Research Article

Preliminary Phytochemical Screening, Gas Chromatography Mass Spectrum and Fourier Transform Infrared Spectroscopy Analysis of Aerial Part of *Maerua apetala* Roth (Jacobs)

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Abstract

The present study was carried out to characterize the bioactive constituents present in the aerial part of *Maerua apetala* Roth (Jacobs) using FT-IR and GC-MS. Preliminary phytochemical screening of aerial part of *M. apetala* showed the presence of alkaloids, flavonoids, saponins, phenols, quinones, steroids, tannins, glycosides, sugars and fixed oils in the different extracts. The FTIR spectrum confirmed the presence of hydroxyl, alkyl, carbonyl, carboxylic, aldehydes, amine, phenyl nucleus and mono chlorinated compounds. The bioactive components of the ethanol extract of aerial part of *Maerua apetala* were investigated using Perkin-Elmer Gas Chromatography-Mass Spectrometry (GC-MS), while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC-MS analysis revealed that, twenty compounds were detected in ethanol extract of aerial part of *M. apetala*. Z-1 and 9-Hexadecadiene (35.92) was found to be the major components in the ethanol extract of *M. apetala*.

The findings of the study provided evidences that various solvent extracts of the tested plant contained medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of various diseases as well as for nutritive purposes.

Keywords: *Maerua apetala*, Phytochemical screening, FT-IR, Vitamin E, GC-MS

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Introduction

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value [1]. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [2]. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes [3]. 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. Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents. 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 [4-6].

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials [7]. Knowledge of the phytochemical constituents is very essential to facilitate search of the actual effectiveness of the plant in medicine. The plants are having numerous bioactive components which are identified (at less than 1 ng)

by using GC or LC-MS. Spectroscopic (FT-IR) methods together or separate can be used because of its simplicity, cost-effective and rapid tests for detecting phytocomponents [8-10].

Maerua is an important ayurvedic drug used as one of the ingredients in many Ayurvedic preparations. Ethnomedical survey reveals that *Maerua* is used to cure various diseases such as fever, stomach ache, skin infections, urinary calculii, diabetes mellitus, epilepsy, pruritis, rigidity in lower limbs, and abdominal colic [11]. *Maerua* is an important drug used in diseases like anaemia, diabetes, stomach disorders, typhoid and cough [12].

Maerua apetala is a deciduous tree, upto 5m tall; bark pale. Leaves thick, sub-coriaceous, 3-foliolate, petiole to 7.5cm; leaflets linear-oblong and lanceolate, 2-6x 1.7-3.5cm, base rounded, margin entire, apex acute to acuminate, secondary nerves 3-6 pairs, obscure; petiole to 5cm. Flowers fragrant, creamish tinged with purple axillary or terminal corymbs; stamens many. Berry oblong, 1-2 ridged, 1-3cm long; seeds are embedded in a scarlet pulp, muricate and white.

In the last few years, spectroscopic methods have become firmly established as a key technological platform for secondary metabolite profiling in both plant and non plant species [13-14].Therefore, the present study was conducted to investigate the phytochemical constituents of whole plant of *M. apetala* using FT-IR and GC-MS.

Experimental

Materials and methods

The aerial parts of *Maerua apetala* Roth (Jacobs) were collected from Vattakottai, Kanyakumari District, Tamil Nadu. The plant was identified with help of local flora and authenticated in Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu.

Preparation of extracts for phytochemical screening

Freshly collected aerial part of *M. apetala* was dried in shade, and then coarsely powdered separately in a willy mill. The coarse powder (100g) was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered though Whatman No.41 filter paper. All the extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures [15-17].

FT-IR analysis

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermoscientific Nicot iS5 iD1 transmission, between 4000 − 400 cm^{-1} [18].

Preparation of extracts for GC-MS

Aerial part of *M. apetala* was cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of aerial part of plant powder was weighed and transferred to stoppered flask and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first six hours and then it was kept aside and again shaken after 24hours. This process was repeated for three days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The ethanol extract thus obtained were used for GC-MS analysis.

GC-MS Analysis

GC-MS analysis of ethanol extract of aerial part were performed by using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1 µMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl 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 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % 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. 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.

Result and Discussion

Preliminary phytochemical screening is an important initial step to find out the phytoconstituents present in the plant extract, which further leads to the isolation of active compounds, responsible for many pharmacological actions. Phytochemical screening is usually carried out to screen and characterize the constituents available in a given plant sample. Generally in the phytochemical screening of any plant one normally identifies secondary metabolites that have been accumulated to some extent at specific organ of the plant. These metabolites that are mainly used by the plant for protection against herbivores may have pharmacological activity when tested on animals [19]. Phytochemical screening of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of aerial part of *M. apetala* was presented in **Table-1**. Result of phytochemical screening of aerial part of *M. apetala* various extracts showed the presence of alkaloids, steroids, coumarins, catechins, tannins, phenols, flavonoids, saponins, glycosides and xanthoprotein.

Table 1 Preliminary phytochemical screening of aerial part of *M. apetala*

Bioactive components	Nature of extract				
	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol
Alkaloids	+	+	+	+	+
Anthroquinones	-	-	-	+	+
Catechin	+	-	-	-	-
Coumanin	+	-	+	+	+
Flavonoids	+	+	+	+	+
Phenols	+	+	+	+	+
Quinones	+	+	+	+	+
Saponins	+	+	+	+	+
Steroids	+	+	+	+	+
Tannins	+	+	+	+	+
Terpenoids	-	-	-	-	-
Glycosides	+	+	+	+	+
Xanthoprotein	+	+	+	+	+
Sugar	+	+	+	+	+
Fixed oil	+	+	+	+	+

Medicinal use of alkaloids of plants has a long history, and thus when the first alkaloids were synthesized in the 19th century, they immediately found application in clinical practice [20]. Many alkaloids are still used in medicine, usually in the form of salts. Alkaloids are known to exhibit emetic amoebicides, expectorant, anesthetics, antipyretics, analgesics, antihelminthic and can be used for the treatment of stomach problems [21]. Preparations of plants containing alkaloids and their extracts, and later pure alkaloids, have long been used as psychoactive substances.

Natural phenolic compounds play an important role in cancer prevention and treatment. Phenolic compounds from medicinal herbs and dietary plants include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others. Various bioactivities of phenolic compounds are responsible for their chemopreventive properties (e.g., antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects) and also contribute to their inducing apoptosis by arresting cell cycle, regulating carcinogen metabolism and ontogenesis

expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signalling pathways. The most recent literature regarding phenolic compounds to summarize structural categories and molecular anticancer mechanisms of phenolic compounds from medicinal herbs and dietary plants [22]. (Delete this sentebce) Phenol is also a versatile precursor to a large collection of drugs, most notably aspirin but also many herbicides and pharmaceutical drugs. Phenol is also used as an oral anesthetic/analgesic in products. Phenol derivatives are also used in the preparation of cosmetics including sunscreens, hair colorings and skin lightening preparation [22].

Flavonoids are the most common group of polyphenolic compounds in the human diet and are found universally in plants [23]. Flavonols, the original bioflavonoids such as quercetin, are also found universally, but in lesser quantities. The widespread distribution of flavonoids, their variety and their relatively low toxicity compared to other active plant compounds (for instance alkaloids) mean that many animals, including humans, ingest significant quantities in their diet.

Natural or synthetic quinones show a biological or pharmacological activity and some of them show antitumoral activity. These applications include purgative, antimicrobacterial, anti-tumor, inhibition of PGE2 biosynthesis and anti-cardiovascular disease [24].

Coumarins have shown some evidence of many biological activities, but they are approved for few medical uses as pharmaceuticals. Reported coumarin activity includes anti-HIV, anti-tumor, anti-hypertension, anti-arrhythmia, anti-inflammatory, anti-osteoporosis, antiseptic, and analgesic. It is also used in the treatment of asthma [25] and lymphedema [26]. Coumarin is found naturally in many edible plants such as strawberries, black currants, apricots and cherries.

Tannins are a group of natural products which are recognized as health protecting antioxidants. Tannins are plant polyphenolic compounds that are contained in large quantities in food and beverages (tea, red wine, nuts, etc.) consumed by humans daily. It has been shown that various tannins exert broad cancer chemoprotective activity in a number of animal models. An increasing body of evidence demonstrates that tannins act as both anti-initiating and antipromoting agents. In view of the fact that tannins may be of valid medicinal efficacy in human clinical trials, attempts are made to integrate results from animal studies, and consider their possible application in humans [27, 28].

Saponins are a mild detergent used in intracellular histochemistry staining to allow antibody access to intercellular proteins. In medicine, it is used in hypercholesterolaemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight lose etc. It is known to have anti-fungal properties.

Plant steroids are known to be important for their cardiotonic activities, possess insecticidal and antimicrobial properties. Plant derived natural products such as flavonoids, terpenoids and steroids etc., have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity [29]. Plant steroids are known to the important for their cardio tonic activities, possess insecticidal and antimicrobial properties [30]. (Delete this sentence)These observations cited on phytochemical compounds support the present findings on the usefulness of aerial part of *M. apetala* in various medicaments.

The FT-IR spectral studies of aerial part of *M. apetala* gave the following characteristics absorption peaks as shown in **Table 2** and **Figure 1**.

Table 2 FTIR spectroscopic data of aerial part of *M. apetala*

S. No	Group	Stretching Frequency (cm^{-1})
1	O-H	3377.12 3321.19 3199.69
2	C-H(Alkyl)	2923.88
3	C-H(Carbonyl compounds)	2854.45
4	C=O	1730.03 1625.88

5	C=C (Phenyl nucleus)	1593.09
6	C-CHO(aldehydes)	1400.22
7.	C-N(amine)	1114.78
8.	C-Cl(Monochlorinated compounds)	754.12

The uses of FT-IR spectral fingerprinting for herbal preparations tend to focus on the identification and assessment of the stability of the chemical constituents functional group. The functional group identification is based on the FT-IR peaks attributed to the stretching and bending librations. IR analysis of aerial parts of *M. apetala* powder gave peaks that suggest the presence of different functional groups ranging from O-H stretching, hydroxyl (3377.12-3199.69cm^{-1}), C-H stretching, alkyl (2923.88cm^{-1}), C-H stretching, Carbonyl (2854.45cm^{-1}) C=O stretching, carboxylic, Carbonyl (1730.03 and 1625.88cm^{-1}), C=C stretching phenyl nucleus (1593.09cm^{-1}), C-CHO skeletal (aldehydes) (1400.22cm^{-1}), C-N stretching amine (1114.12cm^{-1}) and C-Cl stretching, mono chlorinated compounds (754.12cm^{-1}). Hence, the plant powder subjected to FT-IR analysis is used for identification of chemical constituents present in *M. apetala*. In addition, FT-IR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition.

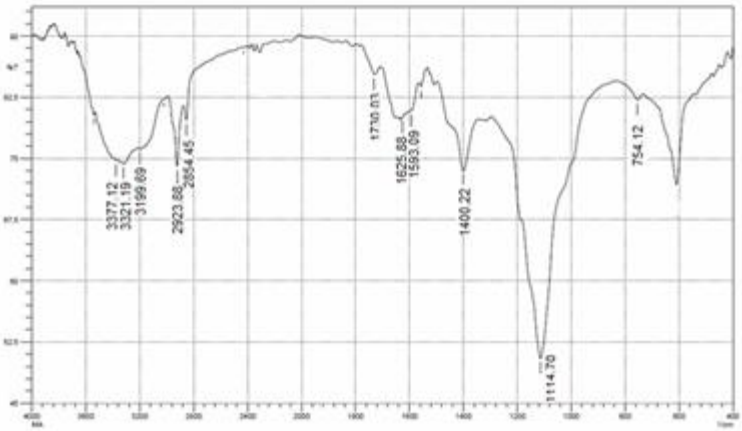


Figure 1 FT-IR Spectrum of aerial part of *M. apetala*

The results pertaining to GC-MS analysis leads to the identification of number of compounds from the GC fractions of the ethanol extract of aerial part of *M. apetala* **(Figure-2)**.

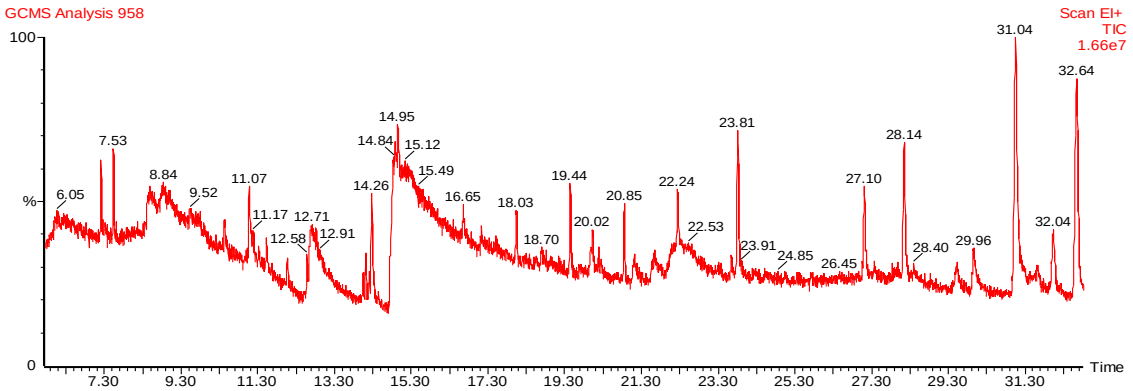
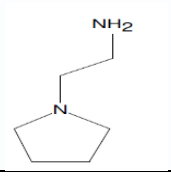
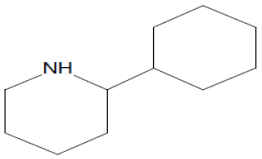
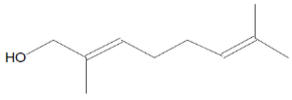
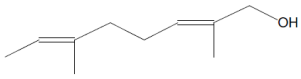
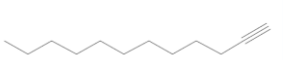
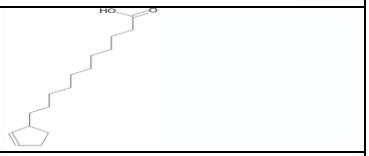
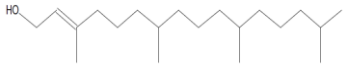
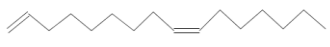
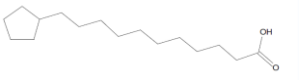
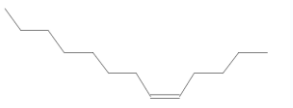
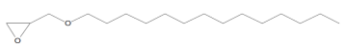
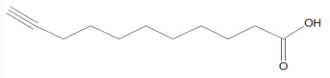
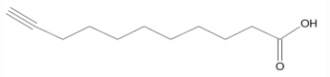
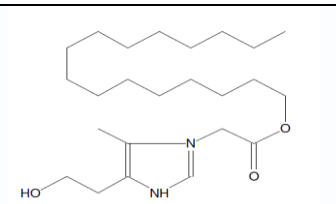
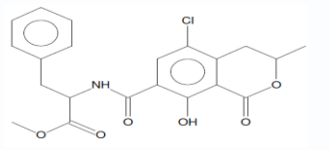
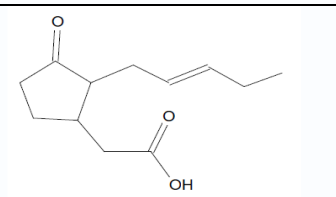
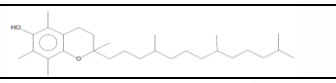
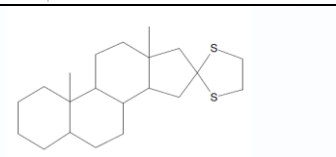
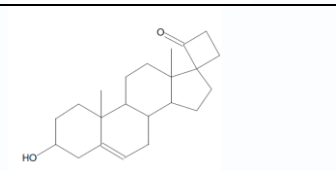
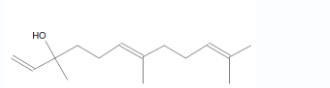


Figure 2 GC-MS chromatogram of ethanol extract of aerial part of *M. apetala*

The compounds were identified through mass spectrometry attached with GC. Twenty compounds were detected in ethanol extract of aerial part of *M. apetala*. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) are presented in **Table 3**.

Table 3 Phytocomponents detected in aerial part of *M. apetala*

S. No.	RT	Name of the compound	Molecular formula	MW	Peak Area %	Structures
1.	6.05	1-Pyrrolidineethanamine	$C_6H_{14}N_2$	114	9.87	
2.	7.21	2-Cyclohexylpiperidine	$C_{11}H_{21}N$	167	4.05	
3.	7.53	2,6-Octadien-1-ol,2,7-dimethyl-	$C_{10}H_{18}O$	154	4.75	
4.	10.43	2,6-Dimethyl-octa-2,6-dien-1-ol	$C_{10}H_{18}O$	154	2.80	
5.	11.07	1-Dodecyne	$C_{12}H_{22}$	166	3.27	
6.	12.71	2-Cyclopentene-1-undecanoic acid, (+)-	$C_{16}H_{28}O_2$	252	3.38	
7.	14.26	Phytol	$C_{20}H_{40}O$	296	1.85	
8.	14.95	Z-1,9-Hexadecadiene	$C_{16}H_{30}$	222	35.92	
9.	16.65	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254	2.21	
10.	18.03	5-Tridecene, (Z)-	$C_{13}H_{26}$	182	0.76	
11.	19.44	Oxirane, [(tetradecyloxy)methyl]-	$C_{17}H_{34}O_2$	270	1.19	

12.	20.02	2-Nonen-1-ol	$C_9H_{18}O$	142	1.27	
13.	20.18	Undec-10-ynoic acid	$C_{11}H_{18}O_2$	182	2.97	
14.	20.85	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	$C_{24}H_{45}N2O_3$	409	1.01	
15.	23.81	L-Phenylalanine, N-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyran-7-yl)carbonyl]-, methyl ester	$C_{21}H_{20}ClNO_6$	417	2.75	
16.	27.10	(3-Oxo-2-pent-2-enylcyclopentyl)acetic acid	$C_{12}H_{18}O_3$	210	1.97	
17.	28.14	Vitamin E	$C_{29}H_{50}O_2$	430	2.91	
18.	29.52	5à-Androstan-16-one, cyclic ethylene mercaptole	$C_{21}H_{34}S_2$	350	0.93	
19.	31.04	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	$C_{22}H_{32}O_2$	328	9.16	
20.	32.64	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	7.00	

The results revealed that Z-1,9-Hexadecadiene (35.92) was found to be the major component followed by 1-Pyrrolidineethanamine (9.87),Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)- (9.16),1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-(7.00), 2,6-Octadien-1-ol, 2,7-dimethyl-(4.75), 2-Cyclohexylpiperidine(4.05), 2-Cyclopentene-1-undecanoic acid, (+)- (3.38), 1-Dodecyne (3.27), Undec-10-ynoic acid (2.97), Vitamin E (2.91) 2,6-Dimethyl-octa-2,6-dien-1-ol (2.80), L-Phenylalanine,N-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyran-7-yl)carbonyl]-, methyl ester (2.75), Cyclopentaneundecanoic acid(2.21), (3-Oxo-2-pent-2-enylcyclopentyl)acetic acid (1.97), Phytol (1.85), 2-Nonen-1-ol (1.27), Oxirane, [(tetradecyloxy)methyl]-(1.19),3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)4methylimidazolium ion (1.01).Very small quantity of 5à-Androstan-16-one, cyclic ethylene mercaptole (0.93), 5-Tridecene, (Z)- (0.76) were reported.

Table 4 listed the major phytocomponents and its biological activities were obtained through the GC-MS study of aerial part of *M. apetala*. The biological activities listed are based on Dr. Dukes Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service, USDA.

Table 4 Activity of phytocomponents identified in the ethanol extract of aerial part of *M. apetala*

No	Name of the compound	Molecular formula	Compound Nature	**Activity
1.	1-Pyrrolidineethanamine	$C_6H_{14}N_2$	Alkaloid	Antimicrobial Anti-inflammatory
2.	2-Cyclohexylpiperidine	$C_{11}H_{21}N$	Alkaloid	Antimicrobial Anti-inflammatory
3.	2,6-Octadien-1-ol, 2,7-dimethyl-	$C_{10}H_{18}O$	Fragrance compound	Used in Perfumery
4.	2,6-Dimethyl-octa-2,6-dien-1-ol	$C_{10}H_{18}O$	Fragrance compound	Used in Perfumery
5.	Phytol	$C_{20}H40O$	Diterpene	Antimicrobial Anti-inflammatory Anticancer Diuretic
6.	2-Nonen-1-ol	$C_9H_{18}O$	Fragrance compound	Used in Perfumery
7.	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	$C_{24}H_{45}N_2O_3$	Nitrogen compound	Antimicrobial
8.	L-Phenylalanine, N-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyran-7-yl)carbonyl]-, methyl ester	$C_{21}H_{20}ClNO_6$	Amino acid	Useful for depression, Cure Parkinson's disease, Used in arthritis
9.	(3-Oxo-2-pent-2-enylcyclopentyl)acetic acid	$C_{12}H_{18}O_3$	Acetic acid compound	Antimicrobial
10.	Vitamin E	$C_{29}H_{50}O_2$	Vitamin compound	Antiageing, Analgesic, Antidiabetic Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary
11.	5à-Androstan-16-one, cyclic ethylene mercaptole	$C_{21}H_{34}S_2$	Sulfur compound	Antimicrobial
12.	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	$C_{22}H_{32}O_2$	Steroid	Antiarthritic Antimicrobial Anti-inflammatory Anti asthma
13.	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	Fragrance compound	Used in Perfumery

**Activity source: Dr. Duke's Phytochemical and Ethnobotanical Database

Among the identified phytochemicals, phytol is detected in aerial part of *M. apetala* which was also found to be effective at different stages of the arthritis. It was found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species promoting substances such as phytol constitute a

promoting novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases [30].

Vitamin E is one among the twenty compounds of the present study. Vitamin E improves skin health as it is an antioxidant agent. Vitamin E is vital for protecting the skin cells from ultraviolet light, pollution, drugs and other elements that produce cell-damaging free radicals. It improves the ability to regulate vitamins in the body, which itself is important for healthy skin. Vitamin E helps in the prevention of skin cancer, because of its sun protection quality and its powerful antioxidant properties. These two factors help to reduce or prevent damage by the sun. Vitamin E lotions help in preventing and treating sunburns. These lotions protect the epidermis layer of the skin from early stages of ultraviolet light damage. A few studies have found that vitamin E helps relieve menstrual pain. Vitamin E supplements may help to reduce PMS symptoms, including anxiety, craving, and depression. Vitamin E keeps the skin young by reducing the appearance of fine lines and wrinkles. Free radicals are believed to play an important role in the ageing of the skin. Hence the antioxidant activity is important to address skin problems.

Conclusion

In the present study, twenty components from whole plant of *M. apetala* were identified by GC – MS analysis. The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners. So that it might be utilized for the development of traditional medicines and further investigation is in need to elute novel active compounds from the medicinal plants which may create a new way to treat many incurable diseases.

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