

## ANTIBACTERIAL ACTIVITY OF *LOBELIA NICOTIANIFOLIA* AGAINST VARIOUS BACTERIAL STRAINS

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### ABSTRACT

Indian tribal folks had used plant based natural products as curative agents for various illnesses, which is evidenced from Rig-Veda dated back 1500 B.C. In this study, powdered plant material, leaves of *Lobelia nicotianifolia* sequentially extracted using methanol, ethyl acetate, acetone, chloroform, petroleum ether and water. Then the extracts were concentrated to dryness by evaporating the solvent at 40°C. In vitro antibacterial activity of these extracts was studied by agar well diffusion method against gram positive *Staphylococcus aureus* and gram negative *Pseudomonas aeruginosa*, *Salmonella typhi* as well as *Escherichia coli*. Ciproflaxin, Cephotoxime and Amoxycillin antibiotic discs were used as standard. This study demonstrated that the methanol extract has higher antibacterial activity against clinical isolated bacterial pathogens than other extracts. All these extracts were able to inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* except petroleum ether extract and ethyl acetate extract. This present study concluded that *Lobelia nicotianifolia* has strong antibacterial activity against clinical micro-organism. However, further studies should be needed for the isolation and characterization of these active compounds from *Lobelia nicotianifolia*.

**KEYWORDS:** *Lobelia nicotianifolia*, Muller Hinton agar, Bacterial infection.

### INTRODUCTION

In recent years, a number of new antibiotics had been produced and introduced in global market by pharmacological industries. The major problem is resistance to these drugs by micro-organisms has been increased (Ponmurugan Karuppiah *et al.*, 2013). As a result, there is a continuous search for new antimicrobial agent from plant sources (Gotep *et al.*, 2010). The acceptance of traditional medicine as an alternative approach in combating the multiple drug resistance microorganisms had led many researchers to investigate the antimicrobial activity of medicinal plants (Kadi *et al.*, 2011). Resistance of bacterial strains such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* against bactericides and antibiotics has been biggest challenges put forth in the pharmacology field to eradicate it. But some antimicrobial agents are

extremely irritant and toxic causing side effects in human. Thus, formulating new antibiotic without toxic and cost effective is the target put forth to scientists. Hence natural herbal medicines are gaining much attention for their cost effective and eco friendly and no side effects (Bocanegra Garcia *et al.*, 2009). *Staphylococcus aureus* is the commonest strain responsible for causing skin abscesses, carbuncles and soft tissue infection (Linda *et al.*, 2006). *Pseudomonas aeruginosa* is a gram negative aerobic rod which breaks the host defence system to initiate an infection, latter on leading to pneumonia (Vijaya Chaudhari *et al.*, 2013). *Salmonella typhi* is a gram negative pathogenic bacteria causing typhoid (Sandhya A. Marathe *et al.*, 2012). *Escherichia coli* is a gram negative bacteria causing various infections especially urinary tract infection (Niranjan *et al.*, 2014). Considering the herbal plants as antimicrobial agent, an investigation was done to

screen the antibacterial activity form *Lobelia nicotianifolia* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*.

## MATERIALS AND METHODS

### Source of plant material

*Lobelia nicotianifolia* were collected from the Nilgiri hills, Ooty. They were maintained under optimum green house conditions in Chennai.

### Plant extract preparation

*Lobelia nicotianifolia* leaves were subsequently washed with tap water then with distilled water and dried at room temperature for two weeks. The dried plant materials were powdered with a mixer grinder to 2 mm or smaller particle size. The ground plant species were stored in a storage container at room temperature. Two grams of dried and powdered plant material were separately mixed with 20ml of methanol, ethyl acetate, acetone, chloroform, petroleum ether and water kept on rotary shaker at 190-220 rpm for 24hours. It was then filtered through whattman filter paper no.1 and centrifuged at 5000g for 15minutes. The supernatant was collected and the solvent was allowed to evaporate to make the final Volume to one-fourth of the original, after which it was dissolved in dimethyl sulfoxide (Nair *et al.*, 2007).

### Test microorganisms

The test micro organisms used were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*. These microbial strains included various drug resistant hospital isolates collected and characterized in the central research laboratory, SMV Medical College and Hospital, Puducherry, India. All strains were maintained in the brain heart infusion agar medium kept at 4°C prior to use for antibacterial tests respectively.

### Determination of Zone of Inhibition by Agar Well Diffusion Method

Muller Hinton agar was prepared and poured into petri plate, specific bacterial cultures were spread over the medium and wells are carved out over the medium with cork-borer (0.85 cm) different aliquotes of the test plant extract was added into the well and plates were incubated overnight at 37°C, sterile cotton swab was immersed into the culture until it is thoroughly wet. The surplus suspension was removed from the swab by rotating against the inside of the culture tube and spread the entire surface of each MHA plate with the particular cultures. The cultures were spread evenly in three directions. So that ever confluent growth will be resulted (Murray *et al.*, 1995). The crude extracts of chloroform, acetone, ethyl acetate, methanol and petroleum ether of *lobelia nicotianifolia* were loaded on the well at concentrations 25µL, 50µL, 75µL, 100µL, 150µL and 200µL, allowed to dry. A parallel control was made by placing each antibiotic disc on the agar surface using aseptic condition. Incubated for 24 hours at 37°C and then zone of diameters in mm for each organic extracts were measured.

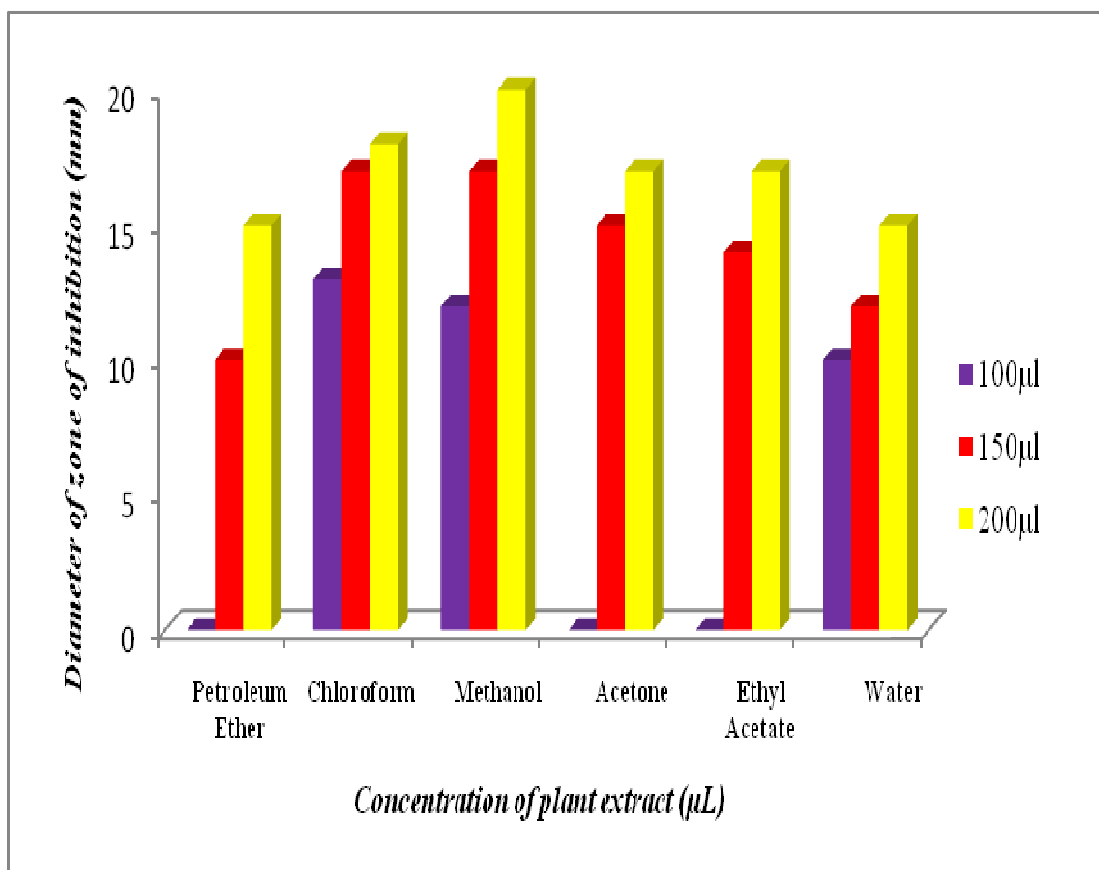
## RESULTS AND DISCUSSION

### Antibacterial activity

In this study, antibacterial activity of the plant *Lobelia nicotianifolia* was investigated. *Lobelia nicotianifolia* was extracted in six different solvents such as petroleum ether, chloroform, methanol, acetone, ethyl acetate, and water. The antibacterial activity of each solvent extract of the *Lobelia nicotianifolia* against four different clinical isolates such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* was analysed using the agar well diffusion method. Of the different concentrations of *Lobelia nicotianifolia* plant extract studied, 200µL showed maximum growth inhibition in all the clinical isolates studied.

**Table 1**  
**Zone of growth inhibition (mm) by Antibiotics**

Bacteria studied	Ciproflaxin	Cephotoxime	Amoxycillin
<i>Staphylococcus aureus</i>	19	18	24
<i>Pseudomonas aeruginosa</i>	16	18	24
<i>Salmonella typhi</i>	8	10	14
<i>Escherichia coli</i>	11	10	12

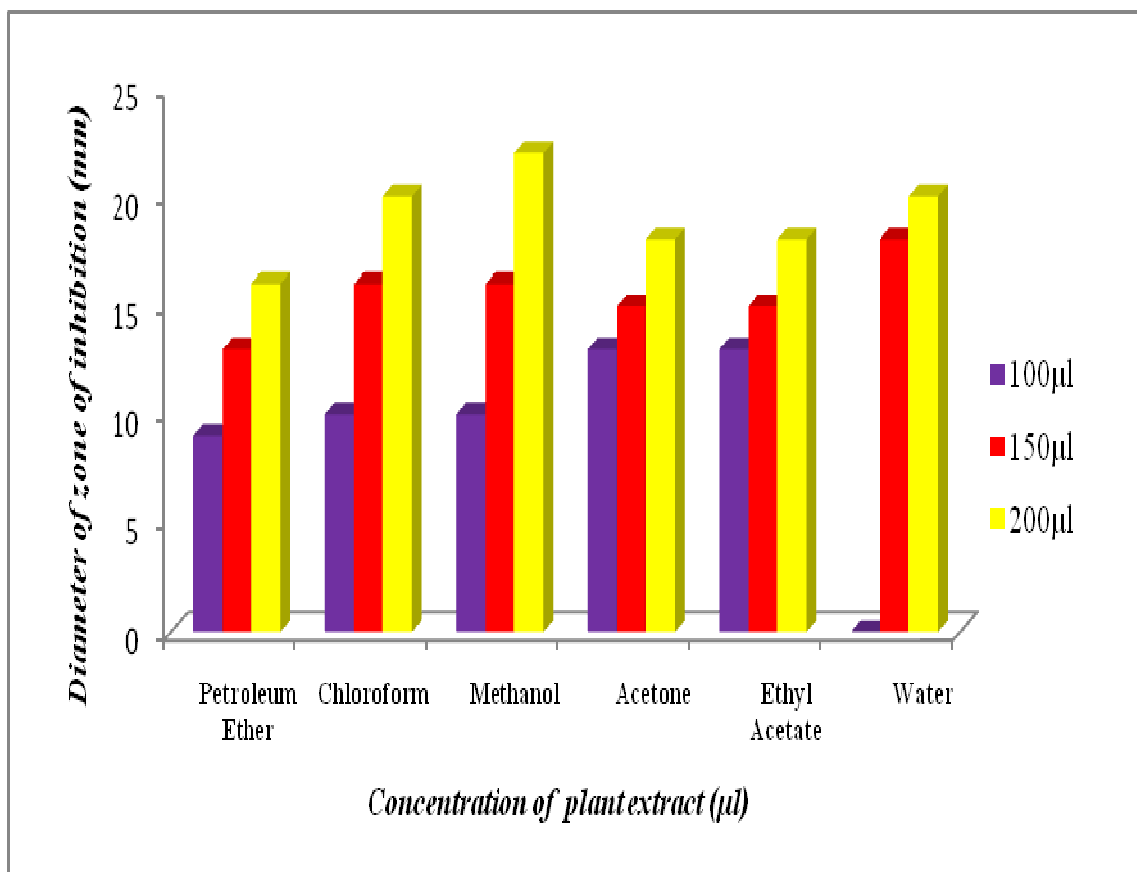


**Figure 1**

***Zone of inhibition (mm) of Staphylococcus aureus by Lobelia nicotianifolia extracted in different solvents***

Figure 1 shows the antibacterial effect of lobelia nicotianifolia extracted in six different solvents on *Staphylococcus aureus*. The methanolic extract showed strong antibacterial activity against *Staphylococcus aureus*. The chloroform, acetone and ethyl acetate extract showed moderate antibacterial activity, while, water and Petroleum

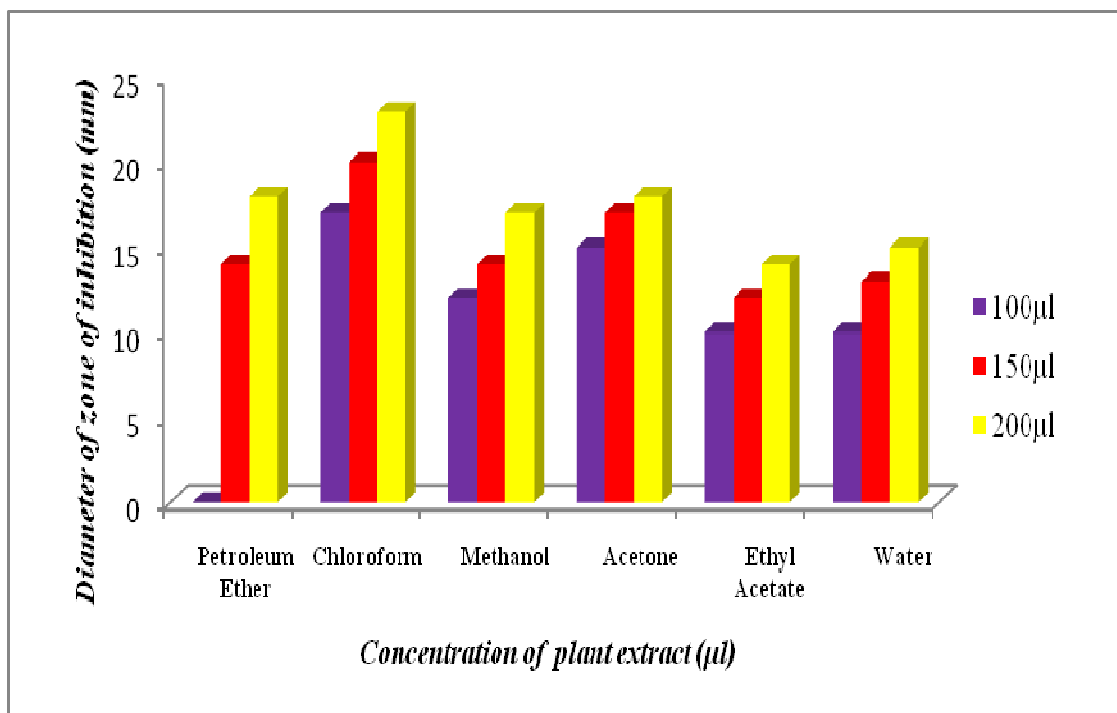
ether extract showed comparatively less antibacterial activity against *Staphylococcus aureus* at concentration of 200µL. The growth inhibition of the methanolic extracts (20mm) which is comparable to that shown by standard antibiotic ciprofloxacin (19mm), cephotaxime (18mm) against *Staphylococcus aureus* (See table 1).



**Figure 2**  
***Zone of inhibition (mm) of Pseudomonas aeruginosa by Lobelia nicotianifolia extracted in different solvents***

Figure 2 shows the antibacterial effect of lobelia nicotianifolia extracted in six different solvents on *Pseudomonas aeruginosa*. The methanolic extract showed strong antibacterial activity against *Pseudomonas aeruginosa*. The chloroform, acetone, ethyl acetate and water extract showed moderate antibacterial activity, while Petroleum ether extract

showed comparatively less antibacterial activity against *Pseudomonas aeruginosa* at concentration of 200µL. The growth inhibition of the methanolic extract (22mm) is slightly better than that shown by standard antibiotic ciprofloxacin (16mm), cephotaxime(18mm) against *Pseudomonas aeruginosa* (See table 1).

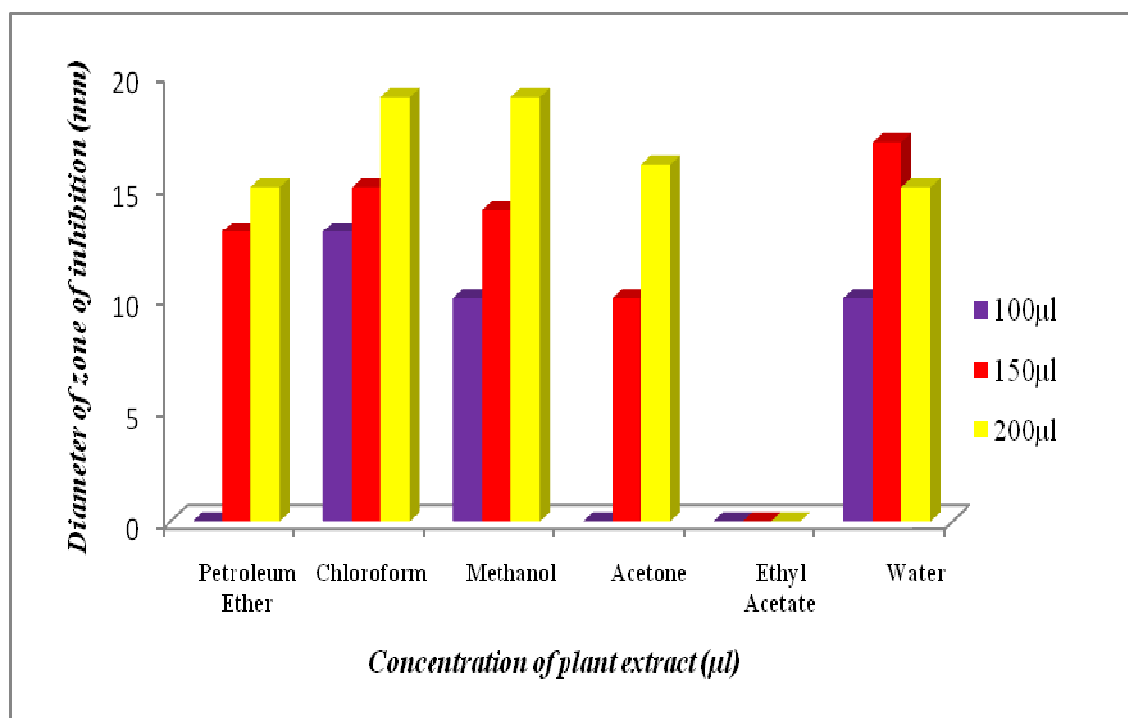


**Figure 3**

**Zone of inhibition (mm) of Salmonella typhi by Lobelia nicotianifolia extracted in different solvents**

Figure 3 shows the antibacterial effect of Lobelia nicotianifolia extracted in six different solvents on Salmonella typhi. The chloroform extract of the Lobelia nicotianifolia showed strong antibacterial activity against Salmonella typhi. The petroleum ether, methanol, acetone and water extract showed moderate antibacterial activity, while ethyl acetate

extract showed comparatively less antibacterial activity against Salmonella typhi at concentration of 200µL. The growth inhibition of the chloroform extract(23mm) is comparatively greater than that shown by standard antibiotic ciprofloxacin(8mm), cephotaxime(10mm) and amoxicillin(14mm) against Salmonella typhi (See table 1).

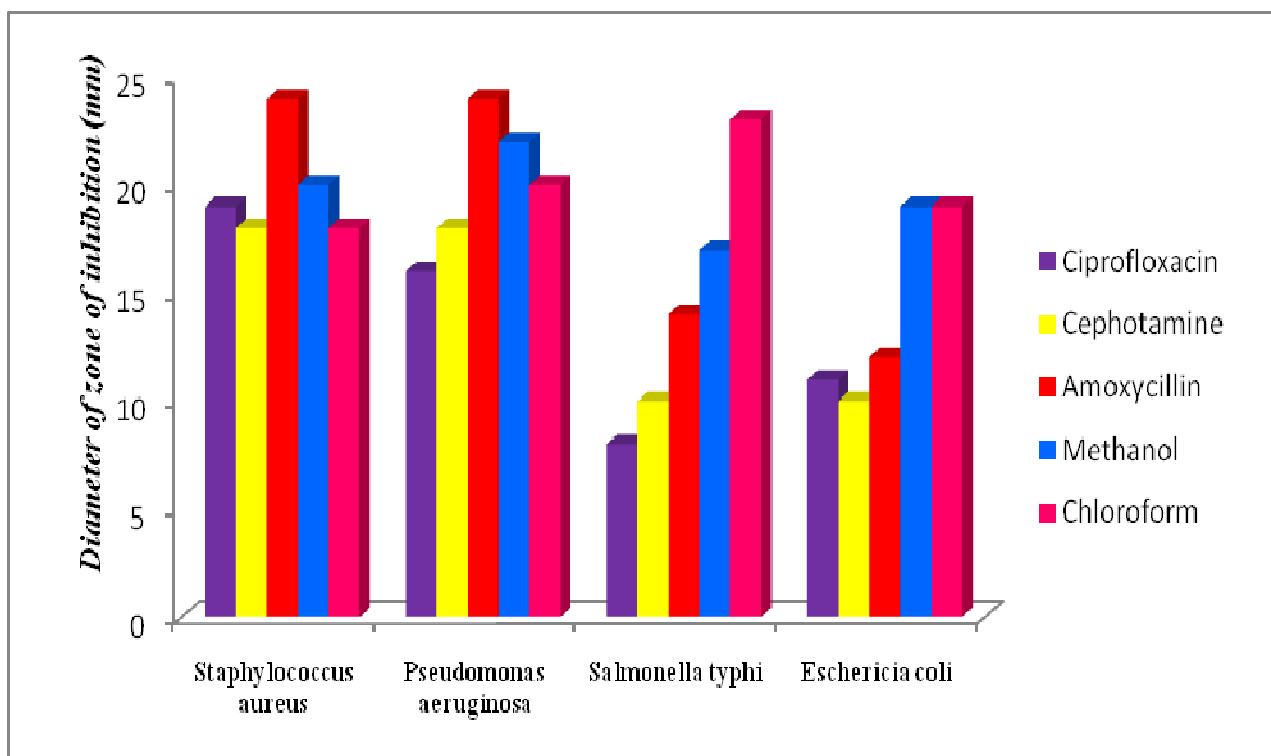


**Figure 4**

**Zone of inhibition (mm) of Escherichia coli by Lobelia nicotianifolia extracted in different solvents**

Figure 4 shows the antibacterial effect of *Lobelia nicotianifolia* extracted in six different solvents on *Escherichia coli*. The chloroform, methanol extract of the *Lobelia nicotianifolia* showed strong antibacterial activity against *Escherichia coli*. The petroleum ether, acetone and water extract showed moderate antibacterial activity, while ethyl acetate extract showed no antibacterial activity against *Escherichia coli* at concentration of 200 $\mu$ L. The

growth inhibition of the chloroform extract(19mm) and methanol(19mm) is comparatively greater than that shown by standard antibiotic ciprofloxacin(11mm), cephotaxime(10mm) and amoxicillin(12mm) against *Escherichia coli* (See table 1). From these studies it is evident that solvents such as methanol and chloroform are more suitable for extracting the antibacterial compound from *Lobelia nicotianifolia*.



**Figure 5**  
*Comparing the zone of inhibition (mm) of various strains by Lobelia nicotianifolia with the standard antibiotic disc*

It is also observed from Figure 5, the methanol extract of *Lobelia nicotianifolia* was found to be as effective as standard antibiotic ciprofloxacin, cephotaxime against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and the chloroform extract of *Lobelia nicotianifolia* was more effective than antibiotics ciprofloxacin, cephotaxime and amoxycillin against *Salmonella typhi* and *Escherichia coli*.

## CONCLUSION

From these observations, it can be inferred that different bioactive compounds present in *Lobelia nicotianifolia* possessing antibacterial activity against different bacterial species and is found to

be soluble either in methanol, a high polar solvent or chloroform, a mid polar solvent. Also, it is observed that, the methanol extract of *Lobelia nicotianifolia* was found to be as effective activity as antibiotics ciprofloxacin, cephotaxime against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and the chloroform extract of *Lobelia nicotianifolia* was more effective than standard antibiotics ciprofloxacin, cephotaxime and amoxycillin against *Salmonella typhi* and *E.coli*. From these findings, it can be suggested that these solvent extracts of *Lobelia nicotianifolia* would appear to provide the scientific basis for the use of dressing of wound, burns, boils, cuts and also in other antibacterial preparations.

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