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Research Article

PHYTOCHEMICAL AND GC-MS STUDIES *ONINDIGOFERA LINNAEI* LINN.

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Abstract
Xanthine oxidase is a highly versatile enzyme that is widely distributed among different *Indigofera linnaei* Linn.is a potential folklore medicinal plant (Fabaceae) used for Aurveda and Siddha systems of medicine. In this study Alkaloids, Carbohydrate Glycoside, Saponin, Flavonoids, tannins and Phytosteroids were identified as the major phytochemical constituents in the methanol, acetone and toluene fractions of *Indigofera linnaei* Linn. leaf extract. Their structures were elucidated, on the basis of GC-MS data.2,4,6-Octanerione (9.24%), 4, (methyl cyclopropyl)-1-butene(8.87%), non-ionic acid methyl ester(5.78%), trans-N-methyl-3-oxo-5,6-dimethoxy morphian (9.63%). (IRS, 2SR) 2-Dimethyl (Phenyl) silylpentane-3-ol (7.39%), 2, 2-bis (t-phenyl 3, 4" dimethyl phosphate) (5.84%), 2-cyclopropylenetic acid(6.49%) these different active phytochemicals have been found to possess a wide range of activities. In conclusion *Indigofera linnaei* Linn. contains biologically active compounds that may serve as candidate for the discovery of new drugs in the treatment of antimicrobial activities.

Keywords: GC-MS, Phytochemicals, *Indigofera linnaei* Linn.,antimicrobial activities

1. Introduction:

Plants have great potential uses, especially as traditional medicine and pharmacopoeial drugs. A large proportion of the world population depends on traditional medicine because of the scarcity and high costs of orthodox medicine[1]. Medicinal plants have provided the modern medicine with numerous plant-derived therapeutic agents[2]. Many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases[3]. The use of plants with pharmaceutical properties has received increased interest nowadays from bothhomeopathic and allopathic branches. These medicinal plants play an important role in public health,especially in developing countries, where it is believed that the intense utilization of plants with therapeutic action does not lead to intoxication [4]. The cost of drugs in use today is too expensive for themajority of the population in the third world countries and therefore the search for some cheap sources ofantimicrobial substances in nature become inevitable. Plants are good sources for new safe, biodegradableand renewable drugs. The use of plants as therapeutic agents in addition to being used as food is age long.Though the therapeutic uses of plants by the primitive people lack scientific explanations [5], there is agreat awareness in the use and significance of these medicinal floras by the World Health Organization inseveral resource- poor nations [6]. This has led to intensified efforts on the documentation of medicinal plants [7].

***1.2 Morphological Description*:** Shrublets or perennial herbs, usually prostrate, sometimes ascending, 20-90 cm tall. Stems with appressedmedifixed sub-symmetrically 2-branched trichomes.Stipules ovate, 3-4 × ca. 2 mm, apex acuminate. Leaves 1.5-3 Cm, 5-9 foliolate; petiole and petiolules with appressedmedifixed trichomes; stipels not visible; leaflet blades alternate, obovate to narrowly obovate, 5-10(-15) × 2-3(-5) mm, both surfaces with medifixed 2-branched trichomes, secondary veins not visible, base cuneate, apex obtuse to truncate and with a ca. 0.1 mm mucro. Racemes 0.5-2.5 cm, sessile; bracts ovate to triangular, 2-2.5 x ca. 1 mm. Pedicels, bracts, and calyces with medifixedtrichomes. Pedicel ca. 0.5 mm. Calyx 2-2.5 mm, with appressedmedifixedtrichomes; tube ca. 1 mm; teeth narrowly triangular, 1-1.5 mm. Corolla red; standard red to orangish red, broadly

ovate to orbicular, 3-4.5 × 2.5-4 mm, outside hairy; wings 3-4 × 1-1.5 mm, glabrous, margin shortly ciliate; keel 3-4 × ca. 1.5 mm, glabrous, margin shortly ciliate, lateral spur ca. 0.5 mm. Stamens 2.5-4 mm; anthers glabrous.

2. Materials and Methods

2.1 Collection of plant material: The leaves of *Indigofera linnaei Linn.*werecollected from the Bharadhidasan university herbarium, Thiruchirappalli, Tamil Nadu, India. They were identified and authenticated by the Bharadhidasan university herbarium, Trichirappalli, Tamil Nadu, India.

2.2 Preparation of powder and extract: Leaves of *Indigofera linnaei Linn*. (500g) was shade dried, powdered and extracted with ethanol for 8 hours using soxhletapparatus.The extract was then filtered through Whatmann filter paper No.41 along with 2g sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate is wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and reduce the volume to 1ml.The extract contains both polar and non-polar phytocomponents.

2.2 GC-MS Analysis: The GC-MS analysis of *Indigofera linnaei Linn.* powder leaves extract with in absolute alcohol, was performed using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary column (5% phenyl 95% dimethyl polysiloxane) (30nm X 0.25mm ID X 0.25µmdf) and mass detector turbomass gold of the company which was operated in EI mode. Helium was the carriers gas at a flow rate of 1ml/min. and the injector was operated at 290ºC and the oven temperature was programmed as follows; 50ºC at 8ºC/min to 200ºC (5min) at 7ºC/min to 290ºC(10min).

2.3 Identification of components: Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST), WILEY8, FAME having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the (NIST) ,WILEY8, FAME library. The name, molecular weight and structure of the components of the test materials were ascertained.[8-9]

3. Results and Discussion

3.1 Ultra Violet - Visible Spectroscopy: The plant sample extracts of two solvents (methanol and toluene) has been taken for UV-vis study.

The plant extracts of methanol and toluene is been tested for UV-vis spectrum. The principle of UV-spectral analysis is for separation of functional group and electron transition compound respectively. The functional group of active compounds by UV-visible spectrum by position of peak values ranges from 404.17 to 666.27 in methanol extract and 412.12 to 669.96 in toluene extract.

Table 1: Ultra Violet - Visible Spectroscopy data *Indigofera linnaei Linn*

S. No.	Methanol	Toluene
1.	404.17	412.12
2.	534.04	534.60
3.	607.76	609.31
4.	666.27	669.96

3.2 Fourier Transformed Infrared: Performing the next advanced phytochemical analysis technique of FTIR the presence of various functional groups of different compounds was found.

Table 2:*Indigofera linnaei* Linn

S. No.	Methanol
1.	3819.71
2.	3937.71
3.	3410.62
4.	2925.22
5.	2362.12
6.	2134.44

7.	1641.82
8.	1388.09
9.	1054.34
10.	621.03

The FTIR method is the radiation passed through sample to be separated the functional group of compounds, the FTIR analysis is done in methanolic extract, the peak area ranges from 3819.17 to 621.03.

The FTIR and UV spectrum was used to identify the functional group of the active components based on the peak values in the infrared radiation. The methanol extract of *G. kollimalayanum* was passed through FTIR, the functional groups of the components were separated based on its peak ration and the same was passed into UV spectroscopy for electron transition of compounds.

The FTIR analysis confirmed the presence of the carboxylic acid, and Alkenes-CH_2, CH_3, Aromatic strecting which shows major peaks at 1019.87 and 2922.33 *etc.* (Yuvarajan*et al*.).

3.3 Gas Chromatography Mass Spectrometry: The plant sample taken on subjecting to the GC-MS provided the result of different peaks determining the presence of 19 compounds it's found that most of compounds showed various therapeutic properties revealing its medicinal properties.

The GC-MS analyses of 19 bioactive compounds were identified in the methanolic extracts of *I. linnaei* Linn. they were 2,4,6-Octanerione, 4, (methyl cyclopropyl)-1-butene, non-ionic acid methyl ester, trans-N-methyl-3-oxo-5,6-dimethoxy morphian. (IRS, 2SR) 2-Dimethyl (Phenyl) silylpentane-3-ol, 2,2-bis (t-phenyl 3,4" dimethyl phosphate), 2-cyclopropylenetic acid *etc.* has the following peak areas.

Table 3: Phytocompounds identified in the *Indigofera linnaei* Linn. whole plant extract (GC-MS study).

S.No.	RT	Name of Compound	M	MW	Peak Area
1.	5.33	2,4,6-Octanetrion-E	$C_8H_{12}O_3$	156	3.99
2.	18.18	4-Methyl Cyclopropyl)-1-butene	C_8H_{14}	110	2.62
3.	20.99	Nonanoic acid, methyl ester	$C_{10}H_{20}O_2$	172	7.86
4.	24.55	Trans-N-Methyl-3-Oxo-5,6-dimethoxy morphian	$C_{19}H_{25}O_3$	315	4.17
5.	27.08	(1RS, 2SR)-2-methyl (Phenyl) silypentane-3-ol	$C_{13}H_{22}OSi$	222	3.67
6.	27.51	2,2.bis [t-phenyl-3"-4"-dimethyl phosphate)	$C_{24}H_{22}N_4O_2$	438	2.88
7.	27.90	2-Cyclopropylacetic acid	$C_5H_8O_2$	100	6.67
8.	28.26	5-a-andorst-16-en-3-ol-[(t-butyl)dimethylsily] ether	$C_{25}H_{44}O_{59}$	388	4.59
9.	28.90	t-Butyl [(4-methylpropy)-2,5-dioximidazolidin-4-yl] methyl carbonate	$C_{13}H_{23}N_3O_4$	285	2.70
10.	32.04	2,2,Dimethyl-3-hydroxy Propyl 2,2-dimethyl butonate	$C_{11}H_{22}O_3$	202	2.06
11.	32.83	Dichloroquinolin-8-olatoaluminium (3)	$C_9H_6ACl_2NO$	241	2.97
12.	33.59	2-[2-bromo-4-(1-methyl ethyl) phenyl]amino-5[6-(3-pyridinyl) hexyl] Pyridine	$C_{25}H_{30}BrN_3$	451	2.04
13.	35.24	2-tert-butoxy-3-methyl-5-(trimethylsilyl) Cyclohexa-2,5-diene-1,4-dione	$C_{14}H_{22}O_3Si$	266	2.19
14.	36.54	Diethyl-2,6-dimethyl-4-(3-Pyridazinyl) 1,4-dihydropyridine-3,5-carboxylate	$C_{17}H_{21}N_3O_4$	331	2.33
15.	39.28	N-(tert-Butoxycarbonyl)-2-(4-methoxyphenyl)allylamine	$C_{15}H_{21}NO_3$	263	2.45
16.	41.88	(5a, 6a)4,5-Epoxy-6-acetoxyl-17b hydroxyl-17-cyclopropylmethyl-3a- pthalimidomorphinan	$C_{30}H_{30}N_2O_6$	514	4.00

S.No.	RT	Name of Compound	M	MW	Peak Area
17.	42.11	7,16-Dichloro-7,16-di(phenyl sufinyl) diocosane	$C_{34}H_{52}C_{12}O_2S_2$	626	1.81
18.	43.05	(2S, 3S) - 2, 3 - Epoxy-l-hexanol	$C_6H_{12}O_2$	116	2.80
19.	44.47	(1R*, 2R*, 6S*)-2-(tert-Butyldimethylsiloxy)-6,9,9-trimethyl bicycle [4.2.1] no, nun-8-one	$C_{18}H_{34}O_2Si$	310	7.15

Table 4 : Preliminary phytochemical activities of*Indigofera linnaei Linn*

S. No.	Name of Compound	Nature of Compound Group	Activity
1.	2,4,6-Octanetrion-E	Polyketone	Antiinflammatory response, provides functional support against leukemia
2.	4-Methyl Cyclopropyl)-1-butene	Alkane	Promotes growth reduction of mutation rate, antitoxicity against compounds.
	Nonanoic acid, methyl ester	Ester	The antihelmeththic properties
4.	Trans-N-Methyl-3-Oxo-5,6-dimethoxy morphian	Alkene	Antitumour activity.
5.	(1RS, 2SR)-2-methyl (Phenyl) silypentane-3-ol	Pentane	Cytotoxicity and efficacy of allergenic extracts
6.	2,2.bis [t-phenyl-3"-4"-dimethyl phosphate)	Alkene	Antidiabetic activity.
7.	2-Cyclopropylacetic acid	Propionic acid	Tumour and antiseptic activity on lesion of skin
8.	5-a-andorst-16-en-3-ol-[(t-butyl)dimethylsily] ether	Ether	Anti-leper against the skin
9.	t-Butyl [(4-methylpropy)-2,5-dioximidazolidin-4-yl] methyl carbonate	Propane	Provides control liver damage against antisceptic activities
10.	2,2,Dimethyl-3-hydroxy Propyl 2,2-dimethyl butonate	Butane	Anticancerous activity shows presence of compounds.
11.	Dichloroquinolin-8-olatoaluminium (3)	Ketone	Anti-tumour activity
12.	2-[2-bromo-4-(1-methyl ethyl) phenyl]amino-5[6-(3-pyridinyl) hexyl] Pyridine	Aldehyde	Antihyperplasmic activity of growth reduction in intestinal enzymes.
13.	2-tert-butoxy-3-methyl-5-(trimethylsilyl) Cyclohexa-2,5-diene-1,4-dione	Isohexobutane	Antiheoplasmic activity
14.	Diethyl-2,6-dimethyl-4-(3-Pyridazinyl) 1,4-dihydropyridine-3,5-carboxylate	Diethyl butane	Control hypersensitive reaction
15.	N-(tert-Butoxycarbonyl)-2-(4-methoxyphenyl)allylamine	Allyl amino butane	Phytocompound having liver susceptibility of reactions
16.	(5a, 6a)4,5-Epoxy-6-acetoxyl-17b hydroxyl-17-cyclopropylmethyl-3a-pthalimidomorphinan	Cyclopropane	Antiallergenic reactions.

S. No.	Name of Compound	Nature of Compound Group	Activity
17.	7,16-Dichloro-7,16-di(phenyl sufinyl) diocosane	Sulfohydroxydiene	Antitumour activity
18.	(2S, 3S) - 2, 3 - Epoxy-l-hexanol	Epohexane	Antiinflammatory response against skin lesions
19.	(1R*, 2R*, 6S*)-2-(tert-Butyldimethylsiloxy)-6,9,9-trimethyl bicycle [4.2.1] no, nun-8-one	Methyl butane	Anti anaesthetic properties.

3.4 Phytochemical Studies

3.4.1 Preliminary Phytochemical Analysis: Qualitative phytochemical studies of different extracts of leaves of *Indigofera linnaei Linn*. were performed on its alcoholic and water extracts to identify its Alkaloid, Carbohydrate and Glycoside, Saponin, Protein & Amino acid, Phenolic compounds & Flavonoids and Phytosterols by using suitable chemicals and reagents (Table 2).Alkaloid test results of leaf showed slightly positive in all four tested reagents. Qualitative phytochemical studies of Carbohydrate & Glycoside showed a good characteristic colour and precipitate in all five tested reagent. Slight presence of Saponin was confirmed by foam test in leaf in all extracted solvents. Protein and amino acid was found absent in all tests. However in Millon's test alcoholic extract showed slight presence of protein. Phenolic compounds and Flavonoids were abundantly present in all the extracts. However alkaline test showed the moderate result in comparison to other two tests [10-14]. Libermann-Burchards test showed slight presence of phytosterol in all the extracts. The above qualitative phytochemical screening showed that the whole plant is a rich source of Glycosides, Phenols & Flavonoids. However, presence of protein and alkaloids is limited in leaves. [15-16]

Table 5: Qualitative Phytochemical Screening of leaves of *Indigofera linnaei Linn.*

	Cold Maceration		Sohxalation
Phytochemical test	**Alcoholic Extract**	**Alcoholic Extract**	**Ethanolic Extract by Sohxalation**
1. Alkaloids			
Mayer's test	+	-	+
Wagner's test	+	-	+
Hager's test	+	+	-
Dragendorff's test	+	+	+
2. Carbohydrates & Glycosides			
Molish's test	+++	+++	+++
Fehling's test	+++	+++	+++
Barfoed's test	+++	+++	+++
Benedict's test	+++	+++	+++
Borntrager's test	+++	+++	+++
3.Saponins			
Foam test	+	+	+
4. Proteins & amino acid			
Millon's test	-	-	-
Biuret's test	-	-	-
Ninhydrin test	-	-	-
5. Phenolic compounds & flavonoids			
Ferric chloride test	++	+++	+
Lead acetate test	++	+++	+
Alkaline test	++	++	++
6. Phytosterol			
Libermann-Burchard's test	+	+	+
-,Negative; +, Slight; ++, Moderate;+++,Frequent;			

The phytochemical screening of whole plant extract*Indigofera linnaei Linn.*revealed the presence of alkaloids, flavonoids, Phytosteroids, glycosides, carbohydrates, saponins *etc.*

The phytocompounds which exhibits the properties of antitoxic and antibacterial activity, so the plant extracts are subjected to further studies.

The phytochemical analysis of the passiflora incarnate leaf extract shows the presence of tannins, alkaloids, flavonoids and carbohydrates *etc.* Tannins have been found to form irreversible complexes with proline rich proteins resulting in the inhibition of the cell protein synthesis (Hagerman *et al.*).

Conclusion:

The several secondary metabolites were present in the plant extracts of solvents methanol, acetone and toluene. The phytocompounds were alkaloids, flavonoids, glycosides, Phytosteroids, carbohydrate, saponins, tannins *etc.* were found in plant extracts.The UV-visible spectrum which shows the peak area having functional groups in methanol, and toluene solvents.The FTIR analysis which shows distinct peak areas of functional group. This functional groups having N-acetyl, alkene, forming of groups *etc*.There are 19 compounds is separated through GC-MS analysis. The 19 compounds were listed and their compounds, their nature, biological functions of that particular compounds. This GC-MS analysis which exhibits certain new compounds also but their biological properties were not found.The phytocompounds of the plant *Indigofera linnaei Linn.*Linn. can be detected through the qualitative, UV-Vis spectrum, FTIR-analysis and GC-MS. This detection of compound and its structure and activities will lead to the number of new drugs invention for various incurable diseases.

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References:

1. Hill AF. Economic Botany. A textbook of useful plants and plant. 2nd edn. 1952McGarw-Hill Book Company Inc, New York. Products
2. Okwu DE. Flavouring properties of spices on cassava Fufu. *Afr. J. Roots Tuber Crops* 1999; 3(2): 19-21.
3. Tagboto S, Townson S .Antiparasitic properties of medicinal plants and other naturally occurring products. *Adv. Parasitol*., 2001; *50*: 199-295.
4. Mossi, A.J. Mazutti, Paroul, M., Corazza, N., Dariva, M.L., Cansian, C. & Oliveira, R.L. Chemical variation of tannins and triterpenes in Brazilian populations of *Maytenusilicifolia* Mart. *Ex Reiss Brazilian Journal of Biology* 2009: (2). 69 http://dx.doi.org/10.1590/S1519-69842009000200015
5. Dutta, A.C. Botany for degree students. Oxford University Press, London, 1994; 73.
6. WHO. WHO traditional medicine strategy 2002- 2005. WHO, Geneva, 2002.
7. Perumal, S.R &Ignacimuthu, S. Antibacterial activity of some folklore medicinal plants used by tribes in Western chats of India. *J. Ethanopharmacol*. 2000, 69: 63-71.
8. Nezhadali A, Nabavi M , Akbarpour M, Chemical composition of ethanol/n-hexane extract of the leaf from *Tanacetum polycephalum* subsp. duderanum as a herbal plant in Iran *Der Pharmacia Sinica*, 2010: 1, 147.
9. Sathyaprabha G , Kumaravel S , Panneerselvam A, Bioactive Compounds Identification of *Pleurotus platypus* and *Pleurotus eous by GC-MS Adv. Appl. Sci. Res*., 2011, 2, 51.
10. Nandkarni AK. Materia medica. Edn 2, Vol.1, Tarun Enterprises, 2000, pp. 266.
11. Khandelwal KR. Practical Pharmacognosy. Edn 5, Niraliprakashan, Pune, 2005, pp.149-154.
12. Kokate CK. Practical Pharmacognosy. Edn 4, 2003; VallabhPrakashan, New Delhi, 2003, pp. 122-126.
13. Harborne JB. Phytochemical Methods. Springer (India) Pvt. Ltd., New Delhi, 2005, 17.
14. Wagner H, Bladt S. Drug Analysis. Springer, Newyork, 1996, 3-335.
15. V. Usnale,*et.al*., Pharmacognostical studies on *Ipomeareniformischois. International Journal of Pharmaceutical and Clinical Research*, 1(2), 2009, 65-67.
16. Sadasivam S, Manickam A. Biochemical Methods. New Age International (P) Limited, New Delhi, 1997, 10-197.