INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

ISSN: 2395-6429, Impact Factor: 4.656
Available Online at www.journalcmpr.com
Volume 8; Issue 05(A); May 2022; Page No.203-208
DOI: http://dx.doi.org/10.24327/23956429.ijcmpr20220049

Research Article

PHYTOCHEMICAL STUDIES ON AN UNEXPLORED MEDICINAL HERB -*INDIGOFERA VISCOSA* LAM. LEAVES

Sudheer Mohammed MM*, Mani J and Narayanasamy A

Pharmacognosy & Phytochemistry Laboratory, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-18, Tamil Nadu, India

ARTICLE INFO

Article History:
Received 10th February, 2022
Received in revised form 2nd March, 2022
Accepted 26th April, 2022
Published online 28th May, 2022

Key words:
Indigofera viscosa, Extracts, Secondary metabolites, FTIR, GC-MS.

ABSTRACT

Species of *Indigofera* (Leguminosae) genus used as traditional medicines in different parts of the world, some of them are not studied for its chemical diversity. The present study was aimed to characterize the phytochemical potential of *Indigofera Viscosa* leaf. Nonpolar to polar solvents were used to successively extract the phytochemicals by using soxhlet apparatus. The extracts were tested for the secondary metabolite screening. Major bioactive compounds such as alkaloids, flavonoids, tannins, steroids, triterpenoids were detected from various extracts. Ethanol extract showed high positive results in the test of such biochemicals, so this extract only used for further study. High amount of total phenolic content was noted in the quantification of secondary metabolites. FTIR analysis indicated the presence of metabolically active alcohols, phenols, carboxylic acids, amino acids, alkynes, esters, nitro compounds, aromatic amines, etc. GC-MS analysis revealed that presence of 15 major compounds in leaf, most of them are well-known for their biological activities. The compound milbemycin B may highly present in the leaf sample because of its large area percentage.The present study will be helpful to the quality assessment of herbal remedies containing *Indigofera* species and chemotaxonomic justification of the species. Further investigations are required to study the biological activities of the plant in crude as well as elute form of extracts.

Copyright © 2022 ***Sudheer Mohammed MM, Mani J and Narayanasamy A****. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

INTRODUCTION

Many herbaceous plants are being used as food and medicine in different parts of the world since a long time ago. The medicinal herbs are useful to healing and curing of various human illnesses because of their phytochemicals[1]. The chemical components are deposited in the specific parts of the plants such as leaves, flowers, stems, roots, fruits, seeds, etc. These phytochemicals are categorized as primary metabolites and secondary metabolites based on their functions. The secondary metabolites are well known for its diverse pharmacological actions. The most important bioactive secondary metabolites are alkaloids, terpenoids, tannins, saponins, glycosides and phenolic groups[2]. Plant based drugs have recently become great interest among human population, because they are easily available, low cost, safe and more efficient, and have no or less side effects. Approximately 20% of known plants have been used in pharmaceutical research to treat cancer and other harmful diseases because of their great impact in the treatment of human healthcare system in the positive way[3].

There are many plant genera helping a human body in a variety of ways. The genus *Indigofera* belongs to the family Leguminosae (sub family Fabaceae), it is the third largest genus in that family, with approximately 750 species [4]. Genus *Indigofera* is highly familiar for the reason of its economically important indigo dye producing species. They are also used as a remedy in the treatment of several diseases across the world. Species of *Indigofera* used to treat skin diseases, swellings, wounds, digestive disorders, snakebites, headaches, chest pain, neurological disorders, diarrhea, dysentery and used as a diuretic, coolants, demulcents and pain killers[5-10].Around 200 compounds have been identified so far, including phenolics, terpenoids, alkaloids and glucose esters of nitropropanoic acids. Crude extracts and purified fractions obtained from various parts of *Indigofera* species studied for their wide range of pharmacological activities[11].

Rusty Indigo (=*Indigofera Viscosa* Lam.) (figure 1) has been extensively used as folk medicine in various countries where it is found. This species is mainly used to treat jaundice, stomach pain, diarrhea, dysentery, scabies, cuts and wounds [12-14].Whole plant and aerial parts of the plant was previously studied for its phytochemical nature and antioxidant properties[15, 16].The objective of this paper was planned to provide phytochemical profiling of leaves of *Indigofera viscosa*.

Corresponding author:* **Sudheer Mohammed MM
Pharmacognosy & Phytochemistry Laboratory, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-18, Tamil Nadu, India

Figure 1 Morphological features of *Indigofera viscosa*

MATERIALS AND METHODS

Collection and extraction of the plant parts

The plant was collected from Thondamuthur region, Coimbatore district, and the plant was authenticated by Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu. The plant twigs were collected from the same place and the leaves separated and cleaned by running water, "the nair" dried at room temperature. The dried leaves was powdered manually by using mortar and pestle, the fine powder was stored in clean glass beaker for phytochemical studies. 100g of powder was used for successive solvent extraction[17] through soxhlet apparatus. Least polar to most polar solvents such aspetroleum ether, dichloromethane, ethanol and water were successively ran for 72 h for proper extraction. Each extract was stored in labelled sterile containers and kept at room temperature.

Secondary metabolite screening

Alkaloids, flavonoids, tannins, steroids, triterpenoids, saponins, glycosides, gum and mucilages and fixed oils were screened in variousextracts by using standard procedures[18-25].

Estimation of secondary metabolites

Total phenolics content

The total phenol content was determined by the standard procedure [26]. 500 µl aliquots of ethanol extract was taken in the test tubes and made up to the volume of 1 ml with distilled water. A test tube with 1 ml of distilled water served as the blank. Then, 500 µl of Folin–Ciocalteau reagent (1N) was added in both the test tubes including the blank. After 5 min 2.5 ml of sodium carbonate ($NaCO_3$) solution (5%) was added in the test tubes. Immediately vortexing the reaction mixture, then the test tubes were placed in the dark room for 40 min, after that the absorbance was recorded at 725 nm using ultraviolet-visible spectrophotometer against blank. The analysis was performed in triplicates, and the results were expressed as Gallic Acid Equivalents (GAE).

Total tannins content

The total phenolics contain both tannin and non-tannin phenolics. The amount of tannins is calculated by subtracting the non-tannin phenolics from the total phenolics. For the determination of non-tannin phenolics[26], 500 µl of plant samples was incubated with 100 mg of polyvinyl polypyrrolidone (PVPP) and 500 µl of distilled water taken in a 2 ml eppendorftube at 4 °C for 4 h. Then the sample was centrifuged at 4000rpm for 10 min at room temperature and the supernatant was collected. The supernatant contains only non-tannin phenolics since the tannins would have been precipitated along with PVPP. The non-tannin phenolics was determined by the same method described for the quantification of total phenolics. The analysis was also performed in triplicates and the results were expressed in Tannic Acid Equivalents (TAE). From the above results, the tannin substance of the example was calculated as takes after:
Tannin (in percentage) =Sum of phenolics (in percentage)-Non tannin phenolics (in percentage).

Total flavonoids content

Total flavonoids content was estimated the standard method [27]. Initially, 500 µl of plant sample was taken in a test tube and 2 ml of distilled water was added to the test tube. A test tube containing 2.5 ml of distilled water served as blank. Then, 150 µl of 5% sodium nitrite ($NaNO_2$) was added in both the test tubes followed by incubation at room temperature for 6 min. After incubation 150 µl of $AlCl_3$ (10%) was added in all the test tubes. The test tubes were incubated for 6 min at room temperature. Then 2ml of 4% sodium hydroxide (NaOH) was added to all the test tubes which were made up to 5 ml using distilled water. The contents in all the test tubes were vortexes well and they were allowed to stand for 15 min at room temperature. The pink colour was developed due to the presence of flavonoids and it read at 510 nm in a spectrophotometer. The amount of flavonoids was calculated as Rutin Equivalents (RE) and the experiment was done in triplicates.

Fourier Transfer Infra-Red (FTIR) analysis

KBr (potassium bromide) pellet method was adopted for FTIR analysis [28] in the powder form of ethanol leaf extract. About 2 to 3mg of leaf sample was mixed with 200mg of perfectly dried KBr pellets for good results. Shimadzhu FTIR spectrometer was used, the absorption spectra range was recorded between 4000 to 400cm^{-1} at room temperature. This study was repeated thrice for spectrum confirmation.

Gas Chromatographic and Mass Spectroscopic (GC–MS) analysis

GC–MS analysis of ethanol extract was carried out using a Clarus-500 gas chromatograph system equipped with Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II (Thermo Scientific Co.). GC-MS experimental condition was as follows: DB 35-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, film thickness: 0.25µm.The oven temperature was programmed from 70–260°C gradually change at 6°C/min. Total run time was 31.68 min.The components were separated using helium (carrier) gasat a flow rate of 1.0 ml/min. Sample released at 3rd minute of the total run time. The electron ionization mode with ionization energy of 70 eV; ion source temperature of 220°C; fragments range from 50 to 650 m/z were set for the MS analysis. About 1 µlof the plant sample was injected manually in a split less mode.

Data analysis and Software used

MS office 2013 Excel software was used to calculate the gallic acid, tannic acid and rutin equivalents. For GC/MS analysis, the raw data were compared with the mass fragmentation patterns of standards of NIST (National Institute of Standard Technology) library using Turbomass software (ver.5.4.0).

RESULTS

Secondary metabolite screening

This study was carried out to test 10 major secondary metabolites, and it revealed that presence of all the tested compounds in various extracts of the plant (table 1). Except dichloromethane, all the remaining extracts showed significant positive results in this screening. Ethanol extract showed the presence of 8 compounds which is higher than other extracts. Dichloromethane showed the presence of least number of compounds (only 2 compounds). Flavonoids and anthraquinones detected only in ethanol extract, while glycosides detected only in petroleum ether extract. Alkaloids and terpenoids were abundantly present in the ethanol extract which is noted as '++' in the table 1. Tannins, triterpenoids

and saponins showed positive result in ethanol and water samples.

Table 1 Secondary Metabolite Screening of *I. viscose* leaf

S. No.	Compounds	Tests	P	D	E	W
1	Alkaloids	Dragendorff's test	_	_	++	+
2	Flavonoids	10% HCl & 5% NaOH test	_	_	+	_
3	Tannins	5% $FeCl_3$ test	_	_	+	+
4	Steroids	Liebermann-Burchard's test	+	+	_	_
5	Triterpenoids	Liebermann-Burchard's test	_	_	++	+
6	Saponins	Foam test	_	_	+	+
7	Glycosides	Keller - Kiliani test	+	_	_	_
8	Gum &Mucilages	Whistler andBeMiller test	+	_	+	+
9	Fixed oils	Spot test	+	+	+	_
10	Anthraquinones	Sanker and Nahar test	_	_	+	_

P – Petroleum Ether; D – Dichloromethane; E – Ethanol; W – Water
' + ' indicate presence of compounds ; ' - ' indicate absence of compounds

Estimation of secondary metabolites

The total amount of phenolics, tannins and flavonoids of the present study was analyzed and the results are tabulated (table 2). Folin-ciocalteu method has been used to estimate the total phenolics, the same method is used in the estimation of total tannin content. Results showed that the total phenolic content of leaf sample was 76.49±5.35 mg GAE / g extract and total tannin content was 37.46 ±3.81 mg TAE / g extract. The total flavonoid estimation of the ethanol extract was 59.66 ±8.87 mg RE / g extract, it was calculated with the aluminium chloride colorimetric assay.

Table 2 Total phenolics, tannins and flavonoids content of ethanol leaf extract

Total phenolics (mg GAE / g extract)	Total tannins (mg TAE / g extract)	Total flavonoids (mg RE / g extract)
76.49±5.35	37.46 ±3.81	59.66 ±8.87

GAE- gallic acid equivalents, TAE- tannic acid equivalents, RE- rutin equivalents.
Values are mean of triplicate determinations (n=3) ± standard deviation

FTIR analysis

The FTIR analysis showed the presence of H–bond, O-H, N-H, C–O, C=O, –C≡C–, C–N, C-H, C–Br and N–O asymmetric stretches based on the peak values. These bonds indicated the occurrence of various functional groups such as alcohols, phenols, carboxylic acids, amino acids, alkynes, esters, alkenes, nitro compounds, aromatic amines, aliphatic amines, ethers and alkyl halides in the ethanol leaf extract (table 3). Which are confirmed the existence of bioactive compounds such as alkaloids, flavonoids, tannins, flavonoids, terpenoids and saponins in *I. viscosa*.

Table 3 FTIR Peak values and functional groups of ethanol leaf sample

Peak value (Wave number cm^{-1})	Type of Bond	Functional group
3753.48	O-H stretch	Alcohols
3356.14	O–H stretch, H–bonded	Alcohols, phenols
2927.94	O–H stretch	Carboxylic acids
2376.30	N-H stretch	Carboxylic acids
2341.58	N-H stretch	Amino acids
2299.15	N-H stretch	Amino acids
2222.00	–C≡C– stretch	Alkynes
2140.99	–C≡C– stretch	Alkynes
1874.81	C=O stretch	Ester
1651.07	–C=C– stretch	Alkenes
1543.05	N–O asymmetric stretch	Nitro compounds
1423.47	C–C stretch	Aromatics
1404.18	C–C stretch	Aromatics
1381.03	C-H stretch	Alkanes
1319.31	C–N stretch	Aromatic amines
1242.16	C–N stretch	Aliphatic amines
1060.85	C–O stretch	Carboxylic acids, esters
894.97	C–H "oop"	Aromatics
775.38	C–H "oop"	Aromatics
532.35	C–Br stretch	Alkyl halides

GC-MS analysis

In GC-MS analysis, the compound name, retention time (RT), molecular formula, molecular weight and area percentage of the test material was ascertained by the library search results. Figure 2 depicted the gas chromatogram of *I. viscosa* ethanol leaf extract. The mass spectrum of each peak was compared with compounds stored in the NIST library (table 4). Totally fifteen characteristic peaks were observed in the gas chromatogram, among them six are major peaks (RT 9.27, 10.72, 11.25, 14.67, 16.60 and 28.11). Among these 15 compounds, alkaloids and terpenoids having three compounds and amines having two components in their groups. Cyclohexenones, alkanes, benzoate ester, cardiac glycosides, phenols, sesquiterpene lactones and macrolides having one compound in each category. The compounds sempervirone (alkaloids), burnamicine (alkaloids), phytol (terpenoids), roemerolidine (alkaloids), digitoxin (cardiac glycosides), toosendanin (terpenoids), sophoracarpan (phenols) and 9,19-cyclolanostane-3,7-diol (terpenoids) are comes under important secondary metabolites. The identified compounds were previously reported as bioactive molecules to treat various diseases (table 5). The present GC-MS analysis proved that medicinal importance of *I. viscosa*.

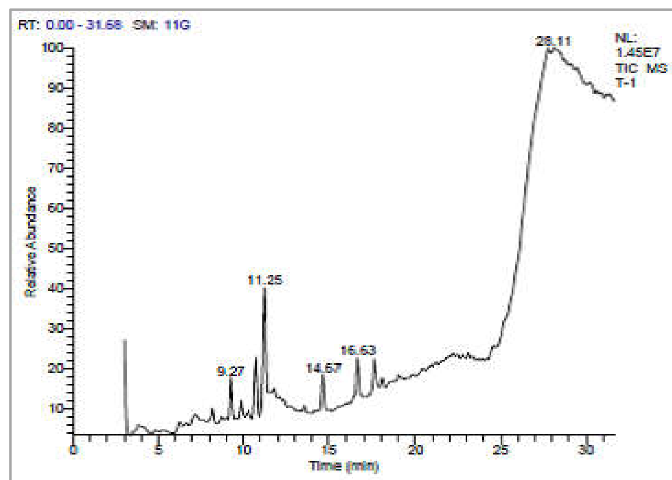

Figure 2 Gas chromatogram of ethanol leaf sample

Table 4 List of compounds identified from GC–MS analysis

S. No.	RT	Compound name	Compoundnature	Molecular formula	Molecular weight	Area %
1	5.24	Sempervirone	Alkaloids	$C_{26}H_{39}NO_2$	397	0.34
2	8.72	à-Cyclohexylbenzylamine	Aliphatic amines	$C_{13}H_{19}N$	189	0.33
3	9.27	3,4-Dihydro-2H-1,5-(3"-t-butyl) benzodioxepine	Amines	$C_{13}H_{18}O_2$	206	2.46
4	9.89	Megastigmatrienone 4	Cyclohexenones	$C_{13}H_{18}O$	190	1.12
5	10.72	bicyclo[3.2.0]hept-2,6-diene-1,2,3,4,4,5-d(6)	Alkanes	$C_7H_2D_6$	92	4.46
6	11.25	Methyl Syringate	Benzoate esters	$C_{10}H_{12}O_5$	212	12.48
7	14.67	Burnamicine	Alkaloids	$C_{20}H_{26}N_2O_2$	326	2.59
8	16.60	Phytol	Terpenoids	$C_{20}H_{40}O$	296	3.19
9	18.06	Roemerolidine	Alkaloids	$C_{18}H_{17}NO_4$	311	0.70
10	18.69	Digitoxin	Cardiac glycosides	$C_{41}H_{64}O_{13}$	764	0.53
11	19.02	Toosendanin	Terpenoids	$C_{30}H_{38}O_{11}$	574	0.69
12	22.01	Sophoracarpan B	Phenols	$C_{17}H_{14}O_6$	314	0.33
13	24.56	9,19-Cyclolanostane-3,7-diol	Terpenoids	$C_{30}H_{52}O_2$	444	0.56
14	25.19	Elephantin	Sesquiterpene lactones	$C_{20}H_{22}O_7$	374	0.36
15	28.11	Milbemycin B	Macrolides	$C_{33}H_{47}ClO_7$	590	53.01

Table 5 Biological uses of the constituents identified from GC–MS analysis

S. No.	Compound name	Uses/ Pharmacological activities
1	Megastigmatrienone 4	Aromatic agent
2	bicyclo[3.2.0]hept-2,6-diene-1,2,3,4,4,5-d(6)	Antimicrobial, Antinociceptive, Antioxidant, Insecticidal activities
3	Methyl Syringate	Antioxidant activity, Suppress hypoxia-induced inflammation, Regulate the food intake
4	Phytol	Antimicrobial, Anti-inflammatory, Anti-cancer, Antiarthritic, Hepatoprotective activities
5	Roemerolidine	Antimalarial, Antiplasmodial, Wound healing activities
6	Digitoxin	Anticancer, Antiviral (HCMV & HSV) activities, Congestive heart failure
7	Toosendanin	Anticancer, Anti-anthelmintic, Anti-inflammatory, Analgesic, Anti-microbial, Antiviral, Cytotoxic, Insecticidal, Anti-botulinum activities.
8	9,19-Cyclolanostane-3,7-diol	Antibacterial, Anti-inflammatory activities
9	Elephantin	Antitumor activity
10	Milbemycin B	Anti-anthelmintic, Anti-parasitic, Anti-infective, Anti-MRSA effect activities

DISCUSSION

Preliminary phytochemical screening in the plant samples is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy [29].The results obtained from phytochemical screening in various extracts of *I. viscosa* leaves revealed that ethanol extract possess high number of bioactive compounds compared to other extracts. Presence of various secondary metabolites in *I. viscosa* leaf sample received attention because of the tested compounds possess extensive medicinal properties, which is proved by scientific research reports [30-33]. In addition, the presence of flavonoids, tannins, sterols, triterpenes and saponins has already been reported in aqueous acetone extract of *Indigofera viscosa* (= *I. colute*a) [15]. But the alkaloids couldn't detected in that study. The current research showed better results compared to the previous study. For this reason, quantification assays, FTIR and GC/MS analysis were carried out only in ethanol sample for the determination of active phytocompounds.

Appreciable level of total phenolic, tannin and flavonoid content were noted in *I. viscosa* ethanol leaf extract. Most of the research reports manifest that the total phenolics have been determined to be the main antioxidant compounds [34]. Several thousands of different compounds have been identified in phenolic group and are the most widely distributed class of secondary metabolites. Many of the polyphenols have been shown to contain higher levels of antioxidant activities [35]. Total phenolics and flavonoids content was already estimated in aerial parts (stems with leaves) of *Indigofera colute*a (= *I. viscosa*) in the extract obtained from the combination of aqueous acetone solvent [15]. In their study, the results were compared with its allied species namely *I. macrocalyx*, *I. nigritana*, *I. pulchra* and *I. tinctoria*. Among them, the highest total phenolic content was recorded in *I. colutea* (54.27±4.87 mg GAE/100 mg extract). But in our current study the total phenolic content was recorded as 76.49±5.35 mg GAE/g extract, which is almost accordance with previous study[15]. Qualitative and quantitative analysis of secondary metabolites are the fundamental studies for the separation and characterization of bioactive molecules.

Occurrence of various functional groups in *I. Viscosa* leaves providing the clue for the occurrence of diverse phytoconstituents. The results were accordance with previous studies. The FTIR analysis of leaves of *I. aspalathoides* and *I. linnaei* were reported to the presence of phenols, alkynes, carboxylic acids, alkenes and aromatics functional groups [36, 37]. Now a days, the FTIR analysis extensively used in many fields, mostly in food applications, pharmaceutical and medicinal researches. This technique is more suitable method to evaluate not only the quality of the powder but also the presence of adulterants if any.

Different class of metabolites were identified in the GC-MS analysis of *I. viscosa* leaves. The mass spectrum of the compound milbemycin B absorb high area percentage (53.01%) in its respective peak time (RT 28.11), it may found to be the major compound of the ethanol leaf extract. The remaining compounds have minimum area percentage.

The compound phytol had previously been reported in GC-MS analysis of *Indigofera aspalathoides, I. suffruticosa, I. tinctoria* and *I. leucocephala*. Unlike this, the remaining 14 constituents are identified only in this study and are not

reported in *Indigofera* species. In 2013, Rajabudeen *et al.* [16] studied GC-MS analysis of methanol extract of *I. Viscosa* whole plant. The authors identified 23 compounds, unfortunately none of the compound is present in our present study. It is due to the selection plant part(s), solvent(s) used for extraction and period of extraction. The identified compounds are regarded as a chemotaxonomic marker for the species *I. viscosa*.

CONCLUSION

In the present study, *Indigofera Viscosa* leaves were subjected to phytochemical profiling. Since this plant is widely used by people across the globe wherever it is distributed due to its medicinal properties. Bioactive compounds showed their presence in various extracts in the qualitative secondary metabolite screening. Significant amount of total phenolic, tannin and flavonoid contents were found in ethanol extract, this suggests that the plant may possess high antioxidant activity. The FTIR analysis confirmed the presence of various functional groups such in the leaf extract, which provide the chemical finger print data of leaf. Further, several compounds were identified from GC/MS analysis. Most of them have been reported as a source of antimicrobial, antioxidant, anti-inflammatory, anti-cancer, antiviral, antiarthritic, anti-botulinum, anti-anthelmintic, hepatoprotective and insecticidal properties and are also as wound healing, analgesic and aromatic agents. We conclude that *I. viscosa* possess valuable bioactive compounds which is proved in our current study. There is enormous scope for future research on *I. viscosa*, especially pharmacological research to investigate unexploited potential of this plant.

Acknowledgement(s)

The authors highly thankful to the Department of Botany, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India, for the utilization of necessary facilities.

Conflict of Interest

None.

References

1. NostroA, Germanò MP, D'angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett ApplMicrobiol. 2000;30(5):379-384.
2. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotech. 2005;4(7):685-688.
3. Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. J Pharm Biomed Anal. 2006;41(5):1523–1542.
4. Schrire B. A review of tribe Indigofereae (Leguminosae–Papilionoideae) in Southern Africa (including South Africa, Lesotho, Swaziland & Namibia; excluding Botswana). S Afr J Bot. 2013;89:281–283.
5. Lev E, Amar Z. “Fossils” of practical medical knowledge from medieval Cairo. JEthnopharmacol. 2008;119(1):24–40.
6. Coe FG, Anderson GJ. Snakebite ethnopharmacopoeia of eastern Nicaragua. JEthnopharmacol. 2005;96(1-2):303–323.
7. Ahmad Jan H, Wali S, Ahmad L, Jan S, Ahmad N, Ullah N. Ethnomedicinal survey of medicinal plants of Chinglai valley, Buner district, Pakistan. EurJ Integr Med. 2017;13:64–74.
8. Dey A, Gorai P, Mukherjee A, Dhan R, Modak BK. Ethnobiological treatments of neurological conditions in the Chota Nagpur Plateau, India. J Ethnopharmacol. 2017;198: 33–44.
9. Manandhar NP. Native phytotherapy among the Raute tribes of Dadeldhura district, Nepal. J Ethnopharmacol. 1998;60(3):199–206.
10. Omprakash K, Subash Chandran G, Velpandian V. *Indigofera aspalathoides*Vahl ex. DC (SivanarVembu): A phytopharmacological review. Int J Pharm Sci Res. 2013;4(10):3775–3781.
11. Gerometta E, Grondin I, Smadja J, Frederich M, Gauvin-Bialecki A. A review of traditional uses, phytochemistry and pharmacology of the genus *Indigofera*. JEthnopharmacol.2020;253:11260.
12. Chifundera K. Contribution to the inventory of medicinal plants from the Bushi area, South Kivu Province, Democratic Republic of Congo. Fitoterapia, 2001;72(4):351-368.
13. Neuwinger HD. African Traditional Medicine: A Dictionary of Plant Use and Applications with Supplement: Search System for Diseases. Stuttgart:Medpharm Scientific Publishers; 2000.
14. Copeland SR. Vegetation and plant food reconstruction of lowermost Bed II, Olduvai Gorge, using modern analogs. J HumEvol. 2007;53(2):146-175.
15. Bakasso S, Lamien-Meda A, Lamien CE, Keindrebeogo M, Millogo JF, Ouedraogo AG, Nacoulma OG. Polyphenol contents and antioxidant activities of five *Indigofera* species (Fabaceae) from Burkina Faso. Pak J Biol Sci. 2008;11(11):1429-1435.
16. Rajabudeen E, Saravana Ganthi A, Sorna Subramanian MP, Natarajan K. GC–MS analysis of biologically active compounds in *Indigofera viscosa* Lam. Int J Plant Sci. 2013;8(1):116-119.
17. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J Med Plant Res. 2010;4(2):104-111.
18. Waldi D. Spray Reagents for Thin Layer Chromatography. In Stahl E (Eds.), Thin layer chromatography - A Laboratory Hand book. New York: Acadameic Press Inc; 1965.
19. Trease GE, Evans WC. Text Book of Pharmacognosy. 15th ed. London: WB Saunders; 2002.
20. SegelmanAB, Farnsworth NR, Quimby MD. Biological and Phytochemical Evaluation of Plants III. False negative saponins test results induced by the presence of tannins. Lloydia. 1969;32(1):52-58.
21. Finar IL. Stereo chemistry and the chemistry of natural products.Volume 2. Singapore: Longman; 1986.
22. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan Publication; 1999.
23. Camporese A, Balick MJ, Arvigo R, Esposito RG, Morsellino N, De Simone F, Tubaro A. Screening of anti-bacterial activity of medicinal plants from Belize (Central America). J Ethnopharmacol. 2003;87(1):103–107.
24. Whistler RL, BeMiller JN. Industrial Gums; Polysaccharides and their Derivatives. 3rd ed. London: Academic Press; 1993.

25. Sanker SD, Nahar L. Chemistry for Pharmacy Students General, Organic and Natural Product Chemistry. England: John Wiley and Sons; 2007.
26. Makkar HPS. Quantification of tannins in tree and shrub foliage: A laboratory Mannual. Dondrecht, The Netherlands: Kluwer academic publishers; 2003.
27. Zhishen J, Mengecheng T, Jianming W. The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. Food Chem. 1999;64(4):555–559.
28. Stuart BH. Infrared Spectroscopy: Fundamentals and Applications. Volume 14 of Analytical Techniques in the Sciences (AnTs). West Sussex: John Wiley and Sons Ltd.; 2004. p. 28-29.
29. Nandagoapalan V, Doss A, Marimuthu C. Phytochemical Analysis of Some Traditional Medicinal Plants. BiosciDiscov. 2016;7(1):17-20.
30. Pulipati S, Babu PS, Naveena U, Parveen SKR, Nausheen SKS, Sai MTN. Determination of Total Phenolic, Tannin, Flavonoid Contents and Evaluation of Antioxidant Property of *Amaranthus tricolor*(L). Int. J. PharmacognPhytochem Res. 2017;9(6):814-819.
31. Mahato SB, Sen S. Advances in triterpenoid research, 1990-1994. Phytochemistry. 1997;44(7):1185-1236.
32. Wang CY, Jang HJ, Han YK, Su XD, Lee SW, Rho MC, Wang HS, Yang SY, Kim YH. Alkaloids from *Tetrastigma hemsleyanum* and their Anti-Inflammatory effects on LPS-induced RAW264.7 Cells. Molecules. 2018;23(6): 1445.
33. Del-Rio A, Obdulio BG, Castillo J, Marin RR, Ortuno A. Uses and properties of citrus flavonoids. J Agric Food Chem. 1997;45(12):4505-4515.
34. Wu SJ, Ng LT. Antioxidant and free radical scavenging activities of wild bitter melon (*Momordica charantia* Linn. var. *abbreviata* Ser.) in Taiwan. LWT - Food Sci Technol. 2008;41(2):323-330.
35. Shirwaikar A, Malini S, Kumari SC. Protective effect of *Pongamia pinnata* flowers against cisplatin and gentamicin induced nephrotoxicity in rats. Indian J Exp Biol. 2003;41(1):58-62.
36. Kumar TT, Salique SM, Ilyas MHM, ThajuddinN, Panneerselvam A,Padusha MSA, Jahangir HS. Phytochemical Screening and Antimicrobial Studies in Leaf Extracts of *Indigofera aspalathoides* (Vahl.). Pharmacogn J.2018;10(6):1208-1215.
37. Yesu Raj Y, Paul John Peter M, Venkatesan M. Phytochemical and GC-MS studies on *Indigofera linnaei* Linn. Int JPhytopharm. 2018;2(5):143-148.

How to cite this article:

Sudheer Mohammed MM, Mani J and Narayanasamy A (2022) 'Phytochemical Studies on An Unexplored Medicinal Herb - Indigofera Viscosalam. Leaves', *International Journal of Current Medical and Pharmaceutical Research,* 08(06), pp 203-208.

****