International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 702-706

Received: 22-09-2017
Accepted: 25-10-2017

R Kavitha
Department of Chemistry, Kunthavai Naacchiyaar Government Arts College for Women (Autonomous) Thanjavur, Tamil Nadu, India

AM Uduman Mohideen
P.G. and Research Department of Chemistry, Khadir Mohideen College, Adirampattinam, Thiruvarur District, Tamil Nadu, India

Correspondence
R Kavitha
Department of Chemistry, Kunthavai Naacchiyaar Government Arts College for Women (Autonomous) Thanjavur, Tamil Nadu, India

Exploration of phytocompounds in *Abelmoschus moschatus* flowers using HPLC, Uv-Vis and ftir techniques

R Kavitha and AM Uduman Mohideen

Abstract
Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. The bioactive components of *Abelmoschus moschatus* flowers have been evaluated using HPLC, UV VIS and FTIR. The phytochemical screening of *Abelmoschus moschatus* flowers showed that the presence of flavonoids, terpenoids, tannin, saponins, glycosides, triterpenoids, phenol, anthroquinones were present in aqueous, ethanol and ethyl acetate extracts. The UV- VIS profile showed the occurrence of peaks at 207-380 nm reveals the presents of flavonoids in the *Abelmoschus moschatus*. The results of FTIR analysis confirmed the presence of phenol, alkanes, Alkenes, alcohol, aromatic, Aliphatic amines and amine compound. The results of this study offer a platform of using Abelmoschus moschatus flower as herbal alternative for various diseases including diabetic, cardiovascular etc. The results of this study offer a platform of using *Abelmoschus moschatus* flowers as herbal alternative for various diseases including cancer and diabetic.

Keywords: GC MS, HPLC, UV-VIS, FTIR, *Abelmoschus moschatus*

1. Introduction
Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments. They are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase in demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as an alternative to allopathic medicines [1]. Phytochemicals simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. In addition, these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures [2]. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants [3]. The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters [4]. Among them some are act as synergistic and enhance the bioactivity of other compounds.
Within a decade, there were a number of dramatic advances in analytical techniques including HPLC, UV, FTIR, NMR and GC-MS that were powerful tools for separation, identification and structure determination of phytochemicals (Roberts and Xia, 1995) [5]. The aim of this study is to determine the bioactive compounds present in the *Abelmoschus moschatus* flower extract with the aid of HPLC, UV-VIS and FTIR Techniques, which may provide an insight in its use of tradition medicine.

2. Materials and Methods
Plant materials
The flowers of *Abelmoschas moschatus* were collected from Thanjavur, Tamil Nadu, India from a single tree.

Preparation of Extracts
The *Abelmoschas moschatus* flowers were first washed well and dust was removed from the flower.

Then the flowers were dried at room temperature and coarsely powdered. The powder was extracted with aqueous, ethanol and ethyl acetate for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used.

Preliminary phytochemicals screening

Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Harborne [6] and Sofowara [7].

UV and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using a high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

HPLC Analysis

Sample preparation: The sample was prepared according to the procedure. The extraction was carried out using 2 ml of fermented broth with 50 mL of 95% ethanol under 80 KHz, 45°C in ultrasonic extraction device for 30 min, repeated twice. The extract was collected and filtered; the filtrate was dried at 50°C under reduced pressure in a rotary evaporator. The dried crude extract was dissolved in the 100 ml mobile phases. After filtering through a filter paper and a 0.45 mm membrane filter (Millipore), the extract was injected into HPLC.

HPLC conditions: Flavonoids were analysed using an RP-HPLC method [8], Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD- 10ATVp UV VIS detector and a loop injector with a loop size of 20 µl. The peak area was calculated by a CLASSVP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6 mm i.d., particle size 5 µm, Luna 5µ C-18; phenomenex, Torrance, CA, USA) at 25°C. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B (methanol). Solvent B was increased to 50% in 4 min and subsequently increased to 80% in 10 min at a flow rate of 1.0 mL/min. Detection wavelength was 280 nm.

3. Results and Discussion

The pharmacological activities of any plant sample are due to the presence of metabolites, secondary metabolites and secretory products in it. These usually consist of the phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, steroids, etc. Most phenolic compounds such as flavonoids, glycosides, triperinoids, flavonons, carbohydrates and anthraquinones are found distributed throughout the plant kingdom [9]. Similarly, the polyphenolic compounds most commonly found in plant extracts are the phenolic acids, flavonoids and tannins [10]. These compounds together with other phenolic structures of plant origin have been reported as scavengers of Reactive Oxygen Species (ROS) and are seen as promising therapeutic drugs for free radical mediated pathologies including diabetic, cardiovascular diseases [11]. Most flavonoidic compounds exhibit antipyretic, analgesic, anti-inflammatory, anti-arthritic, antioxidant and immuno-modulatory properties [12, 13]. These activities of flavonoidic compounds may be due to the presence of gallic acid, ellagic acid, quercitin, tannin acid, vanillin, resorcinol, catechin, etc.

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Abelmoschus moschatus* investigated and summarized in Table-1. The phytochemical screening of *Abelmoschus moschatus* flowers showed that the presence of flavonoids, terpenoids, tannin, saponins, glycosides, triterpenoids, phenol, anthroquinones were present in aqueous, ethanol and ethyl acetate extracts. Among the three extracts, the ethanol extract have high concentrations of phenol, flavonoids and tannin were observed as compared with aqueous and ethyl acetate extract.

Table 1: Qualitative Analysis phytochemicals in different extracts of *Abelmoschus moschatus* flower

S. No	Secondary Metabolites	Aqueous Extract	Ethanol Extract	Ethyl Acetate Extract
1	Tannin	+	++	+
2	Phlobatannins	---	---	---
3	Saponnin	+	+	+
4	Flavonoids	+	++	+
5	Steroids	+	---	+
6	Terpenoids	+	+	+
7	Triterpenoids	+	+	+
8	Alkaloids	+	---	---
9	Carbohydrate	+	---	---
10	Protein	+	+	---
11	Anthroquinone	+	+	+
12	Polyphenol	+	++	+
13	Glycoside	+	+	+

(+) Presence; (-) Absence; (++) Highest concentrations

Determination of phytocompounds using HPLC

HPLC profiles of *Abelmoschus moschatus* were analysed and two phenolic compounds namely Gallic acid (2.77min) and Hypersoide (7.31 min), having different elution times could be obtained (Figure 1 and Table 1) when each compound was analyzed individually using the mobile gradient phase consisting of methanol and 1% acetic acid in water during 30 minutes run time. Earlier review of [14-17] supported the findings of these compounds.

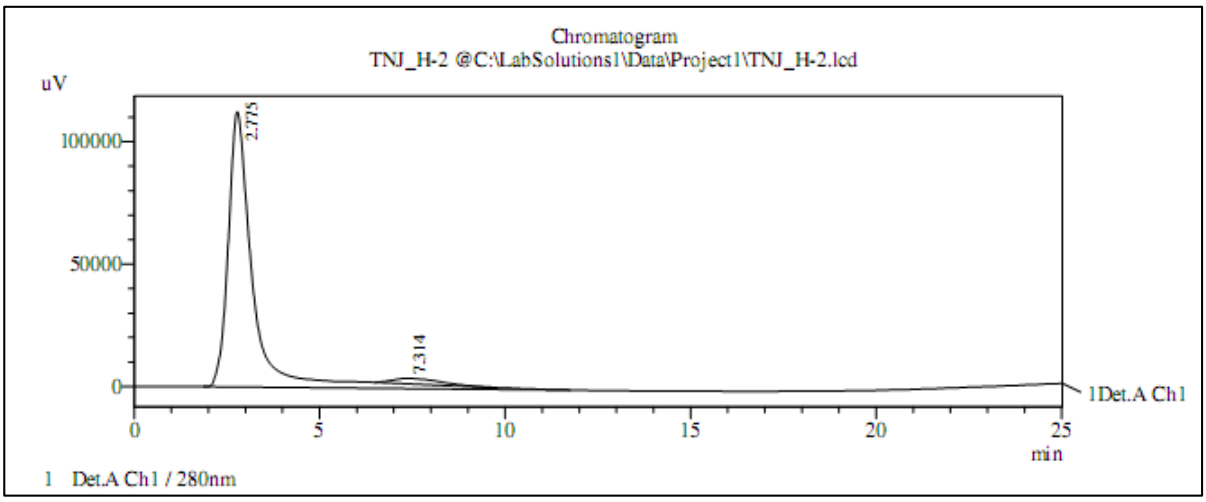

Fig 2: HPLC analysis of *Abelmoschus moschatus* flower extract

Table 2: HPLC analysis of *Abelmoschus moschatus* flower extract

Peak	Retention Time	Area %	Height	Height %	Literature (RT)	Name of the compound
1	2.775	96.171	112430	98.177	2.679	Gallic acid
2	7.314	3.529	2088	1.826	7.39	Hypersoide
Total		100.00	114548	100.00		

Spectrophotometric Analysis

The UV-VIS profile of plant extract was taken at the 200 to 800nm wavelength due to the sharpness of the peaks and proper baseline. The UV-visible spectra were performed to identify the compounds containing σ- bonds, π-bonds, and lone pair of electrons, chromophores and aromatic rings. The profile showed the peaks at 207.6, 260.1 and 381.4nm with the absorption 0.247, 0.106 and 0.003 respectively (Fig-3 and Table 3). The flavonoids spectra typically consist of two absorption maxima in the ranges 230-290 nm (band I) and 300-350 nm (band II). The precise position and relative intensities of these maxima give valuable information on the nature of the flavonoids. Occurrence of peaks at 207-380 nm reveals the presents of flavonoids in the *Abelmoschus moschatus*. On comparison of the seeds and flowers spectra shows that the extracts has similar flavonoids and glycosides compounds reported [18, 19].

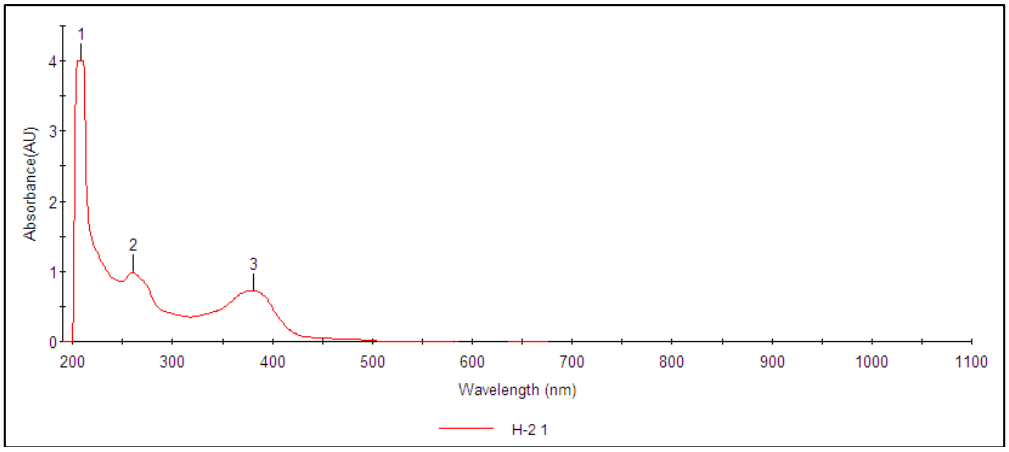

Fig 3: UV-Vis Spectral analysis of *Abelmoschus moschatus* flower extract

Table 3: UV-VIS Peak Values of Extract of *Abelmoschus moschatus* flower

S. No	Wave length (nm)	Absorption Peak
1	207.6	4.00
2	260.1	0.990
3	381.4	0.7299

Functional groups identification

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of phenol, alkanes, Alkenes,

alcohol, aromatic, Aliphatic amines and amine compound (Fig- 4, and Table- 4).

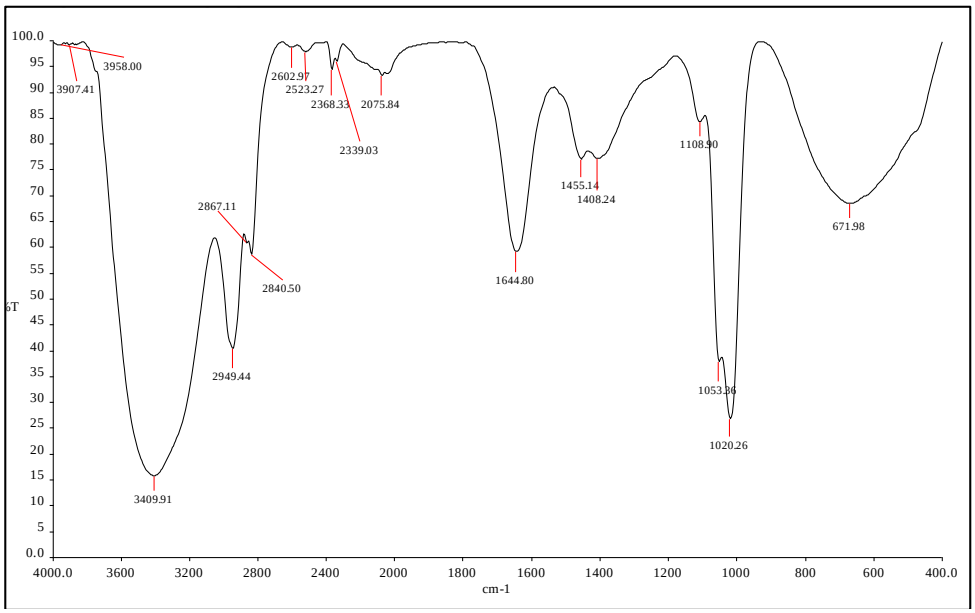

Fig 4: FTIR analysis of *Abelmoschus moschatus* flower extract

Table 4: FTIR Peak Values of Extract of *Abelmoschus moschatus* flower extract

S. No	Frequency (Cm^{-1})	Bond	Functional Groups
1	3438.52	O–H Stretch, H–Bonded	Alcohols, Phenols
2	2949.44, 2867.11	C–H Stretch	Alkanes
3	1644.30	–C=C– Stretch	Alkenes
4	1455.11, 1408.24	C–C Stretch (In–Ring)	Aromatics
5	1108.90, 1020.26	C–N Stretch	Aliphatic Amines
6	671.98	N–H Wag	1˚, 2˚ Amine

4. Conclusion

The phytochemical screening shows that among the three extracts, the ethanol extract has higher concentrations of phenol, flavonoids and tannin were observed as compared with aqueous and ethyl acetate extract. HPLC profiles of *Abelmoschus moschatus* flowers shows the presence of two phenolic compounds namely Gallic acid and Hypersoide. The UV- VIS profile showed the occurrence of peaks at 207-380 nm reveals the presence of flavonoids in the *Abelmoschus moschatus* flower. The results of FTIR analysis confirmed the presence of phenol, alkanes, Alkenes, alcohol, aromatic, Aliphatic amines and amine compound. The results of this study offer a platform of using *Abelmoschus moschatus* flower as herbal alternative for various diseases including diabetic, cardiovascular etc.

5. References

1. Ashis G. Herbal folk remedies of Bankura and Medinipur districts, West Bengal. Indian Journal of Traditional Knowledge. 2003; 2:393-396.
2. Dahanukar SA, Kulkarni AR, Rege NN. Pharmacology of medicinal plants and natural products. Indian Journal of Pharmacology. 2000; 32:S81-S118.
3. Cox P, Balick M. The ethnobotanical approach to drug discovery. Sci American, 1994, 82.
4. Cox PA. Ethnopharmacology and the search for new drugs Bioactive Compounds from Plants Ciba Foundation Symposium, Chichester, John Wiley & Sons. 1990; 154:40.
5. Roberts JKM, Xia JH. High-resolution NMR methods for study of higher plants, Methods Cell Biol. 1995; 49:245-258.
6. Harborne J. Phytochemical methods London. Chapman and Hall, Ltd. Iinuma M, Tsuchiya H, Sato M, Yokoyama J, Ohyama M, Ohkawa Y, 1973.
7. Sofowara A. Medicinal plants and Traditional medicine in Africa.Spectrum Journal Ethnopharmacology, 1993; 90(2-3):P.249-252.
8. Weerasak Samee, Suwanna Vorarat. Simultaneous Determination of Gallic acid, Catechin, Rutin, Ellagic Acid and Quercetin in Flower Extracts of Michelia alba, Caesalpinia pulcherrima and Nelumbo nucifera by HPLC. Thai Pharm Health Sci J. 2007; 2:131-137.
9. Harborne JB. Phytochemical Methods, A Guide to modern Techniques of Plant Analysis. Chapman and Hall, London, 1973, 33-41.
10. Naik GH, Priyadarsini KI, Hari M. Free radical scavenging reactions and phytochemical analysis of Triphala, an ayurvedic formulation. Curr. sci. 2006; 90:1100-1105.
11. Velavan S. Free radicals in health and diseases ─A mini review Pharmacologyonline Newsletter, 2011; 1:1062-1077
12. Balasundram N, Sundram K, Samman S. Analytical, Nutritional and Clinical Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry. 2006; 99(1):191-203.
13. Gill NS, Arora R, Kumar SR. Evaluation of antioxidant, anti-inflammatory and analgesic potential of the Luffa

acutangula Roxb. Var. amara. Res. J. Phytochem. 2011; 5:201-208
14. Gupta Mradu, Sasmal Saumyakanti, Majumdar Sohini, Mukherjee Arup. HPLC Profiles of Standard Phenolic Compounds Present in Medicinal Plants. International Journal of Pharmacognosy and Phytochemical Research. 2012; 4(3):162-167
15. Michael Vagiri, Anders Ekholm, Staffan C, Andersson, Eva Johanssonand, Kimmo Rumpunen. An Optimized Method for Analysis of Phenolic Compounds in Buds, Flower, and Fruits of Black Currant (Ribes nigrum L.). J Agric. Food Chem. 2012; 60:10501.10510.
16. Nadia Alam, Monzur Hossain, Md Ibrahim Khalil, Mohammed Moniruzzaman, Siti Amrah Sulaiman, Siew Hua Gan. High catechin concentrations detected in Withania somnifera (ashwagandha) by high performance liquid chromatography analysis. BMC Complementary and Alternative Medicine. 2011; 11:65.
17. Paranthaman R, Praveen kumar P, Kumaravel S. GC-MS Analysis of Phytochemicals and Simultaneous Determination of Flavonoids in Amaranthus caudatus (Sirukeerai) by RP-HPLC. J Anal Bioanal Techniques. 2012; 3:5
18. Jasper C, Maruzzella JC, Henry PA. The antimicrobial activity of perfume oils, J.Am Pharm. Asso. 1958; 47:471-476.
19. Sofowora A. Medicinal Plants and Traditional Medicine in Afric. John Wiley and son Ltd. 1993, 150-153.