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Research Article

GC-MS ANALYSIS OF CHEMICAL CONSTITUENTS IN THE METHANOLIC TUBER EXTRACT OF *MOMORDICA CYMBALARIA* Hook. F.

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ABSTRACT

The plant *Momordica cymbalaria* belongs to the family Cucurbitaceae. The *Momordica* genus found in the Indian states of Andhra, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu. The plant is traditionally used for the treatment of diabetes mellitus, reumatism, ulcer, skin disease and diarrhea. It is used in the local folk medicine as an abortifacient and for the treatments of diabetes mellitus. In the present study, the powdered tuber of *M. cymbalaria* was successively extracted with Methanol through soxhlet apparatus. The GC-MS analysis has shown the presence of different chemical constituents in the methanolic tuber extract of *M. cymbalaria.* A total of 23 chemical constituents were identified in the methanolic tuber extract of *M. cymbalaria*. At (16.98) retention time 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimeth, Tetrasiloxane, decamethyl, and Methyltris (trimethylsiloxy) silane compound were found to be high (11.17%) and the lowest percentage (1.88%) was found to be Trimethyl (4-tert-butylphenoxy) silane, Silicic acid, diethyl bis (trimethylsilyl) ester and Cyclotrisiloxane, hexamethyl. The present study enhances the traditional usage of tuber of *M. cymbalaria* which possess many chemical constituents it can be used for the treatment of various diseases.

Keywords: GC-MS analysis, Chemical constituents, Soxhlet, Tuber extract and Methanol

INTRODUCTION

Medicinal plant are still major parts of traditional medicinal systems in developing countries many infections disease are known to be treated with herbal remedies throughout the history of mankind. Even today plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries[1]. Medicinal plants which from the backbone of traditional medicine have in the last few decades been the subject of very intense pharmacological studies. This has been brought about by the acknowledgement of the value of medicinal plant as potential source of new compounds of therapeutic value and as source of new compounds in drug development. In many parts of the world medicinal plants are used for antibacterial, antifungal and antiviral activities a plant derived drugs serve as a prototype to develop more affective and loss toxic medicinal.

Medicinal plants, since times immemorial, have been used virtually all cultures as a source of medicine. It is estimated that 70-80% people worldwide relay chiefly on traditional, largely herbal medicines to meet their primary health care needs[2]. Approximately 85% of traditional medicine preparations involve the use of plants or plant extracts. One fifth of all the plants found in India are used for medicinal purpose. The world average stands at 12.5% while India has 20% plant species of medicinal value and which are in use. But, India has about 44% of flora, which is used medicinally. Although, it is difficult to estimate the total number of medicinal plants present worldwide, the fact remains true that India with rich biodiversity ranks first in percent flora, which contains active medicinal ingredient[3]. Medicinal plants are an integral component of ethno-veterinary medicine also. In the past few decades, there has been an ever-increasing global inclination towards herbal medicine, followed by a belated growth in international awareness about the dwindling supply of the world's medicinal plants[4].

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance[5,6]. Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites make up the physical integrity of the plant cell and are involved with the primary metabolite process of building and maintaining of living cells. Secondary metabolites do not seem to be vital to the immediate survival of the plant that produces them and are not an essential part of the process of building and maintaining living cells[7]. These secondary metabolites of plants serve as self-defense mechanism against predation by many microorganisms, insects and herbivores. A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important[8]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, phenolic compounds, steroids, saponins and glycosides[9]. Tribal medicine has not been studied extensively. Infections disease is the number one among all causes of death, accounting approximately one-lady all deaths throughout the world. About 50-75% of hospital deaths are reported due to infections disease. These numbers are still increasing due to development of resistance in microorganisms to the existing first line drug[10].

Many studies have been undertaken with the aim of determining the antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of both topical and systemic microbial infections as possible alternatives to chemical synthetic drugs to which many infectious microorganisms have become resistant[11, 12]. The antimicrobial activity of plant extracts

is due to different chemical agents in the extract. These compounds are usually the secondary metabolites, which function to attract beneficial and repel harmful organisms, serve as phytoprotectants and respond to environmental changes in plants. In humans, however the compounds have beneficial effects[13].

The plant *Momordica cymbalaria* belongs to the family Cucurbitaceae. The *Momordica* genus found in the Indian states of Andhra, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu. It is a wild crop well known as Athalakkai in Tamil. It is available in various parts of India, and it is a highly acceptable wild vegetable across south India. Tubers are reported to possess antioxidant and hepatoprotective activity. Roots are reported to possess anti- implantation activity. Fruits are reported to contain citric acid, malic acid and vitamin C. The fixed oil present in fruits of *Momordica cymbalaria* is reported to contain palmitic acid, oleic acid, stearic acid, α-Eleostearic acid and γ-Linolenic[14]. Upon literature survey, it was revealed that, no work was done in the field of chemical constituents' analysis of tuber of *M. cymbalaria*. Therefore, the present study was undertaken to GC-MS analysis of chemical constituents of the methanolic tuber extract of *M. cymbalaria*.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

The fresh tuber of *Momordica cymbalaria* Hook F. was collected from Mettamalai village, Sattur Taluk, Virudhunager District, Tamil Nadu, India. They were identified and authenticated by Dr. M. Murugan, Assistant professor of Botany, Sri Kaliswari College, Sivakasi and also the herbarium specimen (Specimen no. SKCH136) was submitted at Department of Botany, Sri Kaliswari College, Sivakasi. The collected tubers were washed thoroughly in distilled water and cut in small pieces. The tubers were dried at room temperature under shade condition for 30 days. The dried tubers were uniformly grinded by using mechanical grinder to make fine powder. The powders were stored in air tight containers until further study (Plate 1. A&B).

Extract Preparation

The 50g tuber powder of *Momordica cymbalaria* was serially extracted with 250 ml of Methanol with the help of Soxhlet apparatus. The extraction procedures were continued for 3-4 hours at 60°C -80°C[15]. These extracts were concentration under reduced pressure evaporator and stored in air tight vials at 4°C for further study.

Phytochemical analysis by GC-MS

Gas chromatography-Mass spectrometry (GC-MS) analysis of the methanolic extracts was performed by using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30 m length, 0.25 mm dia. and 0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1.51 ml/min. Injector and mass transfer line temperature was set at 200 and 240°C respectively. The oven temperature was programmed from 70 to 220°C at 10°C/min, held isothermal for l min and finally raised to 300°C at 10°C/min. 2 µ1 of respective diluted samples was manually injected in the split less mode, with split ratio of 1:40 and with mass 18 scan of 50-600 amu. Total running time of GC-MS is 35min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization.

Identification of phytochemical components

The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08s.LIB and WILEY8. LIB library sources were used for matching the identified components from the plant material.

RESULTS

The GC-MS analyses of methanolic tuber extract of *M. cymbalaria* were confirmed the presence of 23 compounds with retention time. Interpretation of mass spectrum of GC-MS was conducted using the database of NIST and WILEY libraries. Out of this 23 compounds 16 compounds were majorly present in the tuber extract of *M. cymbalaria* respectively 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimeth (11.22%), Tetrasiloxane, decamethyl (11.17%), Methyltris (trimethylsiloxy)silane (11.17), 1,4-Bis (trimethylsilyl) benzene (9.48%), 1,2-Bis (trimethylsilyl) benzene (9.48%), N-Methyl-1-adamantaneacetamide (9.48%), Anthracene, 9-ethyl-9,10-dihydro-9, 10 -dimethyl- (9.63%), Arsenous acid, tris (trimethylsilyl) ester (9.63%), Benzenepropanoic acid, tert-butyldimethylsilyl ester (8.25%), 2-Methyl-7-phenylindole (6.09), Propiophenone, 2'-(trimethylsiloxy)- (5.50), Tris (tert-butyldimethylsilyloxy) arsane (5.50%), 1-Methyl-3-phenylindole (5.35%), Pentadecanoic acid (5.30%), Tetradecanoic acid (5.30%) and n-Hexadecanoic acid (5.30%).

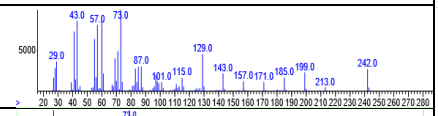
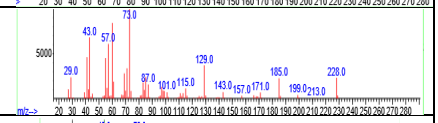
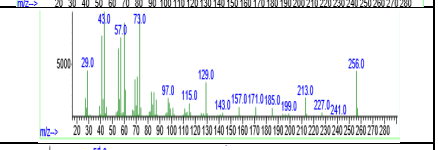
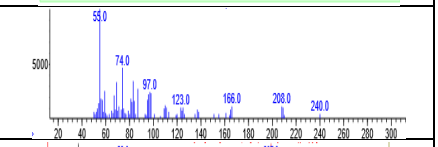
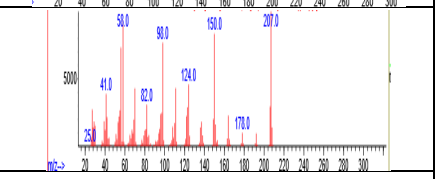
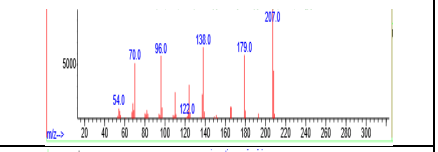
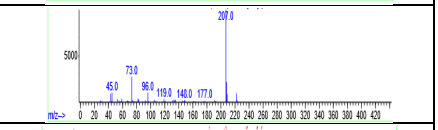
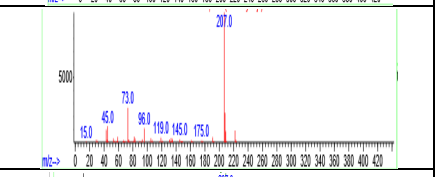
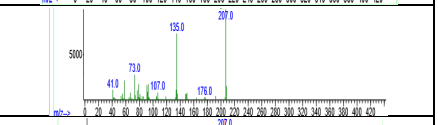
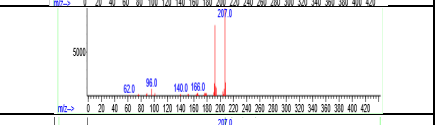
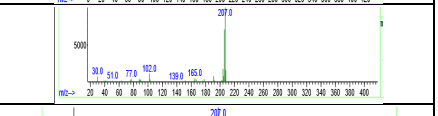
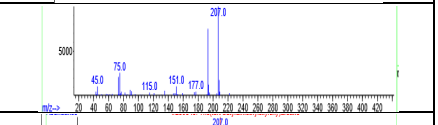
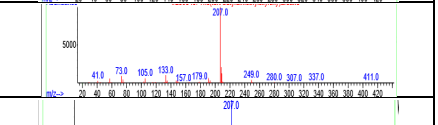
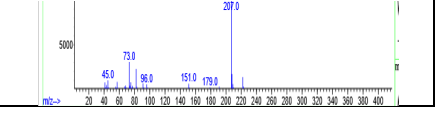
The seven minor compounds such as Methyl 9-tetradecenoate (2.58%), 9-Borabicyclo[3.3.1]nonane,9- [3 (dimethylamino) propyl]- (2.58%), (5S,6aR,10aS)-5-Propyldeca hydrodipyrrolo [1,2-a:1',2'-c]pyrimidine (2.58%) 2-Ethylacridine (3.32%), Trimethyl (4-tert-butylphenoxy) silane (1.88%), Silicic acid, diethyl bis (trimethylsilyl) ester (1.88%) and Cyclotrisiloxane, hexamethyl (1.88%) were also reported from the methanolic tuber extract of *M. cymbalaria*. The chemical constituent's analysis results of *M. cymbalaria* tuber were reported in Table 1 and their GC-MS chromatogram is presented in Plate1-C.

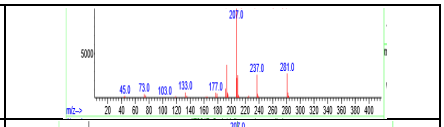
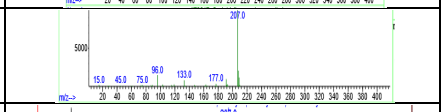
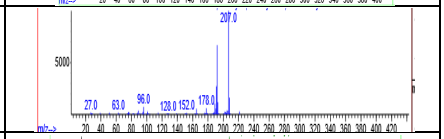
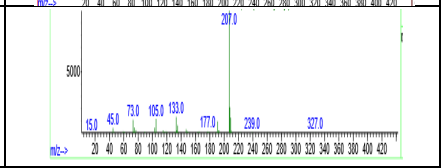
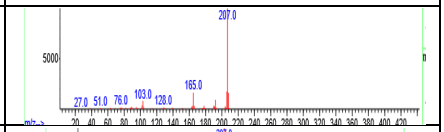
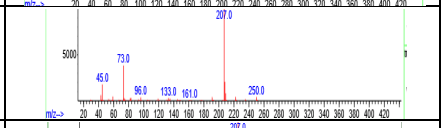
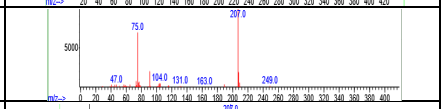
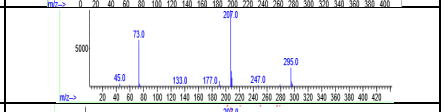
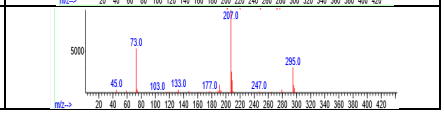
The first compound identified with less retention (11.71 min.) was Pentadecanoic acid, Tetradecanoic acid and n-Hexadecanoic acid whereas Tetrasiloxane, decamethyl- was the last compound which took longest retention time (17.36 min.) to identify. At (16.98) retention time 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimeth, Tetrasiloxane, decamethyl, and Methyltris (trimethylsiloxy) silane compound were found to be high (11.17%) and the lowest percentage (1.88%) was found to be Trimethyl (4-tert-butylphenoxy) silane, Silicic acid, diethyl bis (trimethylsilyl) ester and Cyclotrisiloxane, hexamethyl. The above mentioned isolated compound from the methanolic extract of *Momordica cymbalaria* tuber have a medicinal important.

DISCUSSION

Medicinal plants have been used worldwide for the treatment of various diseases principally in developing countries where infectious diseases are endemic and contemporary health amenities are inadequate[16]. The fixed oil present in fruits of *Momordica cymbalaria* is reported to contain palmitic acid, oleic acid, stearic acid, α-Eleostearic acid and γ-Linolenic acid[14]. In the present study, the GC-MS analysis of methanolic tuber extract of *M. cymbalaria* revealed the presence of 23 compounds. This is the first detailed studies on the GC-MS analysis of chemical constituents of the tuber extract of *M. cymbalaria*.

Table 1: List of chemical compounds identified from methanol tuber extract of *Momordica cymbalaria* through GC-MS analysis

S. No.	RT	Name of the Compound	AREA (%)	Mass spectrum
1.	11.712	Pentadecanoic acid	5.30	
2.	11.712	Tetradecanoic acid	5.30	
3.	11.712	n-Hexadecanoic acid	5.30	
4.	13.386	Methyl 9-tetradecenoate	2.58	
5.	13.386	9-Borabicyclo[3.3.1]nonane,9-[3 (dimethylamino)propyl]-	2.58	
6.	13.386	(5S,6aR,10aS)-5-Propyldeca hydrodipyrrolo[1,2-a:1',2'-c]pyrimidine	2.58	
7.	16.213	1,4-Bis(trimethylsilyl)benzene	9.48	
8.	16.213	1,2-Bis(trimethylsilyl)benzene	9.48	
9.	16.213	N-Methyl-1-adamantaneacetamide	9.48	
10	16.279	2-Ethylacridine	3.32	
11	16.412	2-Methyl-7-phenylindole	6.09	
12	16.487	Propiophenone, 2'-(trimethylsiloxy)-	5.50	
13.	16.487	Tris(tert-butyldimethylsilyloxy)arsane	5.50	
14	16.686	Trimethyl(4-tert-butylphenoxy)silane	1.88	

15	16.686	Silicic acid, diethyl bis(trimethylsilyl) ester	1.88	
16	16.686	Cyclotrisiloxane, hexamethyl	1.88	
17	16.837	Anthracene, 9-ethyl-9,10-dihydro-9, 10 -dimethyl-	9.63	
18	16.837	Arsenous acid, tris(trimethylsilyl) ester	9.63	
19	16.894	1-Methyl-3-phenylindole	5.35	
20	16.989	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimeth	11.22	
21	17.074	Benzenepropanoic acid, tert-butyldimethylsilyl ester	8.25	
22.	17.367	Tetrasiloxane, decamethyl-	11.17	
23.	17.367	Methyltris(trimethylsiloxy)silane	11.17	

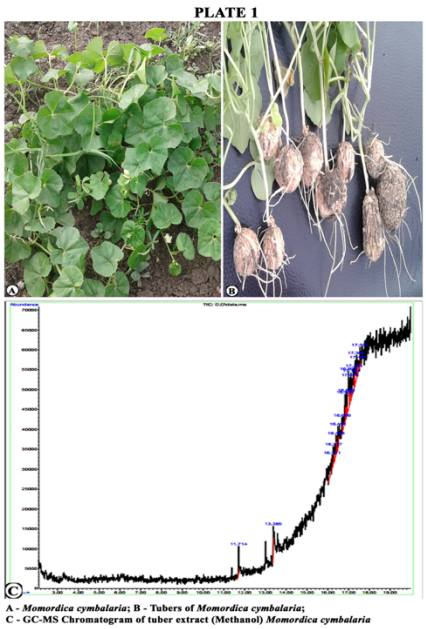


Figure 1: A- ***Momordica cymbalaria;*** **B- Tubers of** ***Momordica cymbalaria*****; C- GC-MS chromatogram of tuber extract (methanol)** ***Momordica cymbalaria***

The GC-MS analysis of the methanolic leaf extract of *Rhododendron campanulatum* revealed the presence of 49 phytochemical compounds. Baccharis oxide (9.99%), betuligenol (8%), alpha and beta-amyrin (7.38 and 2.64%), geranyl acetate (5.91%), (R)-(-)-14-methyl-8-hexadecyn-1-ol (5.19%) and phthalic acid (5.16%) were identified as major constituents[17].

The GC MS analysis of methanolic extract of *Eupatorium triplinerve* showed the eleven different phytochemical compounds namely hexadecanoic acid (14.65%), 2,6,10-trimethyl,14-ethylene-14-pentadecne (9.84%), Bicyclo[4.1.0] heptane, 7-butyl-(2.38%), Decanoic acid, 8-methyl-, methyl ester (3.86%), 1-undecanol (7.82%), 1-hexyl-1-nitrocyclohexane (2.09%), 1,14-tetradecanediol (6.78%), Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (19.18%) and 2-hydroxy-3-[(9E)-9-octadecenoyloxy] propyl(9E)-9-octadecenoate (8.79%)[18].

The leaves of Petroleum ether extract of *Millettia peguensis* by GC-MS analysis clearly showed the presence of 10 compounds. In GC-MS analysis, *Millettia peguensis* showed 3 major and 7 minor compounds. The results revealed that Pentadecane (32.73%), Tetradecane (29.79%) and Octadecane (22.77%) were reported as 3 major components in the Petroleum ether leaves extract of the *Millettia peguensis.* The seven minor compounds such as Eicosane (7.23%), Undecane, 5-methyl- (2.13%), 9-methylheptadecane (1.40%), Sulfurous acid, dodecyl hexyl ester (1.13%), Heptadecane, 2, 6, 10, 15-tetramethyl- (0.99%), 2-Bromo dodecane (0.96%) and Heneicosane (0.87%) were also reported from leaves[15]. The current study of GC-MS analysis of methanolic tuber extract of *M. cymbalaria* showed 16 major and 7 minor compounds. These chemical constituents are good source of biological activities. The methanol has got more chemical constituents compared to the petroleum ether.

Most of the major phytochemical compounds are either pharmacologically active compound useful for various industries. Baccharis oxide is a type of triterpene, known as a precursor of steroids in both plants and animals[19]. A few pharmacological investigations on alpha and beta-amyrin have proven its antioxidant, antimicrobial, anti-inflammatory and anticancer properties[20]. An organic monoterpene, is known to possess antioxidant properties and specific fragrance due to which it is used as cleanser in industries[21, 22]. Monoterpene alcohol found in essential oils and is reported to have anti-convulsant property. It is also used in cosmetic industries[23]. A terpene alcohol is a natural compound being used in toothpaste and gargling solution due to its anti- inflammatory and antibacterial activities[24, 25]. Therefore, the methanolic tuber extract of *M. cymbalaria* can be used for the sourcing of these compounds for various applications.

CONCLUSION

The GC-MS analysis of chemical constituents of the methanolic tuber extract of *M. cymbalaria* clearly showed the presence of 23 chemical constituents. The results indicated that the tuber extracts of *M. cymbalaria* possess many chemical constituents and it can be used for the treatment of various diseases and is recommended as a plant of phyto-pharmaceutical importance.

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