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ORIGINAL ARTICLE

Bioprospecting of *Lobelia nicotianifolia* Roth. Plant Parts for Antioxidant and Cytotoxic Activity and its Phytoconstituents

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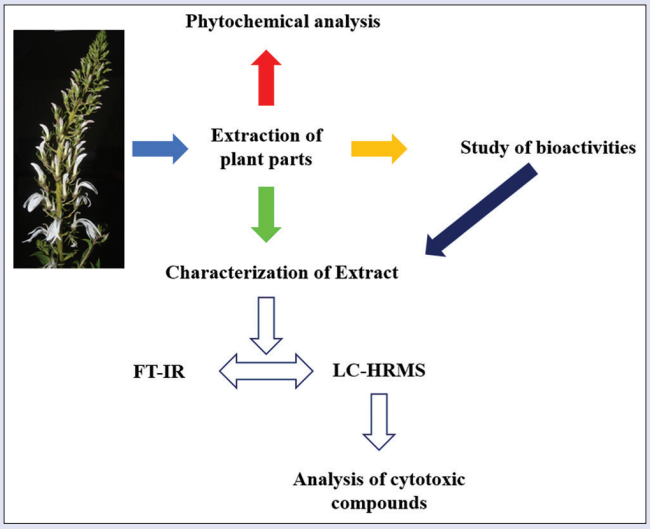
ABSTRACT

Background: Though the *Lobelia nicotianifolia* Roth. is ethnobotanically important plant of India and Sri Lanka its phytoconstituents, antioxidant, and anticancer potential was not yet reported. **Objective:** The objective of this study is to analyze the phytoconstituents of plant parts of *L. nicotianifolia* and to determine its antioxidant and cytotoxic potential. **Materials and Methods:** The plant parts of *L. nicotianifolia* were extracted with different solvents and qualitative analysis revealed the presence of different phytoconstituents. Total phenolic content (TPC) and total flavonoid content (TFC) were recorded in all plant parts. The extracts were subjected to the antioxidant assays and the potent methanolic extracts were used for cytotoxicity study and further characterized by Fourier–transform infrared spectroscopy and liquid chromatography with a high resolution mass spectrometer (LC–HRMS). **Results:** The qualitative analysis showed the presence of a wide array of phytoconstituents in *L. nicotianifolia* plant parts. A significantly higher TPC, TFC, and antioxidant activities were seen in methanolic stem extract. Stem extract showed maximum cytotoxicity against human breast adenocarcinoma (MCF–7) and human cervical adenocarcinoma (HeLa) cell lines whereas, root extract had higher cytotoxicity against human colon adenocarcinoma (HCT–15) cells. The results of cell viability indicated that the methanolic extracts of *L. nicotianifolia* plant parts exhibited a range of cytotoxic activity in a concentration and time dependent manner against selected cancer cell lines. The LC–HRMS showed the presence of cytotoxic compounds comparatively higher in stem. **Conclusion:** The study confirms the antioxidant and cytotoxic potential of *L. nicotianifolia*. To understand the detailed mechanism of cytotoxicity of *L. nicotianifolia*, it is necessary to study the molecular mechanism involved in this study.

Key words: Antioxidant, cytotoxic, HCT–15, HeLa, *Lobelia nicotianifolia*, MCF–7

SUMMARY

- The phytoconstituents, antioxidant, and anticancer potential of *Lobelia nicotianifolia* Roth. plant parts were analysed
- Methanolic extracts of stem showed significantly higher phenolic compounds and antioxidant activity
- Stem and root extracts has higher cytotoxicity against MCF–7, HeLa and MCF-7 cell lines respectively
- Cytotoxicity of *L. nicotianifolia* plant parts were depending on concentrations of extracts and treatment duration
- Chemical characterization of stem by LC–HRMS showed 23 cytotoxic compounds



Abbreviations used: TPC: Total phenolic content; TFC: Total flavonoid content; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); RSA: Radical scavenging activity; HeLa: Human cervical carcinoma; MCF–7: Human breast carcinoma; HCT–15: Human colon adenocarcinoma; ATR–FTIR: Attenuated total reflectance Fourier–transform infrared spectroscopy; LC–HRMS: Liquid chromatography with high-resolution mass spectrometer; HPLC: High-performance liquid chromatography; DMSO: Dimethyl sulfoxide; RT: Room temperature; TAE: Tannic acid equivalent; QE: Quercetin equivalent; MEM: Minimum essential medium; FBS: Fetal bovine serum; $AlCl_3$: Aluminum chloride, CO_2: Carbon dioxide; Abs: Absorbance; RPMI 1640: Roswell Park Memorial Institute media.

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INTRODUCTION

The morbidity and mortality in humans are mainly because of four main non-communicable diseases such as cardiovascular disease, chronic respiratory disease, cancer, and diabetes.[1] In 2018, mortality because of cancer was 9.6 million and increased up to 18 million by 2020.[2,3] There are different types of life-threatening cancers of which cervical (19.6%), breast (12.5%), and colon (23.4%) cancer are most common.[2] Based

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on the type of cancer and its progression, it is treated with different therapies such as hormone therapy, chemotherapy, and radiotherapy.[4] Among all these, extensively employed approach is chemotherapy. The use of chemotherapy for the management of cancer is found to be insubstantial because of the development of multidrug resistance in the cancer cells[5] and other severe adverse effects as reviewed by Aslam *et al.*[6] To overcome the limitations more attention has been paid to the alternatives and complementary plant-based natural products.[6,7] Medicinal plants are known for their wide array of phytoconstituents of which phenolics are recognized for their free radical scavenging and anticancer property.[8-10] In most of the cases, cancerous conditions are related to the overproduction of free radicals in cells.[11] Cells are capable of neutralizing the consequences of free radicals by producing antioxidants.[12] Based on origin, the antioxidants are categorized as endogenous and exogenous. Endogenous antioxidants are produced within the cells while exogenous sources are mainly obtained primarily from different plants. The exogenous antioxidants are in limelight due to lower side effects and their cost. The consumption of different antioxidants in an appropriate amount reduces the chances of morbidity and mortality due to cancer.[13]

Campanulaceae is one of the important family of the tropical and warm temperate regions[14] which is known for its bioactive alkaloids and phenolic compounds.[15] The genus Lobelia is known to have numerous bioactivities like antitumor, immunomodulatory, anti-inflammatory, antioxidant, antiviral, antipyretic, and antidiabetic activities.[16] *Campanulaceae* consist of approximately 300 taxa of which *Lobelia chinensis* and *Lobelia inflata* are native to China and northern America respectively and are well explored for its phenolic compounds and anticancer activity.[17,18] Flavonoids such as apigenin and luteolin are reported from *L. chinensis* which are well associated with the antioxidant and anticancer property.[17] Nevertheless, such a scientific study has not yet been documented for *Lobelia nicotianifolia* Roth. It is a common plant of Indo-Malayan region and the ethnobotanical studies revealed that it is used in the treatment of numerous diseases and disorders.[19] With the help of this ethnobotanical data researchers have documented analgesic,[20] antimicrobial,[21] antioxidants,[22] and antiepileptic[23] activities. Considering the antioxidant and anticancer potential of other over-exploited Lobelia species there is scope to have similar bioactivities in *L. nicotianifolia* which can be used as a substitute.

Considering the medicinal value of *L. nicotianifolia*, in the present study plant parts were extracted with different solvents and preliminary phytochemical analysis was reported to perceive different secondary metabolites. The quantitative analysis was done for the total phenolic content (TPC) and total flavonoid content (TFC). Ultimately our study aimed at determining the *in vitro* antioxidant potential using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. The study was further extended to elaborate the potential cell growth inhibitory effects of crude extracts of *L. nicotianifolia* on different human cancer cell lines such as human cervical carcinoma (HeLa), human breast carcinoma (MCF–7), and human colon adenocarcinoma (HCT–15). The promising cytotoxic effects were tested against cancer cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and the potent extract was characterized with Fourier–transform infrared (FTIR) spectroscopy and liquid chromatography with high-resolution mass spectrometer (LC–HRMS).

MATERIALS AND METHODS

Plant material and authentication

L. nicotianifolia was collected around the Kas lake area of Satara district and was identified and authenticated using Flora of Maharashtra state,[24] India, and the herbarium specimen (NGCPR–1904) was deposited to NGCPR, Shirwal.

Chemicals, reagents, and standard

HPLC and analytical reagent grade organic solvents and chemicals used for extraction were procured from Himedia, India. Tannic acid (GRM7541-100G) and quercetin (RM6191-100G) from Himedia, India, were used as standard for quantification of total phenolics and flavonoids. The HeLa, MCF–7, and HCT–15 cell lines were obtained from National Centre for Cell Sciences, Pune, India. Minimum essential Medium (10370-021), Fetal Bovine Serum (2614079), Roswell Park Memorial Institute (RPMI1640) (11875-085), penicillin and streptomycin (15140-122), dimethyl sulfoxide (D2650) were purchased from Sigma Aldrich. MTT (M6494) was procured from Invitrogen.

Extraction of plant parts

The dried leaf, stem, and root powder (10 g) were extracted with Soxhlet extractor with 100 mL of different solvents. The selection of solvents was based on the polarity where nonpolar (petroleum ether), mid–polar (chloroform), and polar (methanol) solvents were used. The obtained extracts were filtered through Whatman filter paper 1 and concentrated on rotary evaporator under reduced pressure. Obtained viscous extracts were stored at-20°C till further analysis.

Phytochemical analysis

Phytochemical tests for phenolics, flavonoids, anthraquinones, coumarin, terpenes, saponins, and alkaloids (Dragendroff's) were carried out for plant part extracts of *L. nicotianifolia* with some minor modifications.[25]

Quantitative analysis of phenolics and flavonoids

The TPC and TFC in *L. nicotianifolia* extracts were determined using a modified Folin–Ciocalteu method and Aluminum chloride ($AlCl_3$) [26] methods respectively. For TPC 50 microliter (µL) (equivalent to 100 µg) extracts were added to 2 N Folin–Ciocalteu (200 µL) and 1 mL of sodium carbonate followed by incubation of 30 min (min) at 25°C. TFC of different extracts was determined by the addition of an equal volume of extracts with 2% $AlCl_3$. This reaction mixture was incubated for 60 min at room temperature (RT). A UV–Visible spectrophotometer (Shimadzu UV-1900 UV–VIS) was used for the quantification of TPC and TFC at 765 and 420 nm respectively. A graph of absorbance against concentration (0–250 µg/mL) was plotted to obtain the calibration curve and TPC and TFC were expressed as milligrams of tannic acid equivalent (TAE) and quercetin equivalent (QE) per gram extract respectively.

Determination of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity

The percentage radical scavenging activity (% RSA) of *L. nicotianifolia* extracts against DPPH radical was evaluated as described by Zheleva-Dimitrova *et al.*[27] with minor modifications. In brief, 1 ml (mL) of extracts (100-500 µg/mL) was mixed with 4 mL methanolic DPPH (0.2 mM) solution and vortexed thoroughly. This reaction mixture was incubated in the dark for 30 min at RT and the absorbance was measured by spectrophotometer at 517 nm.

The percentage DPPH RSA was calculated using the following equation:

Percentage inhibition = (A0-A1)/A0) ×100 equation 1

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

Two stock solutions were prepared (A) ABTS (7 mM) and (B) potassium persulfate (2.4 mM).[27] The ABTS solution was prepared by mixing equal volumes

of stock A and B and allowed them to react in dark for 14 h at RT. One mL ABTS solution was further diluted with 60 mL methanol to attain a specific absorbance of 0.706 ± 0.01 at 734 nm by a spectrophotometric method. *L. nicotianifolia* extracts (1 mL) was reacted with 1 mL of the ABTS solution and the absorbance was recorded spectrophotometrically at 734 nm after 7 min. The percentage ABTS scavenging activity of the extract was calculated by the following formula:

Percentage inhibition = (A0–A1)/A0) ×100 equation 2

In equation 1 and 2: A0 is the absorbance of control and A1 absorbance of test. The results were compared with Ascorbic acid as reference standard.

In vitro cytotoxicity

Cell line and cell culture

The *in vitro* cytotoxicity of *L. nicotianifolia* extracts was studied against HeLa), MCF–7, and HCT–15 cell lines. The HeLa and MCF–7 cell lines were maintained in T-25 flasks with minimum essential medium (MEM), while HCT–15 cells were maintained in RPMI-1640 media with 10% heat-inactivated fetal bovine serum (FBS) and 100 U/mL and 100 µg/mL penicillin and streptomycin, respectively. These cell lines were maintained under an atmosphere of 5% Carbon dioxide (CO_2) and 95% humidity at 37°C until further study.

Cell proliferation assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide assay)

The *in vitro* cell viability of different human cancer cell lines was determined by MTT colorimetric assay. In total, 1 × 10^4 cells in 200 µL of the respective medium per well were seeded in a 96–well plate and incubated at 37°C under 5% CO_2. After 24 h (h) of incubation, the confluent cells were exposed to different concentrations of plant extracts in their respective culture media without FBS and incubated at 37°C under 5% CO_2. After completion of the treatment at 48 h, the medium was removed and the cells were washed with Hanks' balanced salt solution. Thereafter, 10 µL/well of 5 mg/mL concentration of MTT was added to the cells, and the cells were incubated for another 4 h at 37°C under 5% CO_2. Then, the MTT containing media was removed through aspiration and replaced with 200 µL of dimethyl sulfoxide, which was added to each well to dissolve the formazan crystals. The absorbance of the developed purple color was measured at 560 nm wavelength by using the spectrophotometer (Shimadzu, UV–Vis 1800). The results of cell viability were expressed as the percentage growth inhibition of treated and untreated cells, using the following formula:

Percentage inhibition = 100-(A0 ÷ A1) ×100 equation 3

In equation 3: A0 is the absorbance of treated cells at 560 nm and A1 absorbance of untreated (control) cells at 560 nm.

Cell morphology

Selected cells were seeded at 1 × 10^6 cells/well in a 6-well culture plate and incubated for 24 h at 37°C under 5% CO_2. Later, the cells were treated with different concentrations of plant extracts in their respective culture media without FBS and further incubated up to 48 h. The cytomorphology of the cells was then observed under phase contrast microscope (Primovert Carl Zeiss).

Characterization of extract

Analysis of functional groups by Fourier–transform infrared

The viscous extract was loaded in Attenuated total reflectance Fourier–transform infrared spectroscopy (ATR–FTIR) spectroscope (Shimadzu IRAffinity-1S 00466, Serial No. A221354), with a scan range from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. Prior to every scan, the ATR plate was carefully cleaned with 70% acetone. Further, the obtained results were processed through IR solution software.

Liquid chromatography with high-resolution mass spectrometer analysis of methanolic extracts

The chromatographic system (Agilent, USA) consisted of a binary LC pump (G1312B) with an autosampler (G1329B) and HRMS (G6540B). Extracts were injected onto RPC18 Zorbax (2.1 mm × 50 mm, 1.8-µm) column where the mobile phase for this analysis was two MS grade solvents (A) 0.1% Formic acid in water (95%) and (B) acetonitrile (5%) which was delivered at 0.4 mL/min. The ion source was Dual AJS ESI performing in positive or negative mode with a gas flow set at 8 L/min, spray voltage set at 3500 V, and auxiliary gas and capillary temperatures set at 325°C and 300°C, respectively. The stepwise gradient (A and B) was delivered for a different time duration which was 5% of phase A and 95% of phase B for 0–18 min and 25 min. Whereas, for 0–25 min and 30 min it was 95% of phase A and 5% of phase B. The injection volume was 10 µL and the HRMS full scans were acquired from *m/z* 60–1600 Da with a scanning rate of 2 scans/s. Mass calibration was done before the analysis using the Agilent Q–TOF ESI calibration mix.

Statistical analysis

All experiments were performed in triplicates and the values were expressed as Mean ± standard deviation. One-way analysis of variance followed by Duncan's new multiple range test to evaluate the significance at $P \leq 0.05$. The IC_{50} values of extracts were calculated using the Statistical Package for the Social Sciences – 11 (SPSS 11, IBM, USA) at 95% confidence level.

RESULTS AND DISCUSSION

Phytochemical analysis, total phenolic content, and total flavonoid content

In the present study leaf, stem, and root when extracted with nonpolar, mid-polar, and polar solvents showed the presence of phenolics,

Table 1: Presence of phytochemicals in leaf, stem, and root of *Lobelia nicotianifolia*

Phytochemical	Petroleum ether	Chloroform	Methanol
Leaf			
Phenolics	+	+	+
Flavonoids	+	+	+
Anthraquinone	–	–	+
Coumarin	–	–	+
Terpene	+	+	+
Saponins	+	+	+
Alkaloids	–	+	+
Stem			
Phenolics	+	+	+
Flavonoids	+	+	+
Anthraquinone	–	–	+
Coumarin	–	+	+
Terpene	+	+	+
Saponins	+	+	+
Alkaloids	–	+	+
Root			
Phenolics	+	+	+
Flavonoids	+	+	+
Anthraquinone	–	–	+
Coumarin	–	+	+
Terpene	+	+	+
Saponins	+	+	+
Alkaloids	–	+	+

+: Presence; –: Absence

flavonoids, anthraquinones, and coumarin [Table 1]. It has been reported that the solubility of different phenolics is governed by the polarity of extracting solvents.[8] Previous investigations of *L. chinensis* and *L. erinus* revealed the presence of phenolics and flavonoids.[28] Anthraquinones are active phytoconstituents of various plants and have been reported for the first time for *Lobelia* species [Table 1]. In the present investigation, methanolic extracts of leaf, stem, and root showed the presence of anthraquinones. Another important polyphenol, i.e. coumarin was detected in chloroform and methanolic extracts of leaf, stem, and root [Table 1]. The presence of coumarin in *L. nicotianifolia* has shown a concurrence with the previous study of *L. chinensis*.[29] In our study, nonpolar and polar extracts showed the presence of terpenoids while saponins were recorded in polar extracts only [Table 1]. Terpenoids have a broad range of chemical properties and can be detected in polar and nonpolar solvents whereas saponins are more soluble in polar solvents.[30,31] The occurrences of terpenoids and saponins have been reported from *L. chinensis* and *L. sessilifolia*.[30,32] Alkaloids were detected using Dragendorff's reagent in chloroform and methanolic extracts of leaf, stem, and root. Extensive research has been carried out on alkaloids of *Lobelia* and 46.05% species are known to produce pharmaceutically important alkaloids.[17] Plant phenolics and flavonoids are the important secondary metabolites known for their bioactivities.[8,26] In this study, higher TPC and TFC were seen in methanolic extracts of the stem as compared to leaves and root but with no significant difference among the plant parts used [Table 2]. The efficiency of extraction of TPC (mg TAE/g extract) was in following order methanol (13.46) > chloroform (9.57) > petroleum ether (3.22). For the extraction of TFC, the maximum content was seen in methanol

Table 2: Concentrations of total phenolic content and total flavonoid content in different plant part extracts (leaf, stem, and root) of *Lobelia nicotianifolia*

Extracts	Leaf	Stem	Root
TPC (mg TAE/g extract)			
PE	2.66±0.24^{c}	3.22±0.26^{c}	1.36±0.16^{c}
CHL	8.00±0.08^{b}	9.57±0.41^{b}	7.44±0.06^{b}
MET	11.47±0.14^{a}	13.46±0.48^{a}	10.45±0.47^{a}
TFC (mg QE/g extract)			
PE	1.77±0.05^{c}	2.72±0.27^{c}	1.04±0.04^{c}
CHL	6.91±0.09^{b}	8.05±0.19^{b}	6.54±0.16^{b}
MET	10.21±0.11^{a}	11.66±0.39^{a}	7.88±0.10^{a}

The results represent the mean of three independent experiments ± SD and the columns having different letters as superscripts are significantly differ from each other at P≤0.05. SD: Standard deviation; TPC: Total phenolic content; TFC: Total flavonoid content; TAE: Tannic acid equivalent; QE: Quercetin equivalent; PE: Petroleum ether; CH: Chloroform; MET: Methanol

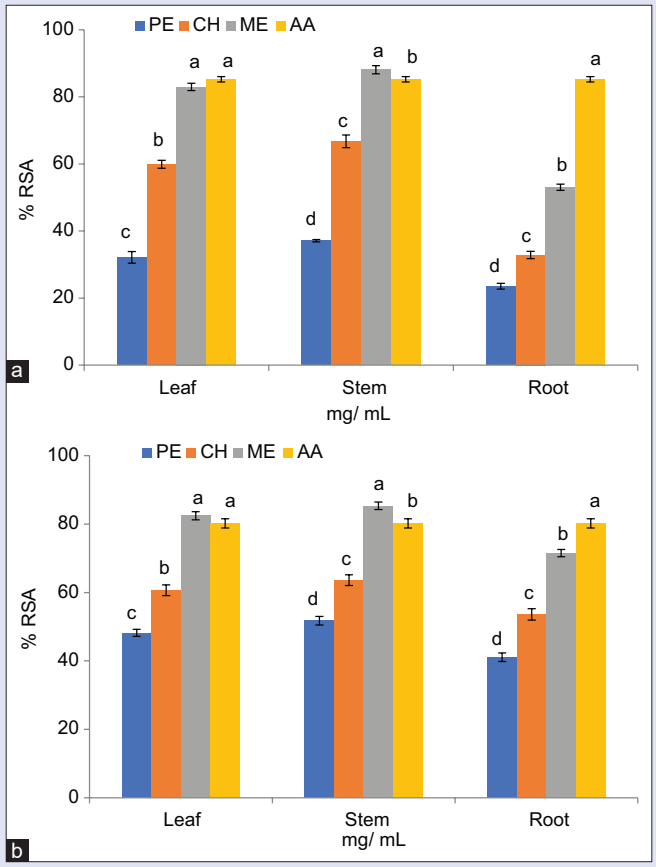

Figure 1: Representative histogram showing radical scavenging activity of *Lobelia nicotianifolia* plant parts (leaf, stem, and root) in (a) 2,2 diphenyl 1 picrylhydrazyl and (b) 2,2′ azino bis (3 ethylbenzothiazoline 6 sulfonic acid) assays at 1 mg/mL. The results represent the means of three independent experiments ± standard deviation and the different letters as superscripts are significantly different from each other at P≤0.05. PE: Petroleum ether; CH: Chloroform; ME: Methanol

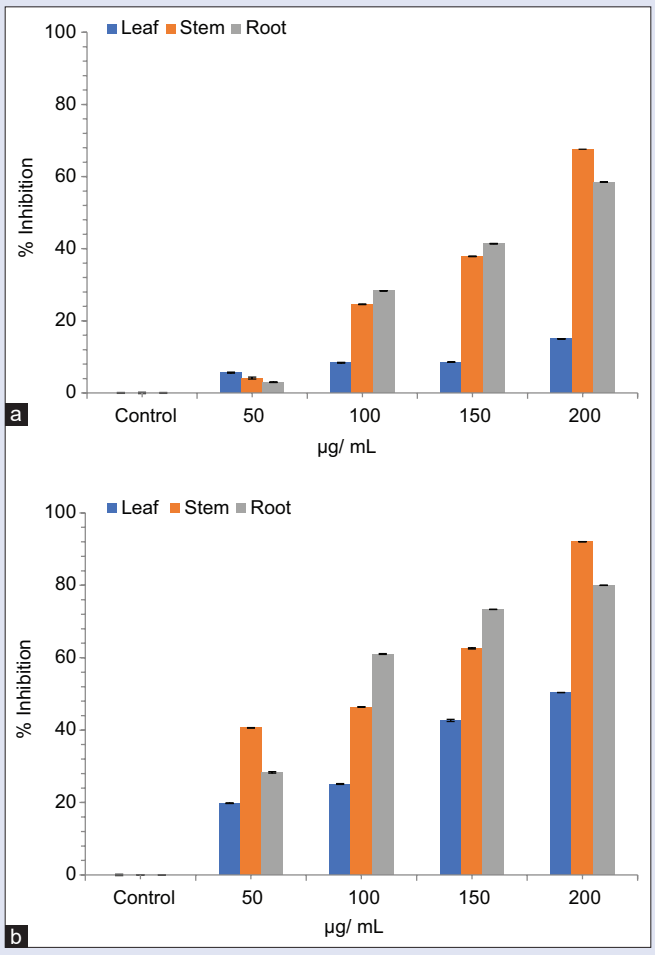

Figure 2: Representative histogram showing in vitro concentration dependent cytotoxicity of methanolic extracts *Lobelia nicotianifolia* plant parts (leaf, stem, and root) on HeLa cells after (a) 24 h and (b) 48 h treatments. The results represent the means of three independent experiments, and error bars represent the standard deviation of the mean

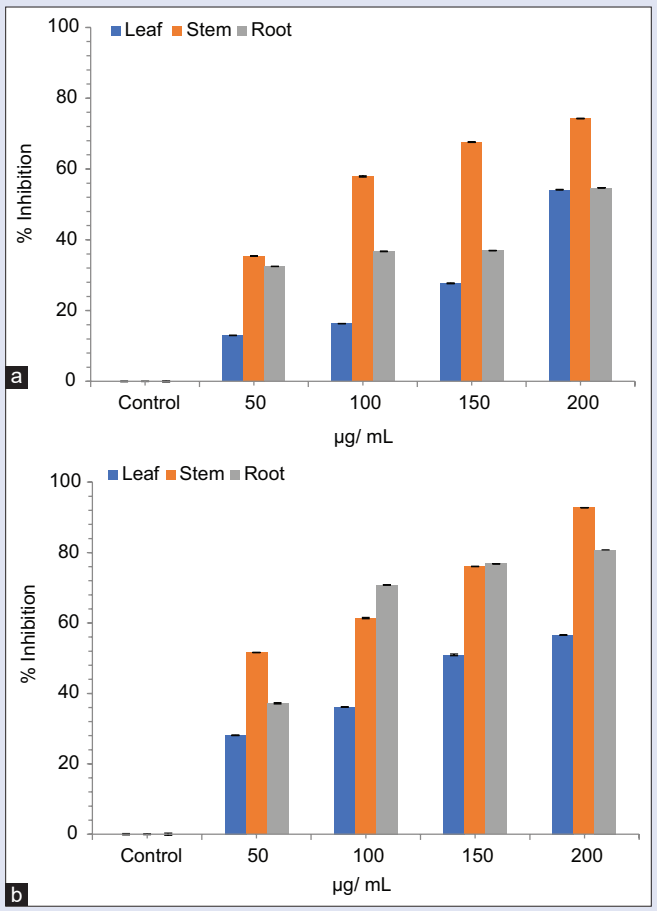

Figure 3: Representative histogram showing *in vitro* concentration dependent cytotoxicity of methanolic extracts *Lobelia nicotianifolia* plant parts (leaf, stem, and root) on MCF–7 cells after (a) 24 h and (b) 48 h treatments. The results represent the means of three independent experiments, and error bars represent the standard deviation of the mean

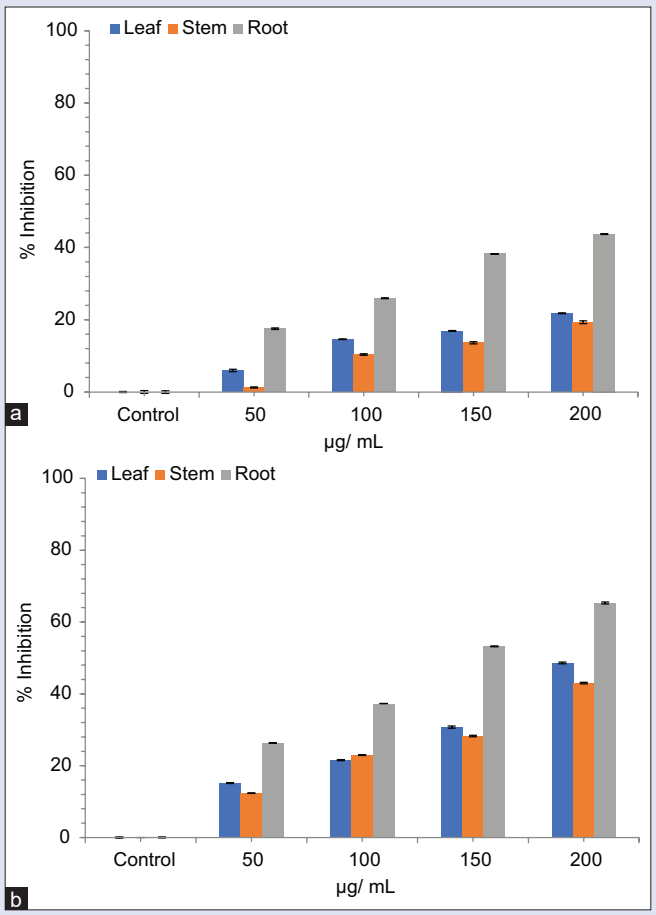

Figure 4: Representative histogram showing *in vitro* concentration dependent cytotoxicity of methanolic extracts *Lobelia nicotianifolia* plant parts (leaf, stem, and root) on HCT–15 cells after (a) 24 h and (b) 48 h treatments. The results represent the means of three independent experiments, and error bars represent the standard deviation of the mean

extract (11.66 mg QE/g extract) followed by chloroform (8.05 mg QE/g extract) and petroleum ether (2.72 mg QE/g extract) and a similar pattern was observed for leaf and root of *L. nicotianifolia*. The results represented in Table 2 express that the increase in solvent polarity linked with increased contents of TPC and TFC which showed the concurrence with previous studies.[33] A wide variety of phenolic compounds showed the presence of polysaccharides, proteins, terpenes, chlorophyll, inorganic compounds that dissolve in polar solvents.[33] Hence, in the present study, a higher amount of TPC and TFC were seen in methanol as compared to chloroform and petroleum ether [Table 2]. The variations of TPC and TFC in different plant parts of *L. nicotianifolia* may be related to the function of these phenolic compounds in plant species, life cycle, and the growth phase as previously reported for different plant species.[8,34]

In vitro antioxidant assays and correlation with total phenolic content and total flavonoid content

DPPH and ABTS % RSA of plant part extracts of *L. nicotianifolia* were evaluated in the present investigation and are shown in Figure 1. The DPPH % RSA was seen in the range of 32.87%–88.08% based on plant parts and extracting solvents [Figure 1a]. The DPPH % RSA of methanolic stem extract (88.08%) was higher as compared to ascorbic acid (85.21%) and other studied extracts. The plant parts extracted with chloroform and petroleum ether has intermediate and lower DPPH % RSA respectively [Figure 1a]. A similar pattern was seen for ABTS % RSA for different plant parts of *L. nicotianifolia* with superiority for methanolic extracts of the

Table 3: Correlation analysis between total phenolic content and total flavonoid content of leaf, stem, and root of *Lobelia nicotianifolia* with 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assays

	DPPH			ABTS		
	Leaf	Stem	Root	Leaf	Stem	Root
TPC						
Leaf	0.995**	0.998**	0.943**	0.958**	0.954**	0.970**
Stem	0.992**	0.995**	0.936**	0.951**	0.948**	0.964**
Root	0.985**	0.993**	0.915**	0.933**	0.929**	0.949**
TFC						
Leaf	0.995**	0.998**	0.942**	0.957**	0.954**	0.969**
Stem	0.993**	0.996**	0.943**	0.957**	0.953**	0.968**
Root	0.958**	0.968**	0.853**	0.879**	0.872**	0.902**

**The correlation is significant at the 0.01 level (two-tailed). TPC: Total phenolic content; TFC: Total flavonoid content; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

Table 4: The putative identification of cytotoxic compounds in different plant parts (leaf, stem, and root) *Lobelia nicotianifolia* by liquid chromatography with high resolution mass spectrometer

Cytotoxic compounds	Formula	Mass	*m/z*	Source	Class	Reference
(+)-α-Tocopherol	$C_{15}H_{28}O_2$	240.2089	258.2426	L, S	P	[44]
(2S)-2-hydroxyphytanic acid	$C_{20}H_{40}O_3$	328.2977	327.2916	S	FA	[45]
11-Cyclohexyl undecanoic acid	$C_{17}H_{32}O_2$	268.2402	267.2339	S	FA	[46]
2-Isoprenyl emodin	$C_{20}H_{18}O_5$	338.1154	337.1067	S	AQ	[47]
Alphitolic acid	$C_{30}H_{48}O_4$	472.3553	471.3464	S	TT	[48]
Anisatin	$C_{15}H_{20}O_8$	328.1158	327.1077	L	SQ	[49]
Apigenin	$C_{21}H_{20}O_{10}$	432.1056	431.0975	L	F	[50]
Benzoquinol	$C_{38}H_{58}O_4$	578.4335	578.4558	S	P	[51]
Bestatin	$C_{16}H_{24}N_2O_4$	308.1736	326.2079	R	PSI	[52]
Ceramide	$C_{30}H_{59}NO_3$	481.4495	499.4815	L	FA	[53]
Cholestane	$C_{27}H_{48}O_2$	404.3654	404.3881	S	TT	[54]
Cryptomeridiol	$C_{15}H_{28}O_2$	240.2089	258.2426	L	SQ	[55]
Cyasterone	$C_{29}H_{44}O_8$	520.3036	520.3276	S	S	[56]
Cycloheximide	$C_{15}H_{23}NO_4$	281.1627	286.1412	L	PSI	[57]
Delcorine	$C_{26}H_{41}NO_7$	479.2883	502.2759	L, S	A	[58]
Dihydroceramide	$C_{19}H_{39}NO_3$	329.293	334.2711	S	SL	[59]
Dodecanol	$C_{12}H_{26}O$	186.1984	191.1779	L	FAL	[60]
Embelin	$C_{17}H_{26}O_4$	294.1831	293.1752	S, R	P	[61]
Euphornin	$C_{33}H_{44}O_9$	584.2985	607.289	L, S	T	[62]
Galangin	$C_{15}H_{10}O_5$	270.0528	269.0449	L	F	[63]
Hernandezine	$C_{39}H_{44}N_2O_7$	652.3149	635.3104	L	A	[64]
Hispidulin	$C_{16}H_{12}O_6$	300.0634	299.0554	L	F	[65]
Ivermectin B1b	$C_{15}H_{23}NO_4$	281.1627	286.1412	L	L	[66]
Methyl oleate	$C_{19}H_{36}O_2$	296.2715	295.2655	S	FA	[67]
Octadecadienoic acid	$C_{18}H_{32}O_4$	312.2301	311.2228	S	FA	[68]
Palmitaldehyde	$C_{16}H_{32}O$	240.2453	258.2782	S	AH	[69]
Palmitic acid	$C_{16}H_{32}O_2$	256.2402	255.234	L, S, R	FA	[70]
Pentamidine	$C_{19}H_{24}N_4O_2$	340.1899	358.2246	R	AM	[71]
Picrocrocin	$C_{16}H_{26}O_7$	330.1679	329.1598	L, S	MT	[72]
Plumieride	$C_{21}H_{26}O_{12}$	470.1424	469.1332	S	IG	[73]
Polygodial	$C_{30}H_{42}O_8$	234.33	233.1547	L	SQ	[74]
Proscillaridin A	$C_{30}H_{42}O_8$	530.6	535.2657	L	CG	[75]
Rottlerin	$C_{30}H_{28}O_8$	516.1784	539.1679	R	P	[76]
Salmeterol	$C_{25}H_{37}NO_4$	415.2723	438.2598	L, S	LABA	[77]
Soyasaponin III	$C_{42}H_{68}O_{14}$	796.4609	801.4409	S	TS	[78]
Stearic acid	$C_{18}H_{36}O_2$	284.2715	283.265	L, S	FA	[79]
Tiliroside	$C_{30}H_{26}O_{13}$	594.1373	593.1279	L, S	F	[80]
Tryptophan	$C_{11}H_{12}N_2O_2$	204.0899	203.0823	L	AA	[81]
Ursolic acid	$C_{30}H_{48}O_3$	456.3603	455.355	S, R	TT	[82]

Classes of cytotoxic compounds observed in plant part extracts of *Lobelia nicotianifolia*. A: Alkaloid; AA: Amino acid; AH: Aldehyde; AM: Amidine; AQ: Anthraquinones; CG: Cardiac glycoside; F: Flavonoids; FA: Fatty acid; FAL: Fatty alcohol; IG: Iridoide glycoside; L: Lactones; LABA: Long acting-β agonist; MT: Monoterpene; P: Phenolics; PSI: Protein synthesis inhibitor; S: Steroid; SL: Sphingolipids; SQ: Sesquiterpenoid; T: Terpenoids; TS: Triterpenoid saponin; TT: Triterpene; UMK: Unsaturated methylated ketone

Table 5: Analysis of inhibitory concentration$_{50}$ (IC_{50}) values of Lobelia nicotianifolia plant part extracts against human cervical adenocarcinoma, human breast adenocarcinoma, and human colon adenocarcinoma at 24 and 48 h

Extracts	IC_{50} values (µg/mL)					
	HeLA		MCF-7		HCT-15	
	24 h	48 h	24 h	48 h	24 h	48 h
Leaf	643.13±1.08	173.02±1.88	171.56±1.27	135.80±1.16	413.23±2.48	210.13±1.65
Stem	193.86±2.77	135.8±1.53	92.16±1.06	66.05±1.84	661.45±2.09	226.82±2.06
Root	144.87±1.31	86.05±2.09	177.58±1.83	73.38±2.01	254.27±1.23	126.96±1.09

HeLa: Human cervical adenocarcinoma; MCF-7: Human breast adenocarcinoma; HCT-15: Human colon adenocarcinoma; IC_{50}: Inhibitory concentration

stem [Figure 1b]. The stem extract of *L. nicotianifolia* quenches a maximum of 85.35% ABTS radicals which was higher as compared to leaf and root extracts. DPPH and ABTS are the most employed antioxidant assays to define the antioxidant potential of food sources and medicinal plant extracts.[35] These assays are based on the capacity to test extracts to donate hydrogen which works as a chain-breaker.[36] The stem of *L. nicotianifolia* extracted with methanol has higher RSA [Figure 1] which might be associated with the array of secondary metabolites [Table 1] and higher phenolics and flavonoids content [Table 2]. Phenolic compounds have hydroxyl groups at the ortho- and para-positions, which contribute to antioxidant activity.[37] We have also studied the correlation between TPC and TFC with DPPH and ABTS assays by using the Pearson correlation coefficient which revealed a significant positive

correlation [Table 3]. A significant correlation between antioxidant assays and of the test extracts is associated with the hydroxyl groups and hydrogen atoms of phenolic compounds which helps in the quenching of free radicals and end the chain formation.[24] This study has indicated that the polarity of extracting solvents and the plant parts influences the antioxidant potential of *L. nicotianifolia* [Figure 1a and b] which showed a concurrence with the previous study.[38]

In vitro cytotoxicity and characterization of extracts

Preliminary phytochemical analysis [Table 1], higher phenolic and flavonoid contents [Table 2], and *in vitro* antioxidant activity [Figure 1] has revealed the superiority of methanolic extracts of *L. nicotianifolia* hence, the extract was used for cytotoxicity study. The cytotoxicity of methanolic extracts of leaf, stem, and root was tested for 24 and 48 h against HeLa, MCF–7, and HCT–15 cells by MTT assay. The cytotoxicity against HeLa cell lines is represented in Figure 2 which revealed that methanolic stem extract at 200 µg/mL has higher inhibition (67.59% and 92.05%). The cell viability for MCF–7 indicated that a higher concentration (200 µg/mL) of methanolic stem extract was lethal and showed 74.28% and 92.77% inhibition at 24 and 48 h, respectively [Figure 3]. In contrast to these cell lines, HCT–15 cells were inhibited by methanolic root extract which was 43.70% for 24 h and 65.33% for 48 h [Figure 4]. Similarly, the IC_{50} values (µg/mL) were also calculated in this study which indicates that the stem and root extracts have potent cytotoxicity against HeLA and MCF–7 cell lines [Table 4]. A lower IC_{50} value (66.05 µg/mL) was seen for stem extract against breast cancer cell line (MCF-7) followed by root extract (73.38 µg/mL). Whereas, the higher IC_{50} was recorded for the leaf extract of *L. nicotianifolia* against HeLA, MCF–7, and HCT–15 cell lines for study duration [Table 4]. Thus, the present observations revealed that the cytotoxicity of *L. nicotianifolia* is specific to plant parts, their concentrations, and study duration. This observation is in agreement with Mazumder *et al*.[39] who has reviewed 99 plants belonging to 57 families and concluded that plant parts and their chemical constituents played a crucial role for a potent cytotoxicity against various cancer cell lines. Further, the cell morphology of HeLa [Figure 5a], MCF–7 [Figure 5d], and HCT–15 [Figure 5g] cells were altered and showed shrunken appearance because of loss of membrane integrity and cytoplasm condensation when treated with higher concentration (100 and 200 µg/mL) [Figure 5b, c, e, f, h and i]. The morphological alterations were because of abnormal accumulation of substances in the cytoplasm and depend on the array of phytoconstituents of plant extracts.[40] For the identification of functional groups and phytoconstituents in the plant parts of *L. nicotianifolia* potent methanolic extracts were characterized with ATR–FTIR, and LC–HRMS.

ATR–FTIR spectroscopy is a rapid, non-invasive, and cost-effective method employed for the analysis of functional groups in crude extracts.[39] The methanolic extract of stem has shown maximum peaks (14) followed by leaf (11) and root (9) [Figure 6]. These variations state the chemical profiles of the plant parts and could be related to the cytotoxicity in this study. The present analysis has revealed the presence of polysaccharides, proteins, lipids amide, alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatics, esters, ethers, alkyl halides[41,42] in different plant parts of *L. nicotianifolia*. The characterization of cytotoxic phytoconstituents in methanolic plant part extracts of *L. nicotianifolia* were done by LC–HRMS. In this technique, a LC along with HRMS was used for the characterization of volatile and nonvolatile phytoconstituents in the complex plant extracts.[43] The methanolic stem extract of *L. nicotianifolia* has maximum 23 cytotoxic compounds followed by leaf (21) and root (6) [Table 4] and could be a reason for higher cytotoxicity of stem extract for MCF–7 and HeLa cells. These compounds are

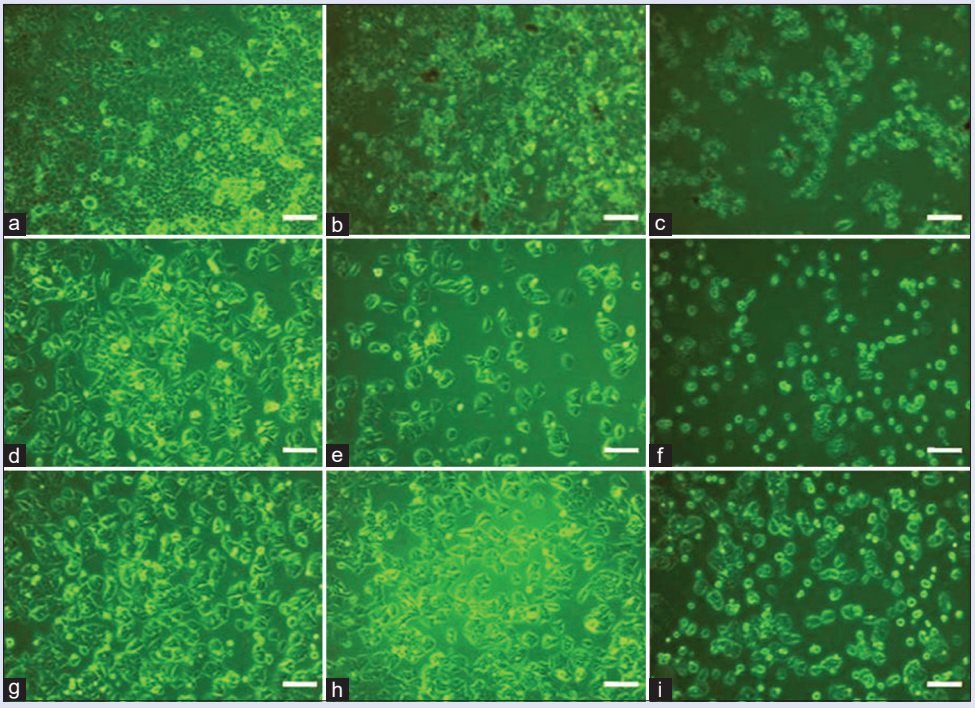

Figure 5: Morphological changes in HeLA cells (a): Control, (b): 100 µg/mL methanolic leaf extract, and(c): 200 µg/mL methanolic leaf extract), MCF–7 (d): Control, (e): 100 µg/mL methanolic leaf extract, and (f): 200 µg/mL methanolic leaf extract), and human colon adenocarcinoma: 15 (g): Control, h: 100 µg/mL methanolic root extract, and (i): 200 µg/mL methanolic root extract) at 48 h exposure. All images are captured at × 20 magnification with phase contrast microscope where a scale bars represent 100 µm

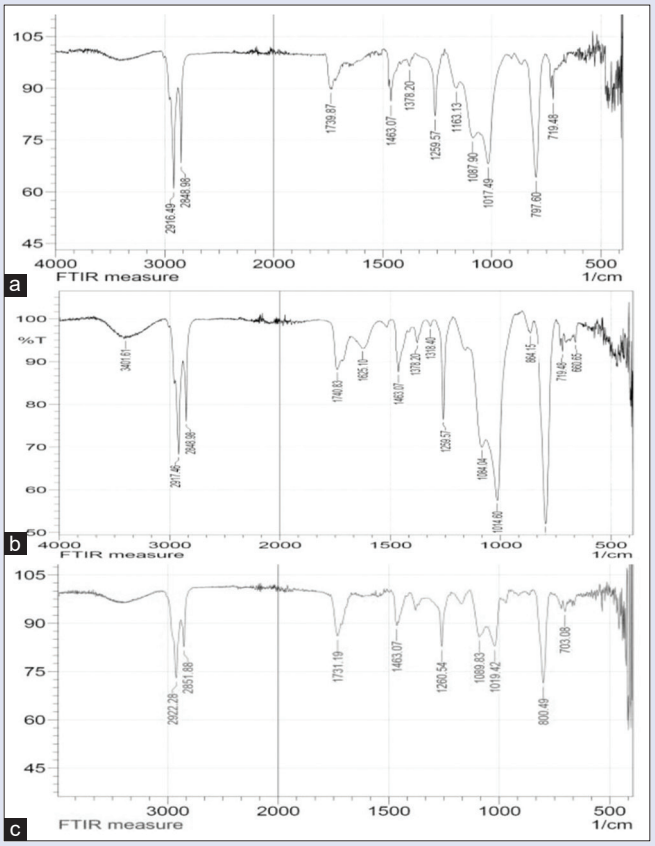

Figure 6: Representative attenuated total reflectance Fourier–transform infrared spectroscopy spectra of observed peaks in methanolic leaf (a), stem (b), and root (c) extracts of *Lobelia nicotianifolia* in the 4000-500 cm^{-1} range

categorized into 21 distinct classes [Table 5] which are well known for its cytotoxicity.[44-82]

CONCLUSION

The present study reveals the phytochemical profiling, *in vitro* antioxidant and anticancer activity of *L. nicotianifolia* plant parts. Based on the results it can be concluded that the extracts prepared in higher polarity solvents were significant radical scavengers than those prepared in less polar solvents. Methanolic extracts showed a large array of different phytochemicals and phenolic compounds. Extracts with higher phenolic and flavonoid contents also had higher antioxidant and anticancer activity (percent inhibition and IC_{50}). However, further studies are needed to understand detailed molecular mechanism responsible for antioxidant and anticancer activity in this plant. Thus, the obtained results could form a good basis for further investigation in the potential discovery of new natural bioactive compounds and molecular mechanism involved in those bioactive compounds from this traditional plant with medicinal value.

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Conflicts of interest

There are no conflicts of interest.

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