Identification Of Bioactive Components And Its Biological Activities Of *Abelmoschas moschatus* Flower Extrtact - A Gc-Ms Study

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Abstract: Most herbal medicines and their derivative products were often prepared from crude plant extracts, which comprise a complex mixture of different phytochemical constituents. The chemical features of these constituents differ considerably among different species. GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in various industries. The aim of this study was to carry out for identification of bioactive compounds from the methanolic extract of Abelmoschas moschatus flower by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of methanolic extract was done by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of various compounds like Caryophyllene, octodeconoic acids, hexadecanoic acid, ascorbic acid and squalene in the methanolic extract of Abelmoschas moschatus may have anticancer, anti-microbial activity, antioxidant and antiinflammatory activity due to the presence of secondary metabolites in the methanolic extract. These findings support the traditional use of Abelmoschas moschatus in various disorders.

Keyword: Gas chromatography and Mass spectroscopy, Abelmoschas moschatus, Phytochemistry

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I. Introduction

Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines [1]. It has been shown that in vitro screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [2].

Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function [3].

Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) [4] Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits [5] Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structural determination of phytochemicals [6].

Abelmoschus moschatus Medic. (English name: Musk mallow; Tamil: Kaattu kasturi) is an annual or biennial plant in the Malvaceae family, which is native to India and distributed in many parts of Asia, including south Taiwan, and in North Australia [7]. It is well known that the volatiles obtained from the seeds of A. moschatus possess an odor similar to that of musk and its aromatic constituents have long been used as a perfume and cosmetic ingredient [8]. This aromatic and medicinal plant has been classified as an 'Herb of Unidentified Safety' by the Food and Drug Administration. Actually, this perennial plant has been applied in Chinese traditional medicine for the treatment of depression and anxiety [9]. In addition to possessing antispasmodic activity in the digestive system, *A. moschatus* is also applied externally to treat cramp, poor circulation and aching joints [9]. Furthermore, a decoction or infusion of *A. moschatus* has been reported to exhibit hypotensive properties [9]. It has also been indicated that aqueous extracts of *A. moschatus* appear to exert a slight antibacterial effect, but not to a clinically relevant degree [9]. The aim of this paper is to determine the organic compounds present in the *Abelmoschas moschatus* flower extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

II. Materials And Methods

2.1 Plant materials

The flowers of *Abelmoschas moschatus* were collected from Thanjavur, Thanjavur District, Tamil Nadu, India from a single tree.

2.2 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. he powder was extracted with methanol 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.. The percentage yield was 4.50% (4g gives 300mg extract).

2.3 GC –MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1 μ Mdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 μ I was employed (split ratio of 10:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min, to 200°C, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0

III. Results and Discussion

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defense mechanisms against, insects and herbivores. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions [10].

3.1 Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The biological activities listed (Table. 2) are based on Dr.Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

3.2 GC-MS Analysis

Twenty compounds were identified in *Abelmoschas moschatus* flower by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table. 1 and Fig. 1). The prevailing compounds were Tetradecane, Tridecanoic Acid, Hexadecanoic acid, 2-hydroxy-, methyl ester and 1-Octadecanol.

Among the identified phytochemicals hexadecanoic acid is suggested to be a fatty acid ester and it may employed as antioxidant, antimicrobial, flavor, hypocholesterolemic agent and larvicidal activities [11, 12]. Compounds like n-hexadecanoic acid, 12-octadecanoic acid, dodecanoic acid, tetradecanoic acid, 1, 2-benzene dicarboxylic acid, butyl octyl ester, hexadecanoic acid, ethyl ester and 9,12-octadecadienoic acid (Z,Z) were

identified in the ethanolic leaf extract of *Vitex altissima*, a Verbenaceae member [13]. Likewise, hexadecane, dodecanoic acid, nonadecane, eicosane, tetradecanoic acid, oleic acid, heptacosane, 9,12- octadecenoic acid, ethyl ester; n-hexadecanoic acid; 1,2-benzenedicarboxylic acid and 9-octadecenoic acid (Z)-ethyl ester were reported in *Clerodendrum inerme* and *C. phlomidis* leaves [14, 15].

Similar results were also observed in the leaves of *Gmelina asiatica* which showed Pregnane – 3,11, 12,14,20 – pentol, 3,12, 20, triacetate 11 (hydroxyacetate), (3a, 11a, 12a, 14a), Tridecanoic acid, methyl ester, 10-Octadecanoic acid, methyl ester, 16-Octadecanoic acid, methyl ester, 2,7- Diphenyl-1,6-dioxopyridazino (4,5:2,3) pyrrolo (4,5,-d) pyridazine, spiro (androstane-3,2- thiazolidine) were anthelminthic, Anti-Inflammatory and Anti-microbial activities and anti-cancerous activity of the leaf extract [16].

Similarly [17] identified twenty-two chemical compounds from the chloroform extract of *G. asiatica* aerial parts, of which six compounds were similar to that of the results obtained in the present study. The compounds were 1, 2 benzene dicarboxylic acid, diisooctyl ester, benzoic acid, 2- hydroxy, phenyl ester; n-hexadecanic acid; octadecanol, 2-bromo; octadecanoic acid and nonadecane.

The investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

Peak	R.Time	Area%	Height%	Molecular	Name Of The Compounds
				Formal	
1	6.572	8.83	16.26	$C_{14}H_{30}$	Tetradecane
2	7.843	2.33	4.83	C ₉ H ₂₀ O ₃	Propane, 1,1,3-triethoxy-
3	9.817	8.44	16.41	C17H36	Heptadecane
4	12.775	6.10	8.98	C ₁₃ H ₂₈	Tridecane
5	15.417	2.87	4.46	$C_{12}H_{24}F_2$	Dodecane, 1,1-Difluoro
6	19.667	3.75	2.00	$C_{13}H_{26}O_2$	Tridecanoic Acid
7	19.758	2.09	2.83	$C_5H_{12}ClN_2O_2P$	2h-1,3,2-Oxazaphosphorin-2-Amine, N-(2- Chloroethyl)Tetrahydro-, 2-Oxide, (R)
8	19.901	3.75	3.41	C ₁₇ H ₃₄ O ₃	Hexadecanoic acid, 2-hydroxy-, methyl ester
9	20.869	4.96	4.77	C ₁₈ H ₃₈ O	1-Octadecanol
10	22.882	2.48	1.14	C ₂₄ H ₃₁ NO ₅ Si	N-[2-(T-Butyldimethylsilyloxy)-2-(3,4- Dimethoxyphenyl)Ethyl]Phthalimide
11	23.742	2.62	0.76	$C_{18}H_{20}N_2O_2$	2(5h)-Furanone, 3,4-Dimethyl-5-[2-[(1- Phenylpropylidene)Hydrazono]Pr Opylidene]-
12	25.428	4.37	2.10	C ₁₁ H ₂₄	Octane, 2,6,6-trimethyl-
13	25.567	1.64	0.96	$C_{16}H_{30}O_4Si_3$	Benzoic Acid, 3,4-Bis(Trimethylsiloxy)-, Trimethylsilyl Ester
14	26.698	2.49	1.49	C ₂₀ H ₂₆ OSi	1,3-Diphenyl-1-Pentenyl Trimethylsilyl Ether
15	27.132	5.96	3.64	C ₁₄ H ₃₁ BO	Diethyl(Decyloxy)-Borane
16	28.284	1.49	1.06	C16H22F12OSi	Propyl-1-[(Tert-Butyldimethylsilyl)Oxy]Perfloroheptene
17	28.782	4.44	3.11	$C_{16}H_{22}O_4$	1,2-Benzenedicarboxylic Acid, Dibutyl Ester
18	28.995	28.07	18.59	$C_{21}H_{44}$	Heptadecane, 2,6,10,15-tetramethyl-
19	29.75	1.66	2.34	$C_{18}H_{32}D_4O_2$	2-Pentadecyl-4,4,5,5-Tetradeutero-1,3-Dioxolane
20	30.045	1.66	0.85	$C_{15}H_{22}O_2$	1,2-Naphthalenedicarboxaldehyde
		100.00	100.00		

Table. 1 Shows the components identified in methanolic extract of Abelmoschas moschatus. (GC MS study)

Table. 2 Activity of phyto-components identified in the methanolic extracts of the Abelmoschas moschatus by

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**Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].



Fig. 1 Chromatogram obtained from the GC/MS with the extract of Abelmoschas moschatus.

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