

Original Research Article

Chemical and biological studies of *Lobelia flaccida* (C. Presl) A.DC leaf: a medicinal plant used by traditional healers in Eastern Cape, South Africa

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Received: 1 October 2015

Revised accepted: 4 July 2016

Abstract

Purpose: To investigate the phytochemical constituents, acute toxicity and biological activities of *Lobelia* herb (dried leaf of *Lobelia flaccida*; family: Campanulaceae), a popular medicinal agent used to manage pain and epilepsy among other indications in Eastern Cape Region of South Africa.

Methods: Essential oil (EO) obtained from the dried leaf was analysed with gas chromatography-mass spectroscopy GC/MS while an infusion extract of the herb was obtained by soaking in hot boiled water (100 °C) for 24 h, filtered and the filtrate dried in vacuo. Phytochemical screening of the infusion extract was performed to detect the presence of secondary plant metabolites and relative abundance of some of the metabolites. The aqueous extract was evaluated for oral (p.o.) acute toxicity (LD₅₀) using the Lorke's method [30]; thereafter the extract was tested for anti-inflammatory activity on carrageenan-induced rat paw oedema at 250 and 500 mg/kg, p.o., normal saline and aspirin (100 mg/kg, p.o.) used as negative and positive controls respectively. Finally, the extract at 500 and 1000 mg/kg, p.o. was tested for anticonvulsant activity on pentylene tetrazol (85 mg/kg, intraperitoneally)-induced convulsion model in mice, normal saline and diazepam (1 mg/kg, i.p.) served as negative and positive control groups respectively.

Results: EO yield was 0.022 % w/w and the two major compounds identified were acetophenone (26.37 %) and caryophyllene (17.35 %). Phytochemical screening showed high concentration of alkaloids, saponins and flavonoids among other constituents. LD₅₀ of the aqueous extract was ≥ 5000 mg/kg per oral while the aqueous extract exhibited significant ($p < 0.01$) anti-inflammatory activity on carrageenan-induced rat paw oedema comparable to aspirin but insignificant anticonvulsant activity on pentylene tetrazol-induced convulsion when compared with diazepam.

Conclusion: *Lobelia* herb is non-toxic, and possesses significant anti-inflammatory and mild anticonvulsant activities. It is suggested that the essential oil of this herb should be screened for pharmacological activities.

Keywords: *Lobelia flaccida*, Essential oil, Gas chromatography/mass spectroscopy analysis, Infusion extract, Acute toxicity, Anti-inflammatory, Anti-convulsant

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The contribution of natural products particularly medicinal plants to discovery of novel drugs have been well highlighted [1] and Newman *et al* [2] reported that research on natural products accounted for about 48 % of all natural product-derived compounds between 1981 and 2002. Furthermore, Jones *et al* [3] revealed that majority of plant-related drugs on the pharmacy shelf as at 1993 actually emanated from ethnomedicinal application of most species that were indicated for the same therapeutic uses. Crude herbs which have long been used as the basis for traditional medicines worldwide are still invaluable now [4]. The role of traditional medicine in mitigating disease burden and poverty in the African continent has been emphasized and extended to include increasing the economic well-being and healthcare accessibility of communities.

Lobelia commonly known as Indian tobacco is a genus of flowering plants comprising about 415 species (family: Campanulaceae) with subtle distribution in tropical-warm temperate regions of the world [5]. *L. inflata* is regarded as the most important variety in this family consisting of several species including *L. cardinalis*, *L. erinus*, *L. spitaca*, *L. siphilitica*, *L. puberula* and *L. appendiculata* [6]. Sixty-nine species occur in South Africa, found throughout the country growing in a variety of habitats [7,8]. One of the commonest species found in the Eastern Cape of South Africa is *L. flaccida* (C. Presl) A.DC, family: Campanulaceae known as itshilizi in Ixhosa language [8,9] and is the specie investigated in this study. Traditionally, different *Lobelia* species has been used for bronchitis and asthma, topically for myositis and rheumatic nodules, as a diuretic, antidote and as carcinostatic agents for stomach cancer in Chinese folk medicine [10,11]. *Lobelia siphilitica* and *L. cardinalis* were historically used in the treatment of syphilis in North America [12]. Although several studies have been undertaken to evaluate lobeline (an alkaloid from *Lobelia*) as anti-smoking drug, the results obtained showed that it was ineffective in helping people quit smoking with conflicting results [13].

Reports on the chemical constituents of *Lobelia* species confirmed alkaloids such as lobeline, norlobelanine; flavonoid compounds (apigenin, luteolin, quercetin), and coumarins; and essential oil [14]. Biological studies on *Lobelia* species showed several activities including antimicrobial [15], antidepressant [16] anticancer [11] and antiepileptic [17] and analgesic and antivenom potentials [18].

The people of the Eastern Cape of South Africa depend largely on the use of medicinal plants born out of strong belief that they are effective to treat many diseases [19]. The Department of Science and Technology, South Africa, initiated the Indigenous Knowledge System (IKS) Project in 2002 in its bid to bridge the gap between the herbal practitioners and the academia, improve the health care system in the rural areas and advance herbal practices in the community.

This is the first study reporting chemical composition and biological activities of this particular species from the Eastern Cape and the aim was to investigate the chemical composition of the essential oil; screen the infusion extract for secondary metabolites; and then determine the acute toxicity profile, anti-inflammatory and anticonvulsant [8,11] activities of the crude infusion extract of *Lobelia flaccida*. Thus, this study is expected to generate new information and to validate the use of this plant in South African herbal practice.

EXPERIMENTAL

Plant collection, and extraction of essential oil and infusion extract

Dried leaves of *Lobelia* were collected from the Herbal Practitioner on 3rd July 2014 at Ginsberg, King Williams Town, Eastern Cape, South Africa. The leaves were used for the extraction of the essential oil and infusion extract. The dried leaves of *Lobelia* were identified by Dr. K Immelman, Herbarium Unit, Department of Botany, Walter Sisulu University (WSU), Mthatha. Essential oil of *Lobelia flaccida* dried leaf was obtained by hydrodistillation using a cleavenger-like apparatus and stored in a vial, protected from light until sent for analysis. Infusion extract of the leaves was obtained according to herbal practitioner's directive. Briefly, 100 g of the dried plant material was grounded into coarse powders and soaked in boiling water with continuous stirring and allowed to infuse for 24 h. Thereafter, it was filtered using Whatman filter paper; a portion was kept for the phytochemical screening, while the remaining portion was dried in an oven at 35 °C.

Phytochemical screening

For qualitative screening, aqueous extract of *Lobelia flaccida* leaf was used while the powdered leaf was used for quantitative screening according to the method described by Mir *et al* [20].

Qualitative screening

The infusion filtrate was used for the qualitative analysis to detect the presence of secondary metabolites including tannins, saponins, flavonoids, terpenoids, alkaloids, phenols, phytosterols, glycosides, anthraquinones, phlobatannins and proteins/amino acids in the aqueous leaf extract of *Lobelia flaccida* [20].

Analysis of essential oil of *Lobelia* leaf

Gas chromatography- flame ionization detector (GC-FID) analysis

GC analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with a FID detector with a SGE BP X5 column that is 30 m in length with a film thickness of 0.25 μm and diameter 0.25 mm ID. The operating conditions were as follows: carrier gas, nitrogen with a flow rate of 3.0 ml/min; column temperature, 60 - 275 $^{\circ}\text{C}$ at 4 $^{\circ}\text{C}/\text{min}$; injector and detector temperature, 280 $^{\circ}\text{C}$ -; volume injected 0.1 μl of the oil; split ratio, 1:50.

GC-MS analysis

GC/MS analysis of the oil was performed on a GCMS-QP2010 Gas Chromatography- mass spectrometer system operating in EI mode at 70 eV, equipped with a HP-5 MS fused silica capillary system with a 5 % phenylmethylsiloxane stationary phase. Capillary column parameter was 30 m by 0.25 mm, film thickness 0.25 μm . The initial temperature of the column was 70 $^{\circ}\text{C}$ and was heated to 250 $^{\circ}\text{C}$ at a rate of 5 $^{\circ}\text{C}/\text{min}$. The final temperature was kept at 450 $^{\circ}\text{C}$ and run time of 68 min. Helium was used as the carrier gas at a flow rate of 1 ml/min. The split ratio was 100:1. Scan time was 68 min with a scanning range of 35 to 450 amu. One microliter (1 μl) of the diluted oil (in hexane) was injected for analysis. N-alkane of C8 to C30 was run under the same condition for Kovat indices determination.

Experimental animals

Mice and rats were obtained from the South African Vaccine Initiative, Johannesburg and kept at the Animal Holding Facility, Zoology Department, Walter Sisulu University WSU. Male and female Wistar rats (200-300 g) were randomly selected (n=6) for the anti-inflammatory test. Swiss mice of both sexes (25-35 g; n=6) were also selected for the acute toxicity and the anticonvulsant tests. They were kept under standard laboratory conditions and had free access to rat chow and water. Food was

however withheld overnight prior to experiments while water was provided *ad libitum*. This study was approved by the Department of Higher Education, WSU (Ref: DVC (AA&R) DRD/SREC) The study was carried out in accordance with the "Principles of Laboratory Animal Care", NIH publication no. 85-23, revised 1985.

Acute toxicity

Acute toxicity effect of the infusion extract of the leaves of *Lobelia flaccida* was assessed in mice using the oral route (p.o.) according to Lorke's method [21]. The method involved using only thirteen (13) animals and was divided into 2 phases. The first phase consists of three (3) sub-groups (n=3) at dose levels of 10, 100 and 1000 mg/kg. The second phase involved four (n=1) dose levels of 1000, 1600, 2900 and 5000 mg/kg respectively. Immediately after treatment, each mouse was placed inside the Plexiglas cage and observed for immediate effects up to 30 min and thereafter for 24 h for lethal effects culminating into death. The LD₅₀ of the extract was estimated as the geometric mean of the lowest dose causing death and the highest dose causing no death according to Eq 1.

$$LD_{50} = \sqrt{(AB)} \dots\dots\dots (1)$$

where A is the maximum dose producing 0 % death and B is the lowest dose that produced 100 % death.

Anti-inflammatory activity studies

The anti-inflammatory activity was evaluated by the carrageenan-induced paw oedema test in rats [22]. The left hind paw was injected with 0.2 ml of normal saline and used the control. Normal saline (10 ml/kg), the extract of *Lobelia* (250 and 500 mg/kg) and aspirin (100 mg/kg) were orally administered 1 h prior to carrageenan injection (0.1 ml 2 % w/v carrageenan in normal saline) to 4 different groups of rats (n=6). The paw oedema sizes were recorded for each rat before carrageenan injection and at 1, 2, 3 and 4 h post carrageenan injection using Vernier Callipers.

Anticonvulsant activity studies

The anticonvulsant effect was evaluated using the pentylenetetrazole (PTZ)-induced convulsion model. Pentylenetetrazole (85 mg/kg) was used to induce tonic-clonic convulsions [23]. Five different groups (n=5) of mice were randomly selected and orally pre-treated for 1 h as follows: group I received normal saline (10 ml/kg), groups II-III received extract (500 and 1000 mg/kg) respectively and group IV diazepam (1 mg/kg).

Thereafter, anticonvulsant assessment was carried out with pentylenetetrazole (85 mg/kg, i.p.). The animals were observed for the onset of convulsion, time of death and mortality. Animals that survived beyond 30 min post-pentylenetetrazole injection were regarded to be protected.

Statistical analysis

The results are expressed as mean \pm standard error of mean (SEM) and analysed using one-way analysis of variance (ANOVA) followed by post hoc test using Dunnett's test for comparison between the treated groups and control at $p < 0.05$; while the ratio of mortality or protection for each group was expressed in percentage. The software used are GraphPad Instant R version 3.0.10.0 (UK) and GraphPad Prism Version 5 2013 (GraphPad Software Inc).

RESULTS

Yield of the essential oil and infusion extract

The yield of the essential oil was 0.22 % w/w, which was light yellowish in colour with characteristic aromatic odour. The infusion extract obtained from the dried leaf of *Lobelia*

weighed 25.54 g (25.50 % w/w). 25.54 g (25.5 % w/w).

Phytochemical profile of the infusion extract of *Lobelia flaccida* leaf

The results obtained from the qualitative screening of *Lobelia* leaf indicated the presence of alkaloids, flavonoids, phenols, phytosterols, proteins, saponins and tannins; while carbohydrates, glycosides, phlobatannins and steroids were not detected. The quantitative analysis of *Lobelia* leaf infusion extract showed that the percentage of alkaloid, saponins and flavonoids constituents were 2.40, 0.14 and 0.40 % w/w respectively while its tannin constituent was not recoverable.

Composition of *Lobelia* essential oil

GC/MS analysis of *Lobelia* essential oil indicated the presence of 25 compounds out of which 23 were fully identified constituting about 98 % of all the constituents (Table 1). The major compounds identified include acetophenone (26.37 %), caryophyllene (8.11 %), borneol (7.46 %) and anethole (6.81 %).

Table 1: Chemical composition of the essential oil of *L. flaccida*

Peak no	Compound ^{a,b}	Eluting time (sec)	KI	Relative % composition
1	α -Pinene	5.205	937	1.40
2	Benzaldehyde	5.710	957	5.36
3	2-Pentylfuran	6.210	989	1.15
4	1,8-cienole	7.225	1030	3.13
5	Acetophenone	8.010	1065	26.37
6	Linalool	8.760	1101	3.92
7	2(10)-Pinen-3-ol, (1S, 3R, 5S)	9.960	1138	0.47
8	Camphor	10.135	1145	6.56
9	D-isomenthone	10.310	1162	0.62
10	Borneol	10.770	1169	7.46
11	α -Terpineol	11.340	1195	2.35
12	(-)-Carvone	12.650	1242	2.60
13	Anethole	13.775	1142	6.81
14	Phenol,2-methyl-5-(1-methylethyl)	14.050	1152	3.67
15	α -Terpineol acetate	15.355	1347	1.31
16	Unidentified	16.720		0.55
17	Caryophyllene	17.345	1436	8.11
18	Geranyl acetone	17.865	1438	1.41
19	β -Humulene	18.240	1483	1.87
20	(E)- α -Ionone	18.750	1492	2.43
21	Cadina-1(10),4-diene	19.740	1533	1.80
22	Caryophyllene oxide	21.330	1588	3.43
23	Guaiol	21.600	1601	3.13
24	Unidentified	22.980		0.62
25	2-Pentadecanone,6,10,14-trimethyl	26.845	1697	3.46

^a[24]; ^b[25]

The classes of compound identified in the oil were sesquiterpenes (8.11%), phenyl alkenes (6.81 %), monoterpenes (7.46 %) and a simple ketone (26.37 %). The major compounds obtained were from different classes and they play different roles in the *Lobelia* but the major compound is an aromatic ketone acetophenone (26.37 %).

Acute toxicity effect of the infusion extract of *Lobelia* dried leaf

There was no mortality at all the doses of the infusion extract up to 5000 mg/kg, p.o. The LD₅₀ was therefore estimated to be ≥ 5000 mg/kg through the oral route in mice.

Effect of infusion extract of *Lobelia* leaf on carrageenan-induced rat paw oedema

Rats pre-treated with normal saline had increased paw sizes throughout the 4-hour period of observation. Infusion extract of *Lobelia*, 250-500 mg/kg caused significant ($p < 0.01$) reduction in paw oedema sizes at 1, 2, 3 and 4 h post-carrageenan injection compared to control group. Also, aspirin (100 mg/kg) similarly caused

significant ($p < 0.01$) reduction in the rats' paw oedema (Figure 1).

Effect of the infusion extract of *Lobelia* leaf against PTZ-induced convulsion

All the mice in the control group convulsed and died within 30 min post-PTZ (85 mg/kg, i.p.). The extract delayed the onset of convulsion and time of death after convulsion compared to vehicle although not statistically significant. Diazepam, (a standard anticonvulsant drug used) caused significant ($p < 0.01$) delay in onset of convulsion and protected the mice against PTZ-induced death. However, *Lobelia* at 500 and 1000 mg/kg offered 40 and 20 % protections against the PTZ-induced mortality respectively compared to the group's 0 % and diazepam's 100 % protection at 30 min observation period (Table 2).

DISCUSSION

Phytochemical screening of *Lobelia* leaf confirmed the presence of several metabolites including alkaloids, saponins, flavonoids, phenols, phytosterols, proteins and tannins.

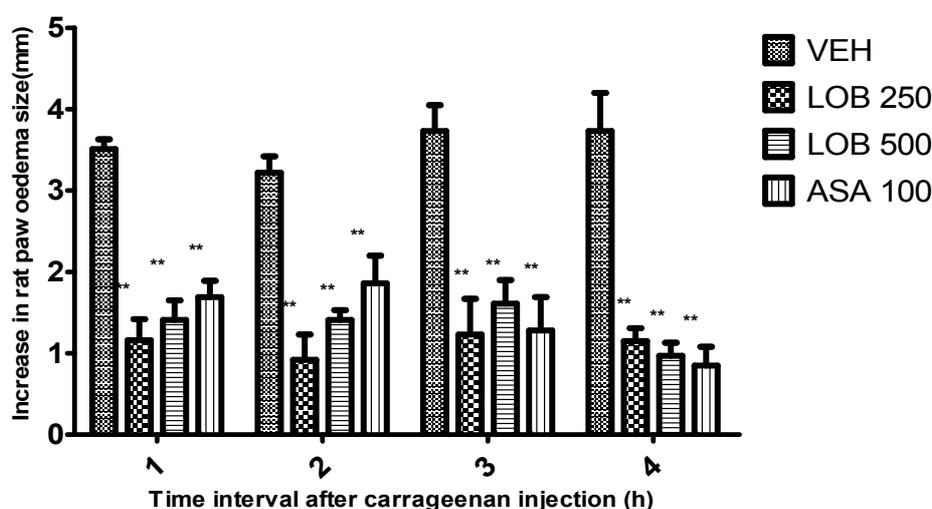


Figure 1: Effect of *Lobelia* leaf infusion on carrageenan induced rat paw oedema. **Key:** LOB 250: *Lobelia* infusion extract 250 mg/kg, LOB 500: *Lobelia* infusion extract 500 mg/kg, ASA: aspirin 100 mg/kg and VEH: normal saline; ** $p < 0.01$; at 1 h, $F_{(3, 20)} = 20$; at 2 h, $F_{(3, 20)} = 14.7$; at 3 h, $F_{(3, 20)} = 16.0$; at 4 h, $F_{(3, 20)} = 22.9$

Table 2: Effect of the infusion extract of *L. flaccida* on PTZ-induced convulsion in mice

Treatment	Convulsion latency(s) Mean \pm SEM	Time of death (s) Mean \pm SEM	% Protection	% Mortality
Normal saline	5.20 \pm 3.69	349.4 \pm 94.4	0	100
Lobelia 500 mg/kg	161.4 \pm 92.30	1038.0 \pm 354.6	40	60
Lobelia 1000mg/kg	91.0 \pm 23.47	919.8 \pm 260.3	20	80
Diazepam 1 mg/kg	1505 \pm 295.0**	1800 \pm 0.0	100	0

** $P < 0.01$, $F_{(3,16)} = 21$; statistically different from other groups; ANOVA, Dunnett's

These phyto-constituents have variously been shown to be responsible for many bioactivities displayed by plants and new compounds can be discovered by probing them further. It must be noted however, that plant extract contains low concentrations of active compounds and a large number of promising compounds especially *Lobelia* species [17,18]. The essential oil analysis revealed the major compounds present in the *Lobelia flaccida* dried leaf to be acetophenone (26.37 %) caryophyllene (8.11 %), borneol (7.46 %) and anethole (6.81 %). There was scanty information in literature on the chemical composition of essential oil of this *Lobelia* specie. However, Joshi *et al* [15] reported that perilla ketone (25.61 %), camphorquinone (12.16 %), dibutyl phthalate (10.66 %) and allylnonanoate (8.47 %) were the main constituents of essential oil of *Lobelia pyramidalis* (Wall) from India. The oil composition in the present study is different in the class of compounds constituting it. Our report showed that there were more monoterpenes and sesquiterpenoids present as compared to that of Joshi *et al* [15].

Acute toxicity profile of the *Lobelia flaccida* dried leaf aqueous extract indicated an LD₅₀ of ≥5000 mg/kg, p.o., indicating that it is non-toxic [21]. This toxicity test result can be used to explain why the plant is a popular medicinal agent that has been used extensively over the years without report of serious adverse effects.

Carrageenan-induced paw oedema continues to be a valuable experimental animal model for acute inflammation which is strongly speculated to be biphasic viz. early phase (1-2 h) mediated by histamine and serotonin, and late phase (>2 h) mediated by bradykinin, leukotrienes and prostaglandins [26]. In this study, *Lobelia* extract significantly inhibited paw oedema induced by carrageenan in both phases, suggesting possible inhibition of release of inflammatory mediators and cyclooxygenase synthesis similarly to nonsteroidal antiinflammatory drugs such as aspirin, whose mechanism of action is inhibition of the cyclooxygenase pathway. The results obtained here support the ethno-medicinal claims ascribed to this particular plant species found in South Africa by the herbal practitioner. The screening of this plant extract showed high flavonoid content (4 % w/w) which has been previously linked to the antiinflammatory activity of *L. chinensis* [10], it can therefore be postulated here that the antiinflammatory activity of this *Lobelia* extract could be associated with the high flavonoids and or saponins present in it [27,28].

The anticonvulsant test indicated that *Lobelia* infusion extract did not significantly alter the onset of convulsion or time of death compared to the vehicle, but it did prolong insignificantly the latency and time of death caused by the PTZ compared to the vehicle. Previous studies have indicated that the extract of this plant showed anticonvulsant potentials in many models of convulsion and lobeline isolated from the leaf of *L. nicotianaefolia* demonstrated significant anticonvulsant activity [29]. Considering the fact that it was just the infusion extract that was used in this study, extraction with other solvents such as ethanol would probably have demonstrated greater anticonvulsant activity because more bioactive substances would be extracted. Also, the essential oil component which was not tested against convulsion due to low quantity obtained in this study may exhibit greater activity since essential oils of many plants demonstrate anticonvulsant activities [43].

CONCLUSION

The major component found in the essential oil of this *L. flaccida* species is caryophyllene. The infusion extract is non-toxic, possesses strong anti-inflammatory activity but mild anticonvulsant activity. Thus justifies the plant's use in traditional medicine to treat pain and rheumatism.

DECLARATIONS

Acknowledgement

This study was funded by NRF research grant no. 82640 (IKS 2012.01.19_10163). We also K Immelman (KEI herbarium) for her assistance in identifying the medicinal plant material.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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