



Evaluation of bioactive constituents in hydroalcoholic extract of *Nelumbo nucifera* by FTIR, HPTLC and GC-MS analysis

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Abstract : The present study was aimed to determine the secondary metabolites which is present in methanolic fraction of hydro alcoholic extract of *Nelumbo nucifera*(G) flowers Gas Chromatography Mass- spectroscopy (GC-MS) method of analysis. Preliminary phytochemical screening of *N. nucifera* was performed for the identification of the bioactive constituents. Methanolic fraction of HPTLC finger print shows three numbers of peaks. Among the observed peaks, Peak No. 2 is having R_f value 0.88 with area 3542.4. Active constituents of Methanolic fraction of hydro alcoholic extract of *Nelumbo nucifera* analysed by GC-MS. Chromatogram-2 shows 19 numbers of peaks among the observed peaks, 5 peaks have greater area with quinoline structure.

Introduction:

Plants play a significant role in the prevention and treatment of diseases and can even prevent and reduce the adverse effects of conventional treatments [1]. They can be a source of chemical compounds of biological and pharmacological importance. History reveals that plants are sources of successful drugs, and will continuously be important for screening of new lead compounds [2]. *N. nucifera* is grown and consumed throughout Asia. *N. nucifera* is a native of China, Japan and India. *N. nucifera* is a large aquatic herb widely found in India [3] *N. nucifera* Gaertn, (sacred lotus) is a large aquatic herb with stout, creeping rhizome. The flowers are hermaphrodite and are pollinated by Insects, beetles. The plant prefers sandy, loamy and clay. The plant prefers acid, neutral and basic soils. It cannot grow in the shade. The Sacred water lotus has been used in the Orient as a medicinal herb for well over 1,500 years[3]. Almost all parts of the lotus plant are eaten as vegetable and also used in the indigenous system of medicine [4]. They are used as astringent, cardiotoxic [5], febrifuge, Hypotensive, resolvent, vasodilator, immunomodulatory[6] antiviral and antitumor activity.

The leaf juice is used in the treatment of diarrhoea and is decocted with liquorice (*Glycyrrhiza* spp.) for the treatment of sunstroke [6]. In particular, the leaves are known for diuretic and astringent properties, and are used to treat fever, sweating and strangury and as a styptic[7].

A decoction of the flowers is used in the treatment of premature ejaculation [7]. The flowers are recommended as a cardiac tonic[8]. A decoction of the floral receptacle is used in the treatment of abdominal cramps, bloody discharges etc. [9]. The flower stalk is haemostatic[10].It is used in treating bleeding gastric ulcers, excessive menstruation, post-partum haemorrhage[11].Hydro alcoholic extract of *Nelumbo nucifera* majorly used for the treatment of diabetes.

The stamens are used in treating urinary frequency, premature ejaculation, haemolysis, epistaxis and uterine bleeding [11].The rhizome is considered to be nutritive, demulcent, diuretic and cholagogue and is used to treat piles, dyspepsia and diarrhoea[12]. A decoction of the fruit is used in the treatment of agitation, fever, heart complaints etc. [8]

The seed contains several medically active constituents, including alkaloids and flavonoids. It is Hypotensive, Sedative and Vasodilator [13].The seed has been shown to lower cholesterol levels and to relax the smooth muscle of the uterus. It is used in the treatment of poor digestion, enteritis, chronic diarrhoea, spermatorrhoea, leukorrhoea, insomnia and palpitations. The plumule and radicle are used to treat thirst in high febrile disease, hypertension, insomnia and restlessness [3]

The root is tonic. The root starch is used in the treatment of diarrhea and dysentery, a paste is applied to ringworm and other skin ailments. It is also taken internally in the treatment of haemorrhages, excessive menstruation and nosebleeds. The roots are harvested in autumn or winter and dried for later use [3].The root nodes are used in the treatment of nasal bleeding, haemoptysis, haematuria and functional bleeding of the uterus [10]. The plant has a folk history in the treatment of cancer; modern research has isolated certain compounds from the plant that show anticancer activity [8].

Almost all the parts are used as a medicine for various diseases .The plant has folk history flower petals has been used for most challenging diseases such as anti diabetic[18] due its antioxidant potential [15]. There are no published literatures that determine the bioactive compounds present in flower petals of various extract.

So the present study was aimed to determine the active constituent present in petals of *Nelumbo nucifera*. This investigation will helpful for the further biological and pharmacological study.

Materials and Methods

Preparation of hydro alcoholic extract

The *Nelumbo nucifera* G flower petals were cut into pieces and shade dried at room temperature. The dried flower petals were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. This powder was packed into soxhlet apparatus and extracted successively with water and ethanol (50:50). Finally residues are dried by lyophilization process.

Isolation of total alkaloid^[14]

Total alkaloids were isolated from supercritical powder of hydro alcoholic extract of *Nelumbo nucifera* flower. 250 gm of hydro alcoholic extract of *Nelumbo nucifera* was mixed with 3:1 ratio of Methanol and Ether by maceration for one week with intermittent stirring, filter, wash the marc with the addition of four successive portions of 100 ml of 3:1 Methanol-Ether solution. Concentrate the combined alcoholic extract to one tenth its volume under vacuum and acidify with sufficient quantity of 1 N H₂SO₄. Allow the Solution to stand for 2 hours. Extract 5 successive 50 ml portion of Chloroform. Concentrate the acidified mixture to half of its volume under vacuum. Filter and basify with Ammonia (pH 12) and allow precipitating. The precipitate was removed carefully. Extract the precipitate with 4 successive 25 ml portions of Chloroform. Combined chloroform extract and distill of the solvent to yield total alkaloid residue [14.]Residue was treated with Mayer's reagent and Dragendroff's reagent for the conformation of total alkaloid

HPTLC analysis of total alkaloids^[17]

Chromatography was performed on a 10 X 3 cm pre-coated HPTLC silica gel 60 F 254 Plate. The plates were washed by Methanol and activated at 60°C for 5 minutes prior to chromatography. Samples were applied on to the plate of 6 mm band using CAMAG Linomat V applicator. The slit dimension is kept at 5 mm X 0.45

mm and 20 mm/s scanning speed is employed. The mobile phase is chosen after trial and error, and 10 ml of mobile phase is used per chromatography. Linear ascending development is carried out in 10 cm X 10 cm twin glass chamber. The mixture is spotted using CAMAG Linomat V applicator[15].

FTIR analysis of total alkaloids[16]

FT-IR spectra of the samples were analyzed using a Perkin-Elmer, FT-IR spectrophotometer, USA within the range of 4000–400 cm^{-1} . About 5 mg of sample was mixed with 100 mg of KBr and compressed into pellet using a hydraulic press. All spectra were corrected against the reference spectrum of KBr pellet.

GCMS analysis of total alkaloids:[17]

The GC-MS system used was the Clarus® 500 GC-MS (PerkinElmer Life and Analytical Sciences), with 30 m \ 0.20 mm I. D.Elite-5 MS column; 50 μm film thickness attached with Clarus 500 MS detector. The samples are introduced *via* EPC pneumatic split/splitless Inlet with Helium as the carrier gas, with the flow rate of 1 ml/min. Temperature program: 75 to 150 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$ for 1 min. 150-250 $^{\circ}\text{C}$ at 8 $^{\circ}\text{C}/\text{min}$ for 2 min., 250 to 310 $^{\circ}\text{C}$ at 15 $^{\circ}\text{C}/\text{min}$ for 3 min. The injector temperature is 290 $^{\circ}\text{C}$. The components are accomplished using computer searches in commercial libraries.

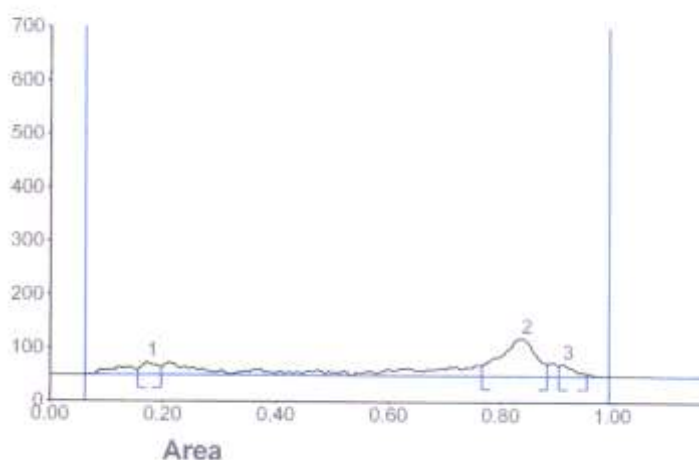
Result and discussion:

HPTLC finger print:

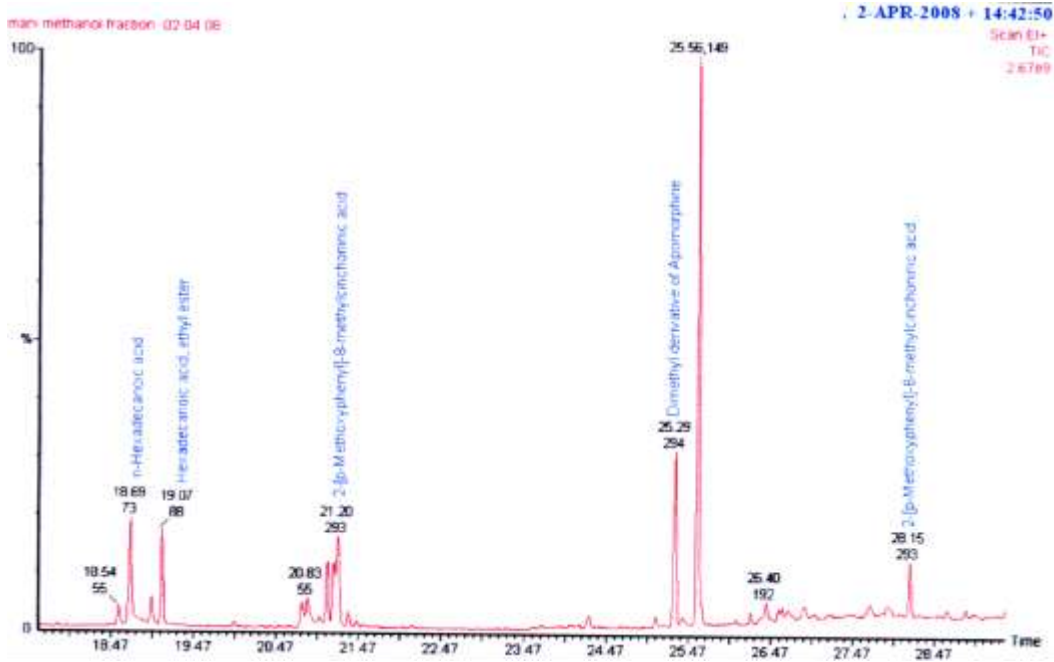
Total alkaloids were separated and conformed to various preliminary tests. The Methanol fraction of total alkaloid contains 3 compounds in Ethylacetate: Benzene (1:1) ratio of mobile phase. Among the observed peaks, Peak No. 2 is having R_f value 0.88 with area 3542.4. This is majorly available compound in methanolic fraction[18]. This has been isolated for further characterization. The below mentioned table shows the R_f value of total alkaloids.

Table.1 HPTLC analysis by isolated total alkaloid from *Nelumbo nucifera*

Peak	R_f value	Height (%)	Area	Percentage area
1	0.20	14.6	521.4	11.47
2	0.88	23.9	3542.4	77.90
3	0.96	5.1	483.6	10.63

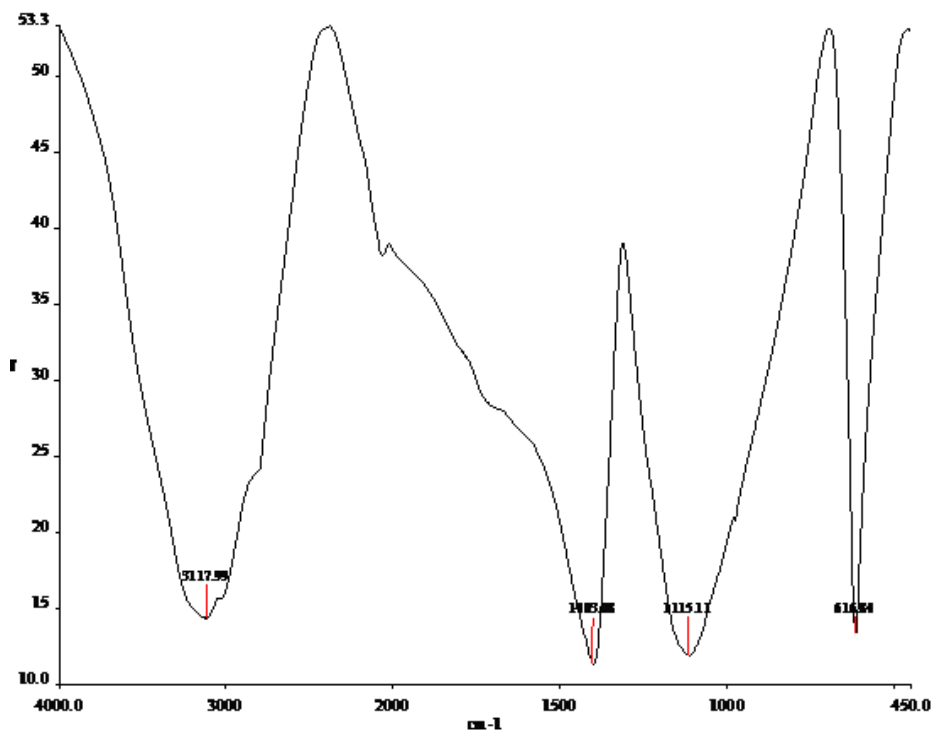


Chromatogram 1: HPTLC finger print of methanol fraction of total alkaloids isolated from *Nelumbo nucifera* petal



Chromatogram 2: GC-MS chromatograms of methanol fraction of hydro alcoholic extract of *Nelumbo nucifera* petal

An active constituent of methanolic fraction of total alkaloid was analysed by GC-MS. Chromatogram-2 shows 19 numbers of peaks. Among the observed peaks 5 compounds have greater area. This area is directly proportional to quantity of the compound present in the extract, of which most of the compound is having quinoline ring. Earlier report also shows the presence of benzo-isoquinoline in leaf extract of *Nelumbo nucifera*. [19]. The presence of these phytochemical constituents justifies the use of this plant for various ailments by traditional practitioners. Isolation of individual phytochemical constituents and subjecting it to biological activities are being undertaken.



Chromatogram 3: FTIR chromatograms of methanol fraction of hydro alcoholic extract of *Nelumbo nucifera* petal.

Fourier Transform Infrared Spectroscopy (FTIR) is a high-resolution analytical tool to identify the chemical constituents and elucidate the structural compounds. FTIR offers a rapid and nondestructive investigation to fingerprint herbal extracts or powders[20]. The frequency of functional group which is present in methanolic fraction of total alkaloid was depicted in Table 2.

Table.2FTIR analysis by *Nelumbo nucifera* isolated alkaloid[21]

S.NO	Peak value in Cm^{-1}	Stretching/bending	Interpretation
1	3117.99	C-H stretching	Aromatic
2	1403.08	$\text{CH}_2\text{-NO}_2$	Either multiple or single bond with strong interaction
3	1115.11	C-O stretching, O-H stretching, C=O stretching	Either alcohol or phenol strong h bonding state
4	616.84	C-Cl stretching	Chlorine

Conclusion:

The result of the present study suggest that the methanolic fraction of total alkaloid of *Nelumbo nucifera* benzylisoquinoline alkaloids the same were reported in earlier studies.In future, several chromatographic techniques and various other purification methods have to perform for isolate and characterize single bioactive compound.

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