SJIF Impact Factor 2022: 8.197| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 7 | Issue: 6 | June 2022 - Peer Reviewed Journal

ISOLATION AND CHARACTERIZATION OF β-SITOSTEROL FROM *LANTANA CAMARA*

Punna Lavanya, Dr.S.Venkata Saibaba, Prathibareddy Nareddy, V.Sandhya, T.Malleshwari

1. K.V.K College f Pharmacy, Surmaiguda, Abdullapurmet, R.R.Dist Telangana,India.
2. Gurram Balanarsaiah College of Pharmacy, Edulabad,Medhcal Dist,Telangana.

ABSTRACT

Objective: *The aim of our study was to investigate chemical constituents of leaves and aerial of Lantana camara since not much phytochemical investigation had been done previously in India.*

Methods: *Phytochemical screening of the extracts obtained from the leaves of L.camara indicated the presence of flavonoids (type of flavonols), saponins, alkaloids, steroids, and terpenoids. Thin-layer chromatography fingerprinting and the spraying reagent (concentrated H_2SO_4 and vanillin in ethanol) were used to identify the hexane extract containing phytosterols.*

Results: *The different chromatographic and spectroscopic results revealed the presence of β-sitosterol isolated from L.camara.*

Conclusion: *The isolation and purification afforded white crystalline powder which was subjected to chemical and spectral identification by infrared and ^1H-nuclear magnetic resonance. The compound was identified as β-sitosterol.*

KEYWORDS: *Lantana camara, β-Sitosterol, ^1H nuclear magnetic resonance.*

INTRODUCTION

Medicinal plants are very ancient and true natural medicines which are useful for the treatment of different diseases. They can be used directly or in extracted forms for the management of various ailments due to the presence of various secondary metabolites [1].

Lantana camara L. (Verbenaceae) is a rambling shrub with a number of flower colors viz. red, pink, white,yellow and violet1. These plants are cultivated as ornamental or hedge plants in India 2. Previously 3,4, we reported the phytochemical studies of Lantana camara L. and the chemical investigation of the stems from the red flowering taxa and pink flowering taxa of this plant. Herein, we report the isolation of compounds from the leaves and aerial parts of another taxa (yellow flower) of this plant, collected at Loacl Herbarium. Plants belonging to this genus are famous for its medical benefits. Recent pharmacological studies have shown that *L.camara L.* has anti-inflammatory, antimicrobial, antioxidant, antitumor, and cardiovascular effect [5]. A large number of phytochemical components have been derived and made this plant a source of flavonoids, alkaloids, saponin ,terpens and complicated sterols and sterols [6] as shown in Fig. 1.

β-sitosterol belongs to the group of phytosterols, which particularly includes stigmasterol and campesterol. Phytosterols are crucial steroid molecules that stabilize the phospholipid bilayers of cellular membranes in plants, having similar structural and biological functions to cholesterol, and are a major group of bioactive constituents with well-proven bioactivity. Phytosterols show a variety of health benefits *in vivo*, in particular, protection against various chronic ailments, such as cardiovascular diseases, diabetes, cancer, and hepatic injury. It is worth mentioning that phytosterols have been attracting much interest because of their well-known cholesterol-lowering property recently [7]. The main objective of this study is to investigate and isolate this compound from *L.camara* cultivated in India since it is an important compound in therapy.

MATERIALS AND METHODS

Plant material

L.camara leaves were collected from local forest area, Babil Province, India, during March and dried in shade at room temperature and grinded as powder and weighed. The plant was identified and authenticated.

Extraction of terpene

Shade-dried pulverized plant material (100 g) from plant leaves was extracted by Soxhlet apparatus with hexane (700 ml), the extract was filtered, and the solvent was evaporated under reduced pressure using rotary evaporator. Hexane extract was analyzed for the presence of terpene using thin-layer

SJIF Impact Factor 2022: 8.197| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 7 | Issue: 6 | June 2022 - Peer Reviewed Journal

chromatography (TLC) with spray reagent and confirmed by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography (LC)-MS analysis.

Isolation of sitosterol by preparative TLC Preparation of stationary phase

Readymade silica gel GF 254 plates with a layer thickness of 0.25 mm, dimension 20 cm×20 cm. The plates were reactivated by heating in the oven at 100°C for 15 min, left to cool, and used for application after allocation of the baseline and the solvent front.

Prepration of solvent system

Mobile phase for sterols (chloroform:acetone) was mixed in a conical flask and introduced in the jar. The jar was lined with a filter paper, closed tightly, and left for saturation.

Application of sample

About 2 g of the sample was dissolved in absolute methanol and applied on the baseline of TLC plates.

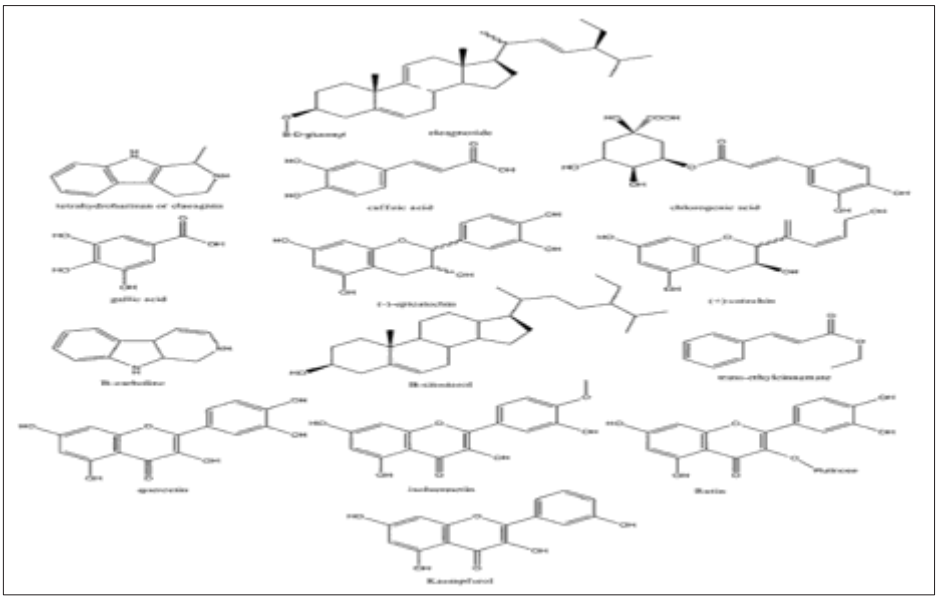


Fig. 1: Important components of Lantana camara β-sitosterol[C29H50O]

Detection of separated spot

Detection was done by spraying side of plate with vanillin-sulfuric acid reagent. The purity of each band was checked by analytical TLC until single spot on TLC plate is obtained for identification with a reference standard.

Method of identification of chemicals

GC-MS

GC-MS was carried out to detect the presence of terpenes in the plant extract. GC-MS conditions for detection of terpenes were: the carrier gas was helium, the injection volume was 1 µL, and the split ratio was 2.0. Injection temperature: 250°C. Column temperatures: From 80°C and rose up to 310°C at a rate of 10°C/min.

LC/MS analysis

LC/MS analysis of phytosterols in the hexane extract, a SHIMADZU LAB solutions using beta-sitosterol as standard, C18 column (2.1 mm×50 mm, 5 µm). An isocratic mobile phase of acetonitrile/ methanol (99:1, v/v) was used at a flow rate of 600 µL/min, and the injection volume was 10 µL. Stock solutions of standard compound were prepared by dissolving compound at 1.000 mg/mL in isopropanol.

Structure elucidation of β-sitosterol

- Fourier transform infrared (FTIR): FTIR spectroscopy is a technique for material analysis; it offers quantitative and qualitative analysis of the sample. FTIR identified chemical bands in molecules, the range of scanning 4000–400 cm^{-1}. IR radiation is passed through a sample. Some of the IR radiation is absorbed by the sample, and some is transmitted. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. IR spectra was done using Nicolet NEXUS 670 FT-IR.
- ^{1}H-nuclear magnetic resonance (NMR): The ^{1}H-NMR spectra were performed at the University of Jordan, Faculty of Science, and Department of Chemistry. Instrument Model: Bruker 500 MHz-Avance III. Chemical shift is in part per million (ppm) with reference to the chemical shift of the deuterated solvent or the internal standard tetramethylsilane.

SJIF Impact Factor 2022: 8.197| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 7 | Issue: 6 | June 2022 - Peer Reviewed Journal

RESULTS AND DISCUSSIONS

Natural products have always been a preferred choice of all as it plays a great role in discovering new medicines. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity [8].

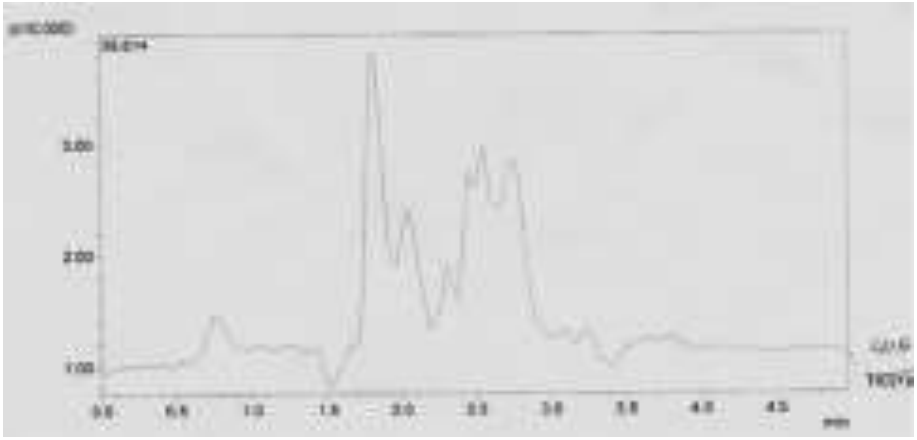


Fig. 2: Chromatogram of β-sitosterol

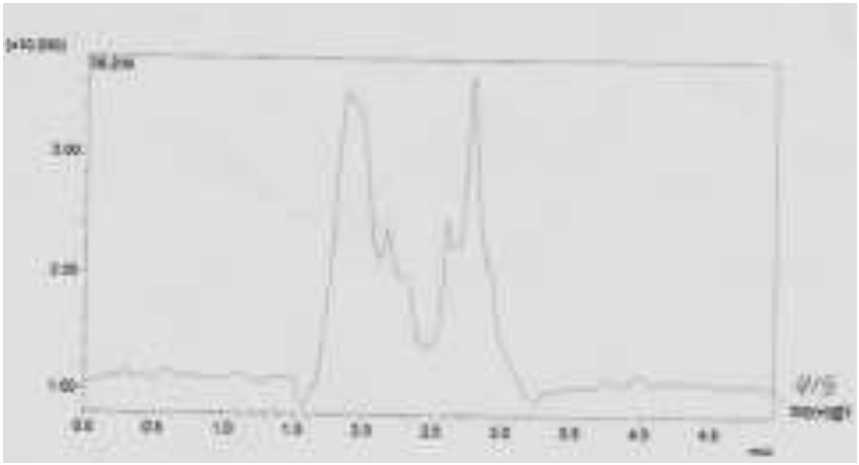


Fig. 3: Chromatogram of unknown

Table 1: R_f value of standard and extract	
Solvent system	**Chloroform:Acetone**
R_f value of β-sitosterol standard	0.280
R_f value of β-sitosterol in extract	0.260

SJIF Impact Factor 2022: 8.197| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 7 | Issue: 6 | June 2022 - Peer Reviewed Journal

Table 2: Characteristic FT-IR Absorption of β-sitosterol

Compound	Bands (cm^{-1})	Interpretation
	3399.85–3328.28	Stretching vibration of OH
	3863.24–2990	Stretching vibration of CH alkane (symmetric and asymmetric)
	3110	Streching vibration of CH alkene
	1640	Stretching vibration of C=C
	1580	Bending vibration of OH
	1460.8–1374.30	Bending vibration of isopropyl
	1312.72	Bending vibration of C-O of 2° alcohol
FT-IR: Fourier transform infrared		

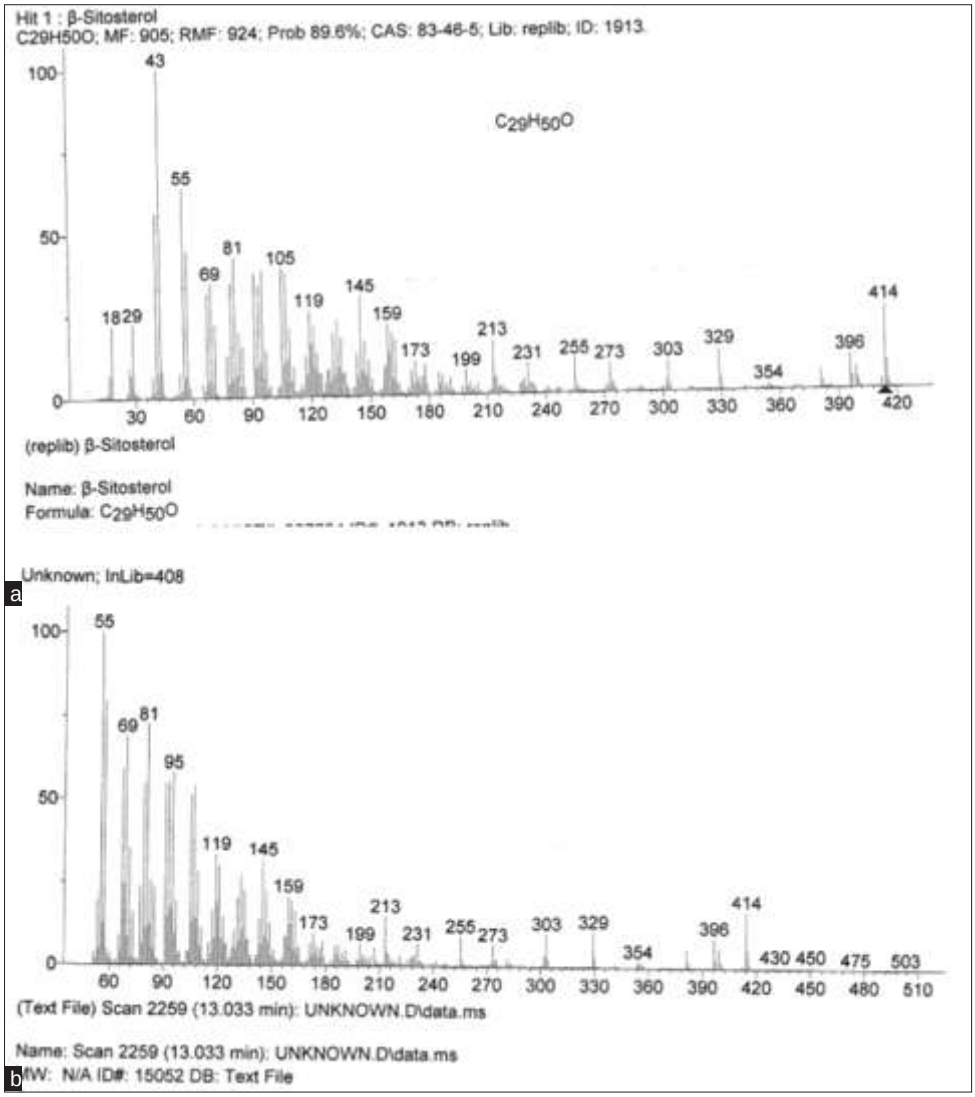


Fig. 4: (a and b) Gas chromatography-mass spectrometry chromatogram of β-sitosterol

Hexane extract of the plant was investigated by TLC which revealed the presence of β-sitosterol that appeared as spot in mobile phase (chloroform:acetone) against β-sitosterol reference standard, and the spot of extract appeared the same R_f value

SJIF Impact Factor 2022: 8.197| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 7 | Issue: 6 | June 2022 - Peer Reviewed Journal

as that in reference standard on TLC plate as shown in Table 1, as indicated by the development of violet spots after spraying by vanillin sulfuric acid spray reagent [9].

LC-MS analysis

The chromatogram shows intensity of ions in mass spectrum ptrhoaptortional to the compound in the sample (β-sitosterol) in database (Figs. 2 and 3).

GC/MS analysis

The chromatogram showed peak with retention time (13.033 min) corresponding to the molecular ion peak at 414 m/e in comparison with NIST83-46-5 database (Fig. 4).

Fragmentation pattern of β-sitosterol

β-sitosterol molecular ion observed at m/z 414 that corresponds to the molecular formula C29H50O. Peak at m/z 396 resulted from loss of water molecule from the molecular ion which will further dealkylated to yield peak at m/z 381.

Peak at m/z 273 is for fragmentation of C17-C20 cleavage. The dehydration of m/z 273 fragment will yield m/z 255 as shown in Fig. 5 [10].

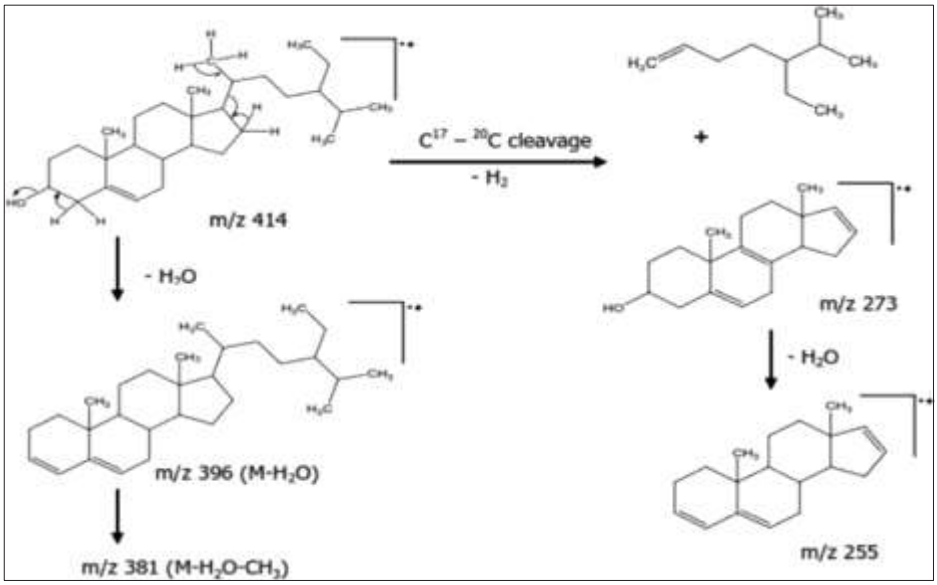


Fig. 5: Fragmentation pattern of β-sitosterol

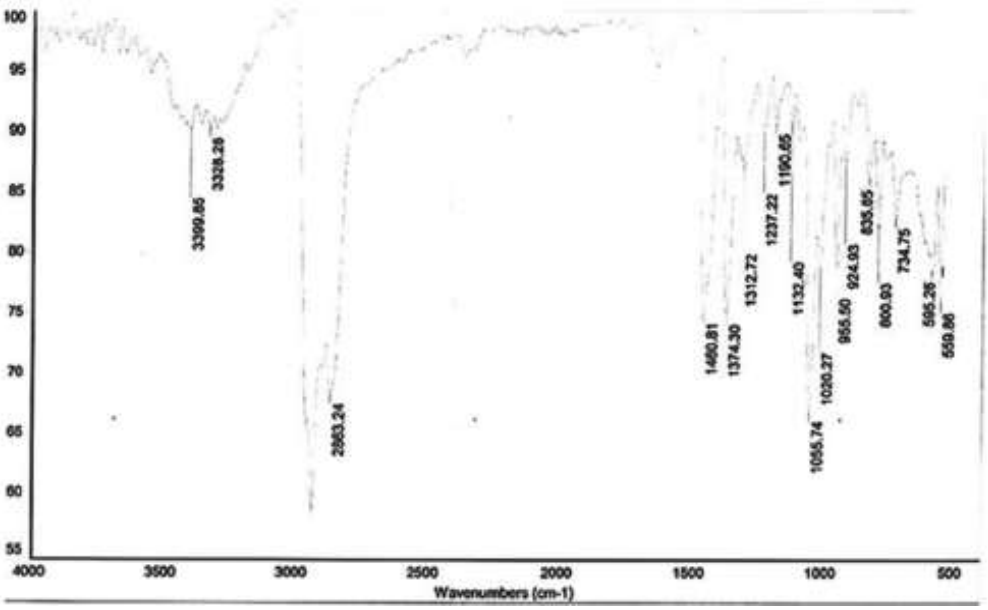
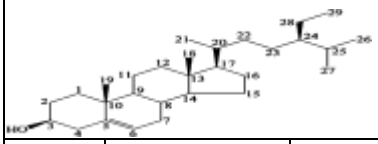


Fig. 6: The Fourier transform infrared spectra of the β-sitosterol

SJIF Impact Factor 2022: 8.197| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 7 | Issue: 6 | June 2022 - Peer Reviewed Journal

Table 3: 1H-NMR chemical shift values for compound recorded in CDCl3 (500 MHz)



Carbon atom	Chemical shift (ppm)	No. of H	Interpretation
C1	1.3151,1.0585		
C2	1.52,1.2793		
C3	3.5283	1H	Singlet, alcoholic proton
C4	2.2143, 1.965		
C6	5.3392	1H	Triplet, C=CH2
C7	2.2878, 1.0765		
C8	1.037		
C9	1.1712		
C11	1.6285, 1.3828		
C12	1.5117		
C14	1.0497		
C15	1.9808, 1.6518		
C16	1.9419, 1.6518		
C17	1.1192		
C18	1.0366	3H	Methyl group
C19	1.2019	3H	Methyl group
C20	1.3151		
C21	0.8976	3H	Methyl group
C22	1.1876	1H	
C23	1.19	1H	
C24	1.10		
C25	1.036		
C26	1.55	3H	Methyl group
C27	0.9912	3H	Methyl group
C28	0.8283		
C29	0.8349	3H	Methyl group
NMR: Nuclear magnetic resonance			

Isolation and purification of β-sitosterol

For isolation and purification of β-sitosterol, preparative TLC plate was conducted on 2 g of hexane extract corresponding to 100 g of the plant to give 47 mg of β-sitosterol (0.5%). The solvent system used was chloroform:acetone. The band of isolated compound was observed by the development of violet spots at plate side after spraying by vanillin sulfuric acid spray reagent.

Structure elucidation of β-sitosterol

FT-IR spectrum

The FT-IR spectra of the β-sitosterol (Fig. 6) showed the characteristic absorption bands by which its functional groups were identified.

The values of the characteristic bands of these spectra have been discussed according to the Lukitaningsih book [10-12], and summarized in Table 2.

SJIF Impact Factor 2022: 8.197| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 7 | Issue: 6 | June 2022 - Peer Reviewed Journal

¹H-NMR

The analysis was used to identify the target compound. The spectra were recorded in chloroform solvent. The values of chemical shifts have been discussed according to the Silverstein *et al*. [11] books and summarized in Table 3.

CONCLUSION

L.camara of India contains significant amount of sitosterol, and further studies needed to investigate the presence of other sterol like stigmasterol which is reported to be found in the plant.

CONFLICTS OF INTEREST

The author declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

1. *Sundaram S, Perumal PC, Gopalakrishnan VK. Chromatographic and spectrophotometric analysis of bioactive compounds from Cayratia trifolia (L.) stem. Int J Pharm Pharm Sci 2018;8:56-64.*
2. *Mohammed FI, Al-Essa MK, Shafagoj YA, Afifi FU. Investigation of the direct effects of the alcoholic extract of Lantana camara L. (Elaeagnaceae) on dispersed intestinal smooth muscle cells of guinea pig. Sci Pharm 2006;74:21-30.*
3. *Katz GL, Shafroth PB. Biology, ecology and management of Lantana camara L. in Western North America. Wetlands 2003;23:763-77.*
4. *Cansev A, Sahan Y, Celik G, Taskesen S, Ozbey H. Chemical properties and antioxidant capacity of Lantana camara L fruits. Asian J Chem 2011;23:2661-5.*
5. *Farzaei MH, Bahramsoltani R, Abbasabadi Z, Rahimi R. A comprehensive review on phytochemical and pharmacological aspects of Lantana camara L. J Pharm Pharmacol 2015;67:1467-80.*
6. *Tehranizadeh ZA, Baratian A, Hosseinzadeh H. Russian olive (Lantana camara) as a herbal healer. BioImpacts: BI 2016;6:155-67.*
7. *Saibaba, S.V.; Kumar, M.S.; Ramu, B. Pharmaceutical impurities and their characterization. European J Pharm Med Res 2016, 3(5), 190-196.*
8. *Trautwein EA, Demonty I. Phytosterols: Natural compounds with established and emerging health benefits. Oléagineux Corps Gras Lipides 2007;14:259-66.*
9. *Ahmed OH, Hamad MN, Jaafar NS. Phytochemical investigation of Chenopodium murale (Family: Chenopodiaceae) cultivated in India, isolation and identification of scopoletin and gallic acid. Asian J Pharm Clin Res 2017;10:70-7.*
10. *Wagner H, Bladt S. Plant Drug Analysis. 2nd ed. Berlin: Springer; 1996.*
11. *Lukitaningsih E. Phytosterol content in bengkoang (Pachyrhizus erosus). Pharmacon: J Farmasi Indones 2012;13:47-54.*
12. *Saibaba SV, Kumar MS, Pandiyan PS. Mini Review on LC/MS Techniques*
13. *Silverstein RM, Webster XF, Kiemle DJ. Spectrometric Identification of Organic Compound. 7th ed. New York: Wiley-Interscience; 2005.*