RESEARCH ARTICLE

Phytochemical Profiling and Gas Chromatography-Mass Spectrometry Analysis of Bioactive Compounds in Methanol Extract of *Indigofera prostrata*

Manjeera K[1,2], Sundararajan R[2*]

[1]*Department of Pharmacology, C.M.R. College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India-501401.*
[2]*Department of Pharmaceutical Chemistry, GITAM School of Pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh, India-530045.*

Received: 23rd Aug 2024; Revised: 30th Oct, 2024; Accepted: 5th Nov, 2024; Available Online: 25th Dec, 2024

ABSTRACT

Background: The contemporary study emphases on identification of bioactive components from methanol extract of *Indigofera prostrata* by Gas chromatography-mass spectrometry investigation and similarly evaluation of phytochemical components that are existing in *Indigofera prostrata* in methanol extract is also done.

Objective: The objective was to accomplish Standardisation, to distinguish, authenticate as well as to prevent the adulteration of *Indigofera prostrata.*

Materials and methods: Standardisation, Preliminary phytochemical screening and GC-MS study was accomplished. A mass spectrophotometer and 7890A gas chromatograph system (GCMSQP2010, SHIMADZU) was utilized to perform the GC-MS analysis. Analysis of Gas chromatography mass spectrometry with methanol extract of *Indigofera prostrata* had revealed the occurrence of diverse kinds of bio active constituents.

Results: Phytochemical screening of the extract of methanol of *Indigofera prostrata* revealed the existence of tannins, flavonoids, compounds of phenol, carbohydrates, amino acids, saponins, triterpenoids, proteins and steroids. Totally 14 compounds had been identified in methanol extract composition of *Indigofera prostrata &* had revealed bio active 1-Butanol, 3-methylformate, β-Acorenol, 9,12-Octadecadienoicacid, Hexadecanoic acid, and d-Mannose, methyl ester, 3-O-Methyl-d-glucose, phytol, Tetradecen-1-olacetate (E-8-Methyl-9), Other ingredients include 2-hydroxy-1-(hydroxymethyl) ethyl ester Campesterol, γ-Sitosterol, Stigmasterol, 2-hydroxy-1(hydroxymethyl) ethyl ester, and luteol. The findings of the experiments mentioned above are as follows: the figures for total ash and loss on drying are 8.35 ± 0.35 and 7.45 ± 0.02 correspondingly, water soluble ash was 3.65 ± 0.04.

Conclusion: These compounds are said to be responsible for the different pharmacological and therapeutic properties. The results from the current study provides insightful information that will help select and carry out future research projects.

Key words: Campesterol, GC-MS, *Indigofera prostrata,* Lupeol, phytocomponents, Stigmasterol, γ-Sitosterol.

How to cite this article: Manjeera K, Sundararajan R. Phytochemical Profiling and Gas Chromatography-Mass Spectrometry Analysis of Bioactive Compounds in Methanol Extract of *Indigofera prostrata.* International Journal of Pharmaceutical Quality Assurance. 2024;15(4):2107-14. doi: 10.25258/ijpqa.15.4.2

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Foods consumed by humans contain a variety of minor, major and bioactive compounds, including glucosinolates, peptides, antioxidants, lipids and carbohydrates.[1] Bioactive molecules are substances with pharmacological or toxicological properties that are produced by plants. High dosages of vitamins, minerals and nutrients were having pharmacological or toxicological effects. Vitamins, minerals and nutrients are typically not described as bioactive substances in plants. Bioactive substances or bioactive molecules are the names given to the secondary metabolites generated by plants. So, secondary plant metabolites are how bioactive molecules in plants are defined in terms of their impact on humans and animals from a pharmacological or toxicological standpoint.[2] The term "bioactive molecules" refers to naturally occurring, food-chain-related substances that have the potential to interact with one or more substances found in living tissue and have a positive impact on human health. Korhonen asserts that extraction or separation procedures must be used, as well as recovery approaches using bio accessibility assessments, in order to identify bioactive substances in plants.[3] The basis for these bioactivity tests, such as those done *in vivo* and *in vitro*, is how bioactive substances interact with biological molecules. Metabolite production is caused by this interaction.

Anticancer, Antibacterial, antifungal, antioxidant and anti-inflammatory characteristics are just limited pharmacological actions that many bioactive components of medicinal plant life exhibit.[4-6] When considering these bioactive chemicals as potential treatments for various illnesses, it is important to evaluate their potential. Drugs made entirely from plants are usually made from basic plant extracts that contain an intricate mixture of diverse

Author for Correspondence: sraja61@gmail.com

phytochemicals. These phytocompounds, whose structures are precise and intricate, are used to cure chronic as well as infectious disorders.[7] Species of diverse plants have a sizable pool of bioactive secondary metabolites, but a tiny portion of those are considered and found to be noteworthy source of bioactive components. In the examination for novel substances, as well as for the best possible control, the development of appropriate screening agents.

Improvement of suitable screening procedures may be crucial in the search for new drugs as well as for better control.[8] The transportation of effective medications with high-pastime profiles has been made possible by the extraction and characterization of such bioactive components from various medicinal plant life.[9]

Basic knowledge regarding chemical as well as pharmacological activity is furnished by initial medicinal plants screening of through chromatographic as well as spectrometric methods, that assist in the choice of active plants biologically.[10] In current years, functional group detection and identification of numerous therapeutically bioactive chemicals present in medicinal plants have been widely performed by using gas chromatography-mass spectrometry as well as Fourier transform infrared techniques.[11,12] One of the fastest, finest and also utmost reliable methods for identifying dissimilar chemicals, like alkaloids, alcohols, detection of nitro group and identification of several therapeutic bioactive chemicals found in medicinal herbs.[13] The gas chromatography-mass spectrometry technique was utilized in current investigation to find and detect the phytochemical components existing in the medicinal plant. In the Fabaceae family, *Indigofera prostrata* is a prostrate spreading branching herb with pubescent stems. Leaves are 3-foliolate, petiole is 0.8-1.3 cm long, stipules are subulate, leaflets are obovate or elliptic-obovate, 0.8-1.8 x 0.4-0.8 cm, with a cuneate base and an obtuse apex. Axillary, 5 mm long, with 4-5 flowered racemes. Flowers colour is brick-red or pink, pedicels measure about 2 mm length. Length of tube of calyx measure about 0.5 mm. Corolla will be 5 mm long, wings are rectangular and keels are up to 4 mm long. long 3mm staminal sheath. Pods are appressed-pubescent, length measures about 1-1.5, slender, terete, deflexed also somewhat winged and have oblong seeds.

Poor pharmacokinetic qualities prevented the commercialization of many medications, which cost pharmaceutical corporations a ton of money.[14] With the development of computational techniques, it is now possible to choose medications from the phytochemicals present in a variety of medicinal plants.[15] Computational predictive models, often known as predictive tools, have also been used to make *in silico* forecasts of toxicological, pharmacological, and pharmacokinetic performance.[16] Computational predictive models also play a significant part in the selection of techniques driving technological and pharmaceutical research. Currently, drug design and testing can be done effectively and economically using molecular docking. This method offers data on drug receptor relations which can be utilised to forecast direction, in order for the medication candidates to connect with the target proteins that they are meant to.[17] Furthermore, by delivering a molecule non-covalently to the target macromolecule's binding site, this method promotes systemic exploration by precisely attaching to the active site. Covalently to the target

Table 1: Physicochemical constants of *Indigofera prostrata* whole plant Powder

Parameters	Values
Organoleptic characters	
Appearance	Powder
Colour	Light Green
Smell	No smell
Taste	pungent
Ash Values	
Acid insoluble ash	0.16±0.06
Water soluble ash	3.65±0.04
Loss on drying	8.35 ± 0.35
Total ash	7.45±0.02

Table 2: *Indigofera prostrata* extractive values

Plant	Part	% Yield of extracts		
		Ethyl acetate	Chloroform	Methanol
Indigofera prostrata	Whole plant	8.9%	9.1%	10%

Table 3: Results of Phytochemical screening

S. No	Phytochemical	Chloroform	Methanol	Ethyl acetate
	Triterpenoids	+	+	+
	Steroids	+	+	+
	Flavonoids	+	+	+
	Alkaloids	+	+	+
	Carbohydrates	+	+	+
	Cardiac glycosides	+	+	+
	saponins	+	+	+
	Proteins	+	+	+
	Phenolic compounds	-	+	-
	Tannins	+	+	+
	Aminoacids	+	+	+
	Gums	-	-	-

+ Designates the presence of Phytoconstituents
- Designates the absence of Phytoconstituents

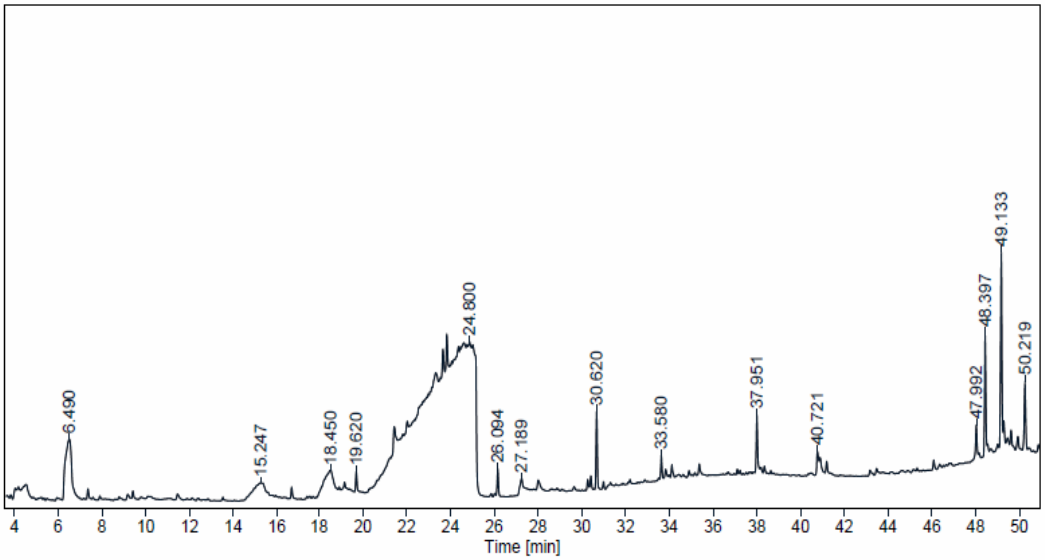


Fig.1: GC-MS chromatogram of methanol extract of *Indigofera prostrata*

macromolecule's binding site, causing each ligand to precisely attach to its active sites.[18] Therefore, the focus of the current work is on GC-MS analysis, identification of bioactive components from a methanol extract of *Indigofera prostrata*.

MATERIALS AND METHODS

Collection and authentication of plant

Indigofera prostrata (Fabaceae) In the month of July, the entire plant was gathered from the Andhra Pradesh region of Chittoor, India. Dr. K. Madhava Chetty, Assistant Professor, Botany Department, Sri Venkateswara University, Tirupati, authenticated the plant material and the voucher specimens (1129) were kept in the herbarium.

Standardization

Determination of loss on drying

5.0 g powder of whole plant was taken in a formerly dried measuring bottle. Until two continuous weights did not fluctuate by more than 5 mg, in an oven material was dried. Then, it was weighed after keeping in a desiccator. The loss of weight was noted with point of reference to initial weight.

Total ash determination

5 gm of plant material was kept in tarred crucible, formerly ignited at 300℃ for 45 min. Thus, material was evenly spread in the plate and the material was burnt with gradual rise of temperature up to 400° up to 4 hours with muffle furnace till that became white, signifying the total removal of carbon. Subsequently, the material was kept in a desiccator and finally measured.

Determination of water-soluble ash

Total ash content was added with 15 mL of water and boiled gently for 20 minutes. Insoluble material was collected by filter paper that was ash less. Subsequently, material was splashed with warm water & for 10 minutes it was burnt on a crucible in muffle furnace up to 400℃. Later, residual matter was permitted to cool down for 20 minutes in a appropriate desiccator & was weighed. When mass of the remainder was deducted from total ash mass, the result was achieved.

Determination of acid-insoluble ash

Hydrochloric acid 15 mL & solid content was placed in to vessel comprising total ash. Material boiled gently for 15 minutes by placing a watch-glass on it. Insoluble matter was composed on filter-paper and splashed through warm water till the residue was unbiassed. Material insoluble was shifted to the vessel, later on it was burnt in a muffle furnace by gradual rise of temperature to 450°c upto 2 hours. The remnant was placed in desiccator for 20 minutes, and without interruption weighed. The calculation was done as mg/g of material air-dried.

Maceration-based successive extraction

The entire plant of *Indigofera prostrata* was washed in water to remove impurities like dirt and were then dried in the shade. To get a consistent, coarse powder, these dry seeds were crushed and sieved. Plant material that has been powdered and weighed (500g) is submerged in methanol.

Phytochemical and preliminary analysis

According to standard procedures, the primary and secondary metabolites of all the extract/fractions of *Indigofera prostrata* were analyzed to authorize the occurrence of numerous primary metabolites, like amino acids, carbohydrates, proteins. Moreover, metabolites (secondary) like resins, steroids, phenols, tannins, saponins, flavonoids, alkaloids, and glycosides.

Analysis by using Gas chromatography-mass spectrometry

A mass spectrophotometer and 7890A gas chromatograph system (GCMSQP2010, SHIMADZU) was utilized to perform the GC-MS analysis. It was provided with an HP-5 MS merged column (silica), which was interfaced through a 5675C inert MSD by Triple-Axis detector. Helium was employed as a carrier gas, was circulated through 1.0 ml/min flow rate. The ion source temperature is 250 °C, and the maintained temperature is 300 °C, also pressure applied -15.4 psi, 1.9 mm of out time, and an injector operational in split manner with a ratio of split i.e 1:50. Applied column temperature increased from 36 °C to 150 °C at 4 °C/min after starting by 36 °C for a more 5 minutes. At the rate of 20 ℃/min, the temperature was elevated up

to 250 ℃ and continued there for 5 minutes. Elution time taken was 37 mins. By comparison, individual component's mean peak area to quantity of whole areas, the comparative percent amount of individual constituent was evaluated.

Compounds' identification

Considering retention indices, the components were detected, and also mass spectrum was interpreted by means of database i.e. National Institute of Standards and Technology's (NSIT's). 62,000 designs and more familiar chemicals are comprised in it. The developed spectra of the *Indigofera prostrata* portion's unknown components were related to the reference mass spectra of identified components kept in the library of NIST.

Table 4: Bioactive compounds found in methanol extract of *Indigofera prostrata*

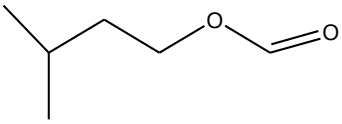
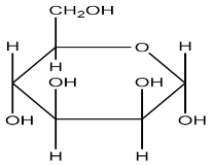
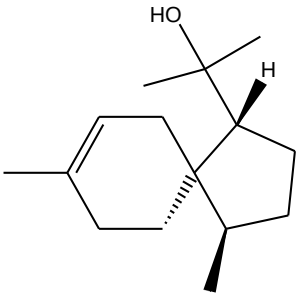
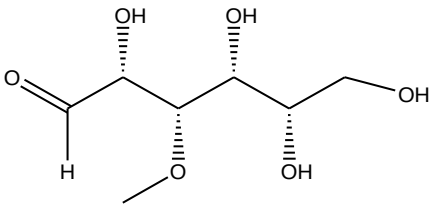
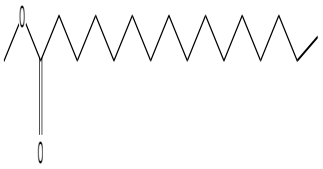
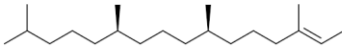
S. No	R. Time	% Area	Name of Compound	Molecular Formula	M.Wt	Compound Structure
1	6.494 min	19.34	1-Butanol,3- methyl-, formate	$C_6H_{12}O_2$	116.16	
2	15.246 min	8.06	d-Mannose	$C_6H_{12}O_6$	180.156	
3	19.621 min	0.59	β-Acorenol	C15H26O	222.37	
4	24.797 min	7.55	3-O-Methyl-d-glucose	C7H14O6	194.18	
5	26.091 min	1.56	Hexadecanoic acid, methyl ester	C17H34O2	270.5	
6	27.191 min	6.49	n-Hexadecanoic acid	C16H32O2	256.42	
7	rt: 30.623 min	4.70	Phytol	C20H40O	296.5	

Table 4: Bioactive compounds found in methanol extract of *Indigofera prostrata*

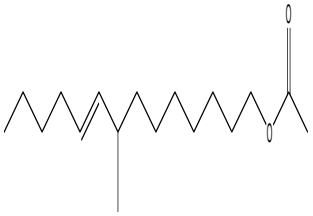
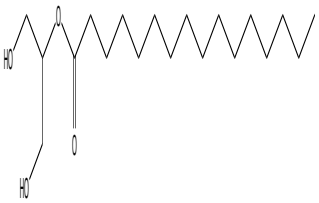
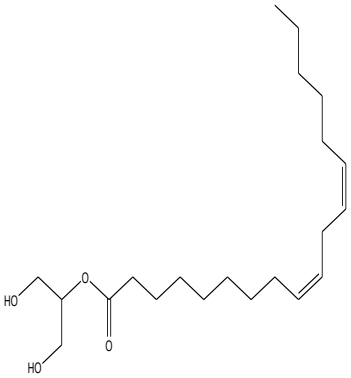
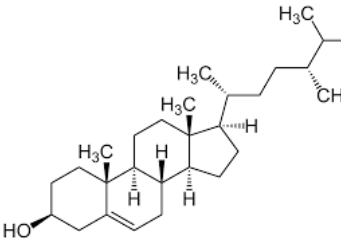
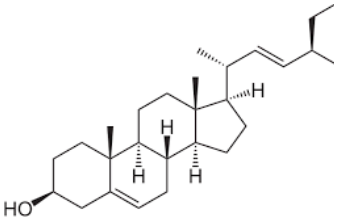
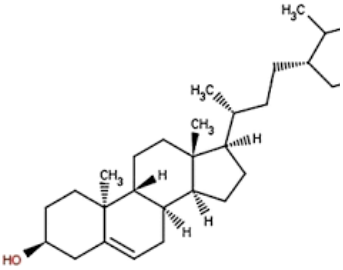
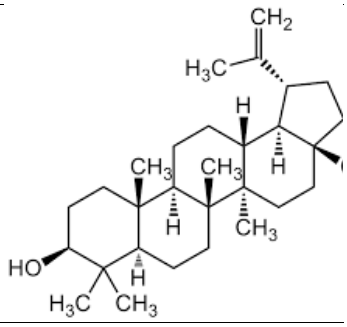
S. No	R. Time	% Area	Name of Compound	Molecular Formula	M.Wt	Compound Structure
8	rt: 33.580 min	0.79	E-8-Methyl-9-tetradecen-1-ol acetate	C17H32O2	268.4	
9	rt: 37.950 min	4.11	Hexadeccanoic acid, 2-hydroxyl-1-(hydroxymethyl)ethyll ester	C19H38O4	330.5	
10	40.719 min	5.43	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C21H38O4	354.5	
11	47.989 min	1.21	Campesterol	C28H48O	400.7	
12	48.395 min	6.42	Stigmasterol	C29H48O	412.7	
13	49.133 min	12.59	γ-Sitosterol	C29H50O	414.7	

Table 4: Bioactive compounds found in methanol extract of *Indigofera prostrata*

S. No	R. Time	% Area	Name of Compound	Molecular Formula	M.Wt	Compound Structure
14	50.214 min	8.35	Lupeol	C30H50O	426.7	

RESULTS

Standardization

Various physicochemical parameters were evaluated 3 times and the average values were found. The value for total ash gave 7.45 ± 0.02. Water soluble ash value was 3.65±0.04. Acid insoluble ash was 0.16±0.06. Outcomes were exhibited below in Table-1.

Solvent extractive values

The extractive values of *Indigofera prostrata* were displayed in Table-2.

The powder of *Indigofera prostrata* which was air-dried & extracted. The yields of dissimilar extracts were 8.9% 9.1% and 10% intended for ethyl acetate, chloroform and methanol correspondingly.

Phytochemical screening

The plant material was subjected to extraction by maceration. Then the extract of selected plant material was subjected to preliminary phytochemical screening. The outcomes revealed that most of the compounds were identified in methanol extract. Flavonoids, terpenoids, saponins and phenolic compounds were detected in methanol extract. So, for further study we have selected methanol extract. The results were displayed in Table.3.

The GC-MS chromatogram is shown in Figure 1 and Table 3 shows the chemical components together with Retention Time, Molecular Weight, Area (%), Atomic Equation within the MEIP. The GC-MS investigation revealed, presence of the following bioactive substances 3-methylformate, 1-butanol, acorenol, d-mannose, 3-O-Methyl-d-glucose, methyl ester, phytol, and 9,12-Octadecadienoic acid, Lupeol, n-hexadecanoic acid, Stigmasterol, Campesterol and 2-hydroxy-1-(hydroxymethyl)ethyl ester.

DISCUSSION

Phytochemical substances institute in medicinal plants are a plentiful supply and can be used to treat a number of chronic disorders.[19] In recent years, a wide range of therapeutic plants have produced numerous powerful biomolecules.[20] Scientists thought that these powerful chemicals could Many illnesses can be treated with minimal negative effects using chemical components derived from natural sources.[21] These powerful substances have the power to stop a variety of chronic diseases in their tracks.[22] There aren't many chronic diseases that are this serious and none of them have specialized medications.[23] Medicinal herbs should be used in these situations since they produce effective outcomes both pharmacologically and phytochemically.[24] A variety of phytoconstituents, comprising carbohydrates, flavonoids, amino acids, tannins, phenols, alkaloids, proteins, steroids, glycosides (cardiac) and even terpenoids were found in *Indigofera prostrata* used in this study. Different extracts of *Indigofera prostrata* may have medicinal benefits because of their bioactive phytoconstituents.

One of the most used methods for separating phytoconstituents is gas chromatography-mass spectrometry. Dissimilar phytochemical components were found in the *Indigofera prostrata* extract after a GC-MS analysis, which may have contributed to the plant's therapeutic qualities. Numerous fruits, vegetables, nuts and seeds contain cholesterol. Canola and corn oils contain the highest concentrations of it. The substance functions as a precursor to several steroid hormones, including anabolic steroids like boldenone and testosterone. Studies conducted since the 1950s have demonstrated that plant sterols such campesterol can lower LDLs and cholesterol. Campesterol also inhibits the absorption of cholesterol in the human gut by competing with it. Contradictory findings have been found when using serum levels of campesterol and the ratio of campesterol to cholesterol as indicators of cardiac risk. Increased intake of plant sterols may lower levels of beta-carotene and lycopene and may cause a vitamin E deficit. Whether high quantities of phytosterols raise the risk of cardiovascular disease is still up for dispute.

Among the sterol components in the diet with the ability to lower the hazard of cardiovascular ailments is stigmasterol.[25] A decline in LDL blood cholesterol of 8-11% is associated to a regular intake of 3 grams of sterols of plant, potentially dropping the danger of cardiovascular disease.[26] As a component of cellular procedures of plants, stigmasterol may play a part in metabolism, enzymes involved in the creation of plant cell membranes, and plant responses to stress. Lupeol has a complex pharmacological activity related to antiprotozoal, antibacterial, anti-inflammatory and anticancer effects. In research laboratory models of skin and prostate cancers, it works well as an inhibitor.[27] Lupeol works largely to reduce inflammation on the interleukin system. It has been discovered that lupeol has a contraceptive effect because it inhibits the calcium channel of sperm.[28] Additionally, it has been confirmed that

lupeol possess anti-angiogenic as well as anti-cancer properties by decreasing both VEGFR-2 and TNF-alpha. With antiprotozoal, antibacterial, anti-inflammatory, anticancer, and chemopreventive effects, lupeol has a complex pharmacology. In research models of laboratory of skin as well as prostate cancers, it works well as an inhibitor. Utilized as a dietary supplement, mannose (D-mannose) helps prevent reoccurring UTIs. According to an analysis published in 2022, consuming mannose to prevent UTIs is just as beneficial as taking antibiotics.[29] While a different study indicated that the standard of the clinical trials was too poor to draw any conclusions concerning the use of D-mannose to treat or prevent UTIs.

CONCLUSION

The intention of the contemporary study was to use GC-MS analysis to recognize different bioactive components from the methanol extract of *Indigofera prostrata* for the first time. These substances are in charge of the various pharmacological and therapeutic activities. phytochemical components that contribute to actions including sedative, antibacterial, antioxidant, and other actions. Hence, the medicinal benefits of phytochemicals are because of their existence. In order to produce advanced medicines utilizing some of the bioactive materials recognized in *Indigofera prostrata*, additional investigation is necessary.

Acknowledgements

The management of GITAM School of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India, is appreciated by the authors for providing the resources needed to complete the research. We also thank C.M.R. College of Pharmacy, Hyderabad, Telangana, India, for their assistance.

REFERENCES

1. Roessner U, Beckles DM. Metabolite measurements. In J. Schwender (Ed.), Plant metabolic networks. Springer 2009; 39-69. DOI:10.1007/978-0-387-78745-9_3
2. Duraisamy G, Manokaran K, Ganesan R, Kanakasabapathy D, Chandrasheakar U. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. Journal of Food Science and Technology. 2015; 52(2): 1212-7. DOI: 10.1007/s13197-013-1105-9
3. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016; 21(5): 559. DOI: 10.3390/molecules21050559
4. Hannu Korhonen. Technology options for new nutritional concepts. MTT Agrifood Research. Food Research. 2002; 55(2): 79-8. DOI: https://doi.org/10.1046/j.1471-0307.2002.00050.x
5. Rakam Gopi Krishna, Raja S. Standardization and Phytochemical Screening of *Bougainvillea glabra*. International Journal of Current Pharmaceutical Research. 2017; 9(5):75-80. DOI: http://dx.doi.org/10.22159/ijcpr.2017v9i5.22143
6. Pandey MM, Rastogi S, Rawat AK. Indian herbal drug for general healthcare: An overview. Internet Journal of Alternative Medicine. 2008; 6:1-4.
7. Malongane F, McGaw LJ, Mudau FN. The synergistic potential of various teas, herbs and therapeutic drugs in health improvement: A review. Journal of the Science of Food and Agriculture. 2017; 97(14): 4679-9. DOI: 10.1002/jsfa.8472.
8. Keskes, H. et al. LC-MS-MS and GC-MS analyses of biologically active extracts and fractions from *Tunisian Juniperus* phoenice leaves. Pharmaceutical Biology. 2017; 55(1): 88-95. DOI: 10.1080/13880209.2016.1230139.
9. Yadav R, Khare RK, Singhal A. Qualitative phytochemical screening of some selected medicinal plants of Shivpuri District. International Journal of Life Sciences. 2017; 3: 844-7. DOI: http://dx.doi.org/10.21276/ijlssr.2016.3.1.16
10. Juszczak AM, Zovko KM, Tomczyk M. Recent trends in the application of chromatographic techniques in the analysis of Luteolin and its derivatives. Biomolecules. 2019; 9: 731. DOI: 10.3390/biom9110731.
11. Satapute P, Murali KP, Kurjogi M, Jogaiah S. Physiological adaptation and spectral annotation of Arsenic and Cadmium heavy metal-resistant and susceptible strain Pseudomonas taiwanensis. Environmental Pollution. 2019; 251: 555-3. DOI: 10.1016/j.envpol.2019.05.054
12. Fan S, Chang J, Zong Y, Hu G, Jia J. GC-MS analysis of the composition of the essential oil from *Dendranthema indicum* Var. *Aromaticum* using three extraction methods and two columns. Molecules. 2018; 23(3): 576. DOI: 10.3390/molecules23030576.
13. Razack S, Kumar KH, Illaiyaraja N, Mahadeva N, Farhath K. Antioxidant, biomolecule oxidation protective activities of *Nardostachys jatamansi* DC and its phytochemical analysis by RP-HPLC and GC-MS. Antioxidants. 2015; 4(1): 185-203. DOI: 10.3390/antiox4010185.
14. Fang J, Liu C, Wang Q, Lin P, Cheng F. In silico poly pharmacology of natural products. Briefings in Bioinformatics. 2018; 19(6): 1153-71. DOI: 10.1093/bib/bbx045
15. Sliwoski G, Kothiwale S, Meiler J, Lowe EW. Computational methods in drug discovery. Pharmacological Reviews. 2014; 66(1): 334-95. DOI: 10.1124/pr.112.007336
16. Loza-Mejia M.A, Salazar JR, Sanchez-Tejeda JF. *In Silico* studies on compounds derived from *Calceolaria*: Phenylethanoid glycosides as potential multitarget inhibitors for the development of pesticides. Biomolecules. 2018; 8(4): 121. DOI: https://doi.org/10.3390/biom8040121
17. Lee K, Kim D. In-Silico molecular binding prediction for human drug targets using deep neural multi-task learning. Genes. 2019; 10(11): 906. DOI: 10.3390/genes10110906
18. Bharathi et.al., In silico molecular docking and *invitro* antidiabetic studies of dihydropyrimido (4,5- a] acridin-2-amines). BioMed Research

International. 2014; 1: 1-10. DOI: 10.1155/2014/971569

19. Adnan et al., Comparative study of *Piper sylvaticum* Roxb. Leaves and stems for anxiolytic and antioxidant properties through *in vivo*, *in vitro* and *in silico* approaches. Biomedicines. 2020; 8(4): 68. DOI: https://doi.org/10.3390/biomedicines8040068
20. Mahmud Z, Emran TB, Qais N, Bachar SC, Sarker M, Uddin MMN. Evaluation of analgesic, anti-inflammatory, thrombolytic and hepatoprotective activities of roots of *Premna esculenta* (Roxb). Journal of Basic and Clinical Physiology and Pharmacology. 2016; 27(1):63-70. DOI: 10.1515/jbcpp-2015-0056
21. Nasir Uddin MM et al., Evaluation of the anti-diarrheal activity of methanol extract and its fractions of *Urena sinuata* L. (Borss) leaves. Journal of Applied Pharmaceutical Science. 2016; 6(12): 56-60. DOI: 10.7324/JAPS.2016.601208
22. Emran TB et al., Effects of organic extracts and their different fractions of five Bangladeshi plants on *in vitro* thrombolysis. BMC Complement. Altern. Med. 2015; 15:128. DOI: https://doi.org/10.1186/s12906-015-0643-2
23. Adnan Md et al., Evaluation of anti-nociceptive and anti-inflammatory activities of the methanol extract of *Holigarna caustica* (Dennst.) Oken leaves. Journal of Ethnopharmacology. 2019; 236:401-11. DOI: 10.1016/j.jep.2019.01.025
24. Kabir MSH et al., Phytochemical screening, antioxidant, thrombolytic, alpha-amylase inhibition and cytotoxic activities of ethanol extract of *Steudnera colocasiifolia* K. Koch leaves. Journal of Young Pharmacists. 2016; 8(4):391-7. DOI:10.5530/jyp.2016.4.15
25. Ferrer A, Altabella T, Arro M, Boronat A. Emerging roles for conjugated sterols in plants". Progress in Lipid Research. 2017; 67: 27-7. DOI:10.1016/j.plipres.2017.06.00
26. Rakam Gopi Krishna and Raja Sundararajan. Screening of antioxidant activity of *Mucuna pruriens* by *in vivo* model. International Journal of Research in Pharmaceutical Sciences. 2019; 10(1): 523-530. DOI: https://doi.org/10.26452/ijrps.v10i1.1875
27. Prasad S, Kalra N, Singh M, Shukla Y. Protective effects of lupeol and mango extract against androgen induced oxidative stress in Swiss albino mice. Asian Journal of Andrology. 2008; 10 (2): 313-8. DOI: https://doi.org/10.1111/j.1745-7262.2008.00313.x
28. Mannowetz N, Miller MR, Lishko PV. Regulation of the sperm calcium channel CatSper by endogenous steroids and plant triterpenoids". Proceedings of the National Academy of Sciences, USA. 2017; 114 (22): 5743-8. DOI: 10.1073/pnas.1700367114S
29. Lenger, Stacy M, Bradley, Megan S, Thomas, Debbie A, Bertolet, Marnie H, Lowder, Jerry L, Sutcliffe, Siobhan. D-mannose vs other agents for recurrent urinary tract infection prevention in adult women: a systematic review and meta-analysis. American Journal of Obstetrics and Gynecology. 2020; 223(2):265.e1–265.e13. DOI:10.1016/j.ajog.2020.05.048.