	8
Aspergillus fumigatus (MTCC 343)	14	12.50	NT	12.5
Candida parapsilosis (ATCC 22019)	17	6.25	NT	0.5

IZ: Diameter of zone of inhibition (mm) including disc diameter of 6 mm
MIC[a] (mg/ml) of essential oil, MIC[b] (µg/ml) of standards, NA: Not active, NT: Not tested, LVF: Levofloxacin, CL: Clotrimazole

The results of the MICs showed that *S. aureus* and *T. mentagrophytes* had the lowest MIC of 3.12 mg/mL, which was correlated with the greatest inhibition zones for *S. aureus* and *T. mentagrophytes*, whereas the highest MIC was 12.50 mg/mL for *P. aeruginosa, E. coli* and *A. fumigatus.*

From the above results it can be concluded that the essential oil of *Lobelia pyramidalis* showed a moderate level of antimicrobial activity. The antimicrobial properties of the essential oil of *Lobelia pyramidalis* may be due to the presence of terpenes. There are several reports on the antimicrobial activity of plant essential oils containing perilla ketone (Yu et al., 2011) and isophytol (Saidana et al., 2008). In addition, the components present at lower amounts such as *α*-phellandrene, *δ*-carene, limolene, and mycrene could also contribute to the antimicrobial activity of plant essential oils (Oliviera et al., 2007; Barros et al., 2009; Liolios et al., 2009). It has been reported that the components present at lower concentrations might be involved in synergism with the other active components (Marino et al., 2001).

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