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# PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIMICROBIAL ACTIVITIES OF *IN VITRO* AND *IN VIVO* GROWN PLANT EXTRACTS OF *LOBELIA INFLATA* L.

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## ABSTRACT

*Lobelia inflata* L. is a medicinally important herb used in the treatment of respiratory problems. The objectives of this present study were phytochemical screening and evaluation of antimicrobial activities of aqueous, ethanolic and methanolic extracts of different parts of the plant such as leaf, root, stem and inflorescence. Extracts of *in vitro* grown callus of the leaf, obtained after 4 weeks of incubation on 1X MS medium containing 1mg/ml 2, 4-D and 1mg/ml Kinetin were also screened. Fifteen extracts revealed the presence of phytochemicals such as alkaloids, carbohydrates and phytosterols. The methonolic extracts of root showed alkaloid (0.50 mg/ml) and stem showed carbohydrate (1.76 mg/ml). The ethanolic leaf extract showed phytosterols (3.68 mg/ml). Antimicrobial activities revealed that among all the extracts, the highest sensitivity was recorded for the methanolic and ethanolic extract of inflorescence against *Klebsiella pneumonia* and *Staphylococcus aureus* respectively. The ethanolic extract of root and stem showed the highest zone of inhibition against *Serratia marcescens.* None of the aqueous extracts exhibited any antimicrobial activity. The methanolic extract of the callus was found to inhibit *Cryptococcus neoformans* among the various fungal pathogens. This study may provide the scientific basis for the use of this plant for various therapeutic purposes. .

**KEYWORDS:** ***Lobelia inflata*** **L., phytochemical screening, antimicrobial activities, callus, extracts, pathogens.**

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# INTRODUCTION

*Lobelia inflata* L. (also known as asthma weed, Indian tobacco, pukeweed, and vomitwort) is a genus of flowering plants found primarily in tropical to warm temperate regions of the world, a few species extending into cooler temperate regions. The species used most commonly in modern herbalism is *Lobelia inflata* L.(Indian Tobacco). It is used mainly as a powerful antispasmodic herb in the treatment of respiratory and muscle disorders [1]. The plant is also taken internally for the treatment of asthma, bronchitis, whooping cough and pleurisy[2]. It also serves as a respiratory stimulant and relaxant[3, 4]. Indian tobacco is a valuable remedy for conditions such as bronchial asthma and chronic bronchitis[1].

*Lobelia inflata* L. acts as a smoking deterrent[5]. The herb is commonly used as an aid to stopping smoking, sometimes in combination with cramp bark. One of the alkaloids in *Lobelia inflata* L., lobeline, has effects on humans similar to those of nicotine and can be helpful in treating the symptoms of nicotine withdrawal[6]. The alkaloids present in the leaves are used to stimulate the removal of phlegm from the respiratory tract[7].

The present study aimed at the phytochemical screening and evaluation of the antimicrobial activities of aqueous, ethanolic and methanolic extracts of different parts of *Lobelia inflata* L. such as root, stem, leaf, inflorescence and *in vitro* grown callus.

# MATERIALS AND METHODS

### *Source of plant material*

*Lobelia inflata* L. plants were collected from the Western Ghats of Karnataka, India. They were maintained under optimum green house conditions in Bangalore.

### *Source of microorganisms*

All the bacterial and fungal cultures were clinical isolates obtained from the Department of Microbiology, Genohelix Biolabs, Bangalore, India. The various bacterial pathogens used in this study were *Klebsiella pneumonia*, *Serratia marcescens* and *Staphylococcus aureus*. The fungal pathogens included *Aspergillus niger*, *Candida albicans* and *Cryptococcus neoformans.*

### *Chemicals*

All the chemicals and reagents were of analytical grade, procured from Sigma, Qualigens, Colloids, Nice and s.d. Fine Chem. All the microbiological media were obtained from Himedia.

### *Sterilization of explant*

Leaves of *Lobelia inflata* L. were used as the explant. The leaves were washed thoroughly under tap water, surface sterilized using liquid detergent 2% (v/v) Savlon, 6-8 drops of Tween-20 for 15 min, rinsed with 70% ethanol for 30 secs, disinfected with 0.05% (w/v) $HgCl_2$ for 6 min and again rinsed in sterile water several times to remove the traces of $HgCl_2$.

### *In vitro callus induction*

The sterilized leaf explants of *Lobelia inflata* L. were inoculated on 1X MS media and Gamborg's media containing 3% sucrose, 1% agar fortified with various combinations and concentrations of growth regulators (NAA, IAA, IBA, 2,4-D, BAP, KIN). The cultures were maintained in the culture room at a temperature of 25±2°C, light intensity of 1000 LUX, relative humidity between 50 - 60%, under photo-periodic regime for 16 hours light and 8 hours dark cycle. The data were collected regularly.

### *Maintenance of callus*

The callus was sub-cultured on the 1X Murashige-Skoog's medium containing 1mg/ml 2, 4-Dichloro phenoxy acetic acid and 1mg/ml Kinetin which showed callus induction. The subcultured callus was used for phytochemical screening and antimicrobial activity studies

### *Preparation of plant extracts*

Different parts of the plant such as inflorescence, leaf, root and stem were washed

with sterile water and dried under shade for 7 days and separately powdered using sterile mortar and pestle. The aqueous, ethanolic and methanolic extracts were prepared by weighing 3 gm of the powdered plant material and dissolving in 50 ml of distilled water, ethanol and methanol respectively. The samples were heated in reflux condenser for 2 hours and centrifuged at 5000 rpm for 15 min at 21$^{o}$C. The supernatants were collected and air dried overnight on a petriplate. The air dried sample was dissolved in 10 ml solvent and stored at 4$^{o}$C until use.

The aqueous, ethanolic and methanolic extracts of the callus were prepared by adding 2 gm of dried and powdered callus to 35 ml of the respective solvent. The samples were heated in reflux condenser for 2 hours and centrifuged at 5000 rpm for 15 min at 4$^{o}$C. The supernatants were collected and air dried overnight on a petriplate. The air dried sample was dissolved in 10 ml solvent and stored at 4$^{o}$C until use.

***Phytochemical screening of Lobelia extracts***

The plant extracts were qualitatively screened for the presence of various phytochemicals such as alkaloids, carbohydrates, proteins and amino acids, saponins, phytosterols, phenolic compounds and gums according to the standard protocols[8]. The phytochemicals that were detected in the extracts were quantitatively estimated using UV–visible spectrophotometer.

***Evaluation of antimicrobial activities***

The antimicrobial activities of the plant extracts were evaluated against various bacterial and fungal pathogens using disc diffusion method on Mueller Hinton agar and Sabouraud Dextrose agar respectively.

# RESULTS

***Callus induction and sub-culturing***

Swelling and curling of the leaf explants were noted at the end of first week, on both the media. After 4 weeks, callus induction was observed on MS media containing 1mg/ml 2, 4-D and 1mg/ml Kinetin. Callus developed fully after 6weeks of incubation. The developed callus were sub cultured and a good mass of callus was obtained after 40 days of incubation Fig.1, following which it was dried and a total of 6 g of dried callus was obtained.

***Phytochemical analysis***

The final concentrations of the plant extracts were 50 mg/ml. The callus extract concentration was 20 mg/ml. The phytochemical screening revealed the presence of alkaloids, carbohydrates and phytosterols in the samples. The significant results of the screening have been presented in Table 1. The detailed results of the quantitative estimations of the phytochemicals, which were detected in the plant extracts, have been represented in Fig 2, 3 and 4.

***Antimicrobial activity***

The antimicrobial activities of the extracts were tested against the respiratory pathogens. Maximum inhibitory activity was recorded for methanolic and ethanolic extracts of inflorescence on *K. pneumoniae* and *S. aureus* respectively**.** Both ethanolic and methanolic extracts of leaf showed minimum inhibition for *S. marcescens, K. pneumonia* and *S. aureus*. Among all the stem extracts, ethanolic extract was most active against *S. marcescens*. Root extract, especially ethanolic extract, was active against *S. marcescens* and *S. aureus*. The results clearly suggested that the ethanolic extracts exhibited better inhibitory activity than the methanolic extracts. Ethanolic extract of callus was also active against *S. marcescens.* The detailed results have been summarized in Table 2. Aqueous extracts showed no zone of inhibition against the bacteria tested.

**Table 1**
***Qualitative phytochemical screening of various extracts of different parts of Lobelia inflata***

| | STEM | | | LEAVES | | | ROOT | | | INFLORESCENCE | | | CALLUS | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Extracts** | **E** | **A** | **M** | **E** | **A** | **M** | **E** | **A** | **M** | **E** | **A** | **M** | **E** | **A** | **M** |
| **Alkaloids** | | | | | | | | | | | | | | | |
| Mayer's | + | - | + | + | - | + | + | - | + | + | - | + | + | - | + |
| Wagners | + | - | + | + | - | + | + | - | + | + | - | + | + | - | + |
| Hager's | + | - | + | + | - | + | + | - | + | + | - | + | + | - | + |
| Dragendroff's | + | - | + | + | - | + | + | - | + | + | - | + | + | - | + |
| **Carbohydrates** | | | | | | | | | | | | | | | |
| Molisch's | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Fehling's | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Barfoed's | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Benedict's | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Saponin** | | | | | | | | | | | | | | | |
| Foam test | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Proteins & Amino acid** | | | | | | | | | | | | | | | |
| Million's | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Biuret | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ninhydrin | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Phytosterols** | | | | | | | | | | | | | | | |
| Liebermann-Burchards | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Phenolic compounds** | | | | | | | | | | | | | | | |
| Ferric chloride | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Gelatin | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Lead acetate | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Alkaline reagent | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Gum and mucilage** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

*Keys: E, ethanolic; A, aqueous; M, methanolic; +, positive; -, negative.*

**Table 2**
***Zone of inhibition for bacterial pathogens***

| | Zone of Inhibition (Diameter in mm) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***K. pneumoniae*** | | | | | | ***S. marcescens*** | | | | | | ***S. aureus*** | | | | | |
| | AE | AC | EE | EC | ME | MC | AE | AC | EE | EC | ME | MC | AE | AC | EE | EC | ME | MC |
| **Inflorescence** | - | - | 8 | 6 | 11 | 7 | - | - | 9 | 8 | 12 | 10 | - | - | 11 | 7 | 7 | 6 |
| **Leaf** | - | - | 7 | 6 | 7 | 6 | - | - | 9 | 8 | 8 | 7 | - | - | 9 | 7 | 9 | 7 |
| **Stem** | - | - | 9 | 7 | 8 | 7 | - | - | 10 | 7 | 8 | 7 | - | - | 8 | 7 | 7 | 7 |
| **Root** | - | - | 8 | 6 | 7 | 6 | - | - | 14 | 11 | 9 | 9 | - | - | 9 | 6 | 7 | 6 |
| **Callus** | - | - | 7 | 6 | 6 | 6 | - | - | 9 | 7 | 9 | 7 | - | - | 8 | 7 | 6 | 6 |

***Keys: AE, Aqueous extract; AC, aqueous control; EE, ethanol extract; EC, ethanol control; ME, methanol extract; MC- methanol control***

The methanolic extract of callus inhibited *C. neoformans,* with a zone of 8 mm. No other extract showed any antimicrobial activity on any fungus. This indicated that the *in vitro* callus sample has some variations in the phytochemical production which may be a parameter for determining the antimicrobial activity. The significant results have been listed in Table 3.

**Table 3**
***Zone of inhibition for fungal pathogens***

| | Zone of Inhibition (Diameter in mm) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A. niger* | | | | | | *C. albicans* | | | | | | *C. neoformans* | | | | | |
| | AE | AC | EE | EC | ME | MC | AE | AC | EE | EC | ME | MC | AE | AC | EE | EC | ME | MC |
| **Inflorescence** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Leaf** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Stem** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Root** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Callus** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 8 | - |

***Keys: AE, Aqueous extract; AC, aqueous control; EE, ethanol extract; EC, ethanol control; ME, methanol extract; MC- methanol control***

**Figure 1**
***a- Callus induction was observed from leaf explants on MS media containing 1mg/ml 2, 4-D and 1mg/ml Kinetin after 4 weeks. b- Fully developed callus after 6 weeks. c- Developed sub-cultured callus after 40 days of incubation.***

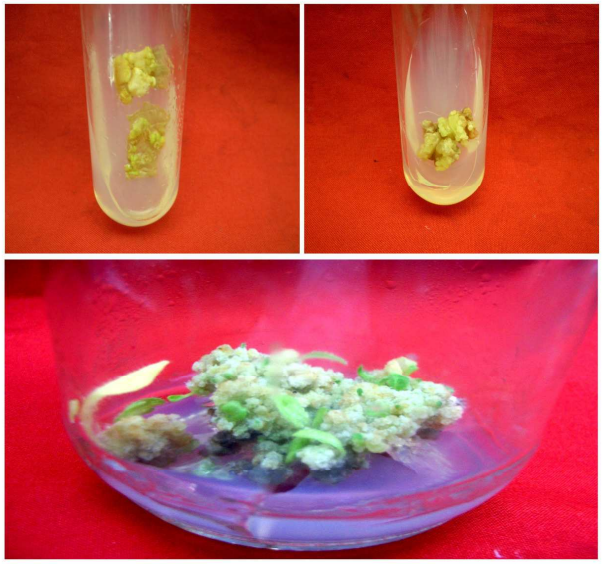


**Figure 2**
***Concentration of alkaloids found in various extracts obtained from different plant parts***

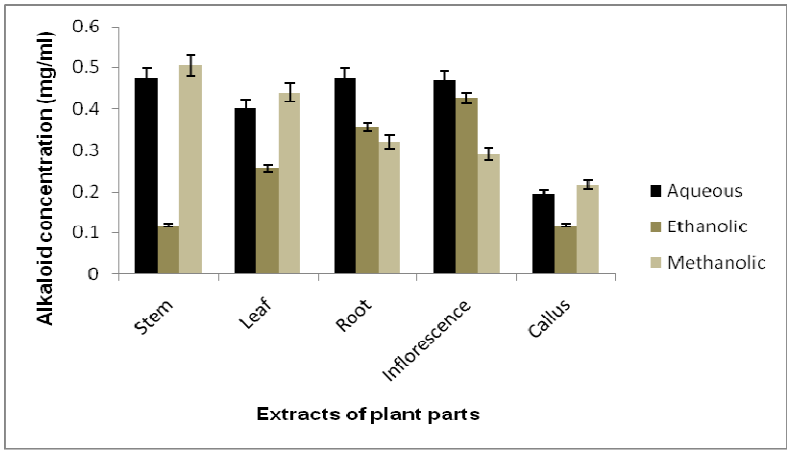



**Figure 3**
***Concentration of carbohydrates found in various extracts obtained from different plant parts***

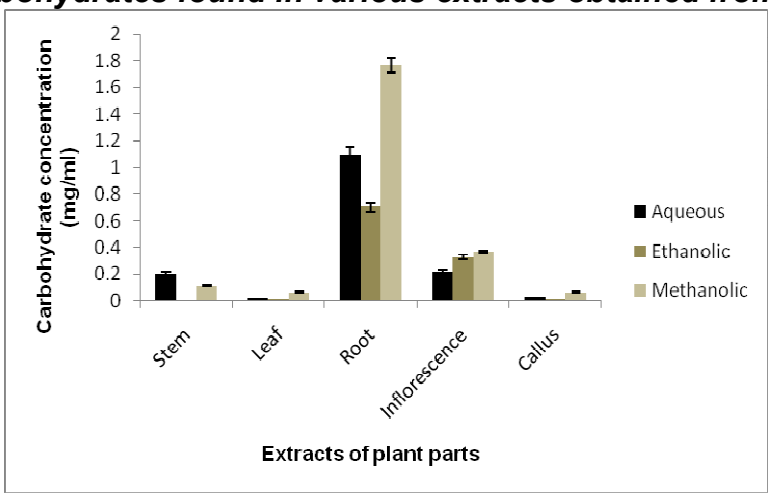
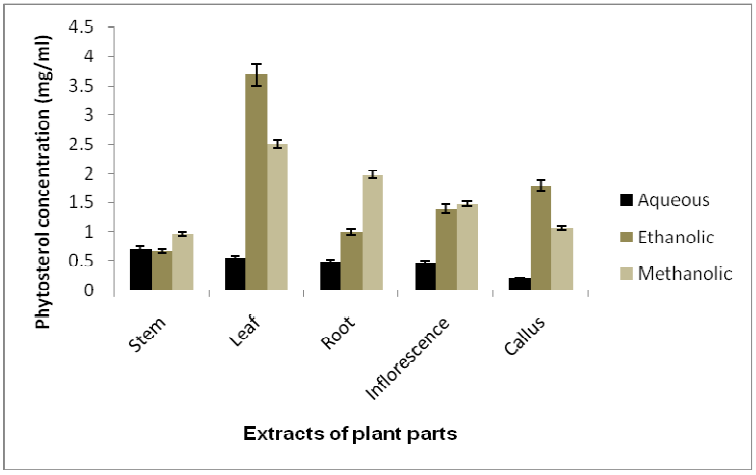



**Figure 4**
***Concentration of phytosterol found in various extracts obtained from different plant parts***



# DISCUSSION

The plants used in folklore medicines are of immense importance for novel herbal drug development and serve as substitutes to synthetic drugs due to minimum side effects after their medications. The study of the phytochemicals, its biological activity, identification of pharmacologically active molecules, detailed biological assays and formulation of dosage forms, followed by several phases of clinical studies is routinely used by pharmaceuticals.

*Lobelia inflata* L. is a wonder herb in certain views while a deadly poison in others. The plant in low doses is an emetic, sedative and used in respiratory disorders. The Encyclopedia of Medicinal Plants states that Indian tobacco is a valuable remedy for conditions such as bronchial asthma, coughs, chronic bronchitis and other ailments of the respiratory system[1].

The current study was inspired by the folklore use of *Lobelia inflata* L.. The plant is not well explored scientifically but known to be of immense potential in various clinical conditions. As the basic investigation on the plant, the current study involved the callus induction from the leaf explants to serve as the *in vitro* sample. The entire plant was dried and used for phytochemical screening and evaluation of antimicrobial activities of different parts of the plant such as inflorescence, leaf, stem, root and callus.

*In vitro* propagation was carried out on two media namely MS media and Gamborg's B5 media[9, 10, 11]. The callus induction was tried out with various concentrations and combinations of growth regulators. The successful callus induction was seen only on MS media containing 1mg/ml 2, 4-D and 1mg/ml kinetin. This result coincides with earlier studies on callus induction[9].

The phytochemical screening of the callus and different parts of the plant revealed the presence of alkaloids, carbohydrates and phytosterols. The presence of alkaloids is in accordance with that reported by[12, 13]. *Lobelia inflata* L. has been found to possess the alkaloid lobeline. Lobeline is used for various purposes such as nicotine substitute for smoking cessation, anti-depressants, respiratory stimulant and treatment for psycho stimulant abuse [12, 14, 15, 16, 17].

The phytochemical screening of *Lobelia inflata* L. for alkaloid, carbohydrate, saponin, phytosterols, proteins, amino acids and gums content is a novel attempt due to the insufficient literatures available on the phytochemical composition of the various parts of the plant. The carbohydrates, alkaloids and phytosterols were also detected in varying quantities in different parts of the plant indicating that each part has its own significant phytochemical content. This indicates that each part of the plant may be specifically used based on clinical necessities, like the use of leaves only as smoking deterrent.

The current study revealed the presence of highest amount of alkaloids in the stem extracts followed by root, inflorescence and leaf. This finding is in perfect accordance with the earlier studies of where it was found that different parts of *Lobelia inflata* L. have rich sources of alkaloids[18]. In our study, the leaf, callus and stem resulted in better extraction of alkaloids with methanol while root and inflorescence with aqueous extract. Thus it can be inferred that the plant can be pharmacologically important since plant parts possess high quantity of alkaloids, which can serve as analgesics, narcotics, CNS stimulants, to reduce hypertension and increase blood pressure[8].

Carbohydrates are one of the most common constituents of a plant. Since carbohydrates are the first products formed in photosynthesis, they are a convenient starting point for any discussion of constituents of vegetable drugs[19]. The metabolism of carbohydrates is of utmost importance to the organism, individually and collectively. Moreover, carbohydrates are the products from which, by subsequent organic reactions, a plant synthesizes a great number of other constituents. Carbohydrates were highest in the

methanolic extract of the root followed by aqueous extract. Following the root, the highest content of carbohydrates was found in the inflorescence organic extract. The high levels of carbohydrates in the roots of the plant suggests the fact that sugars can unite with a wide variety of other compounds to form glycosides and also that vitamin C or ascorbic acid is closely related to certain sugars like xylose[8]. An extensive use of the root of the plant can thus be made as an effective antioxidant, which is indeed novel in its approach since there is no previous scientific literature venturing into this aspect.

Phytosterols were detected in large quantity among all the other phytochemicals. The leaf extract had the highest content of phytosterols followed by the root. This finding may lead to the use of the leaves of the plant as an effective natural analgesic.

The phytochemicals showed better extraction with organic solvents than aqueous. The callus obtained from the leaf explants shows similar results to leaf extracts.

The plant is used in several respiratory disorders mainly asthma and bronchitis[20]. Thus the antimicrobial activity was mainly aimed against the pathogens of the respiratory tract. The aqueous extract showed no antimicrobial activity against any bacterium or fungus. The maximum inhibitory activity was noted for methanolic and ethanolic extracts of inflorescence on *K. pneumonia* and *S. aureus* respectively[21]. Both ethanolic and methanolic leaf extracts showed minimum inhibitory effects against *S. marcescens, K. pneumonia* and *S. aureus*. The antibacterial activity observed with leaf extracts against *S. aureus* is also supported by the study conducted by[22]. Among all the stem extracts, ethanolic extract was most effective against *S. marcescens*. Root extract, especially, ethanolic extract was active against *Serratia marcescens* and *S. aureus*. The results indicated that the ethanolic extracts showed better inhibitory effects than the methanolic extracts. Callus ethanolic extract was most active against *S. marcescens*, whereas, callus methanolic extract was the only sample that inhibited the growth of fungus. It was active against *C. neoformans*, which indicates that the *in vitro* callus sample has some variations in the phytochemical production which may be a parameter for determining the anti-microbial activity.

The antimicrobial study was carried out with a concentration of 50 mg/ml of plant extract and 20 mg/ml of callus. Even at lower concentration than the dried plant parts, the callus shows anti-fungal activity, whereas, the other samples showed no activity. Thus *in vitro* propagation may increase the biological and medicinal properties of the plant. This also indicates that higher concentrations of the extracts may act as more active antimicrobial agents. Further investigations with individually isolated and purified phytochemical component from different plant parts may help in the detailed understanding of their association with the microbiological activity. The present study indicates that the plant *Lobelia* contains large reservoir of phytochemicals and highly potential antibacterial components that may be used for the development of phytomedicine for the therapy and treatments.

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