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Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves

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Abstract

Background: Medicinal plants are of great importance to researchers in the field of pharmacology as most pharmaceutical industries depend on medicinal plant for their raw materials. *Hibiscus asper* belongs to the family Malvaceae and is well known for its medicinal properties. The present study was carried out to evaluate the antioxidant effect and possible bioactive components present in the aqueous methanol fraction of *Hibiscus asper* leaves.

Results: The phytochemical of aqueous methanol fraction of *Hibiscus asper* leaves (AMFHAL) revealed the presence of flavonoids, tannin, phenols, saponins, alkaloids, glycosides, terpenoids, and steroids. The GC-MS analysis revealed the presence of twenty-three bioactive compounds which include 9,12,15-octadecatrien-1-ol, n-Hexadecanoic acid, octadecatrienol acid, methyl palmitate, and phytol.

Conclusion: The phytochemical and GC-MS profiling of aqueous methanol fraction of *Hibiscus asper* leaves revealed the presence of bioactive compounds with important medicinal properties. Hence, the presence of these phytochemicals could be responsible for the therapeutic effects of the plant.

Keywords: *Hibiscus asper*, GC-MS, Phytochemicals, Phytol

Background

Plants are used as medicines in various cultures and serve as a source of many potent drugs due to the presence of certain bioactive compounds for pharmaceutical industries [1]. Plants contain different phytochemicals, also known as secondary metabolites. Phytochemicals are useful in the treatment of certain disorders by their individual, additive, or synergic actions to improve health [2, 3]. Phytochemicals are vital in pharmaceutical industry for development of new drugs and preparation of therapeutic agents [4]. The development of new drugs starts with identification of active principles from the natural sources. The screening of plant extracts is a new approach to find therapeutically active compounds in various plant species [1, 5]. Phytochemicals such as flavonoids, tannins, saponins, alkaloids, and terpenoids have several biological properties which include antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer, and anticancer activities, among others [5].

Hibiscus asper Hook. f. (Malvaceae) is an important medicinal plant widely distributed in tropical Africa and Madagascar. The genus *Hibiscus* is made up of 250 species and is characterized by the presence of bioactive compounds such as phenolic acids, flavonoids, and polysaccharides [6]. This plant is mostly used in folklore medicine for treatment of depression, jaundice, inflammation, anemia, dysmenorrhea, and leucorrhoea and as poison antidote [7]. In addition, the leaves serve as potent sedative, tonic, and restorative agent. It is also used in the treatment of male infertility and skin infection and as an antioxidant [8, 9].

Gas chromatography-mass spectroscopy (GC-MS) is a combined analytical technique used to determine and

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identify compounds present in a plant sample [10]. GC-MS plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active components [11].

Methods

Chemicals

All the chemicals and reagents used for the research were of analytical grade.

Plant collection and adentification

Fresh leaves of *Hibiscus asper* were collected from Isuofia in Aguata Local Government Area of Anambra State. The leaves were identified and authenticated by Mr. Felix Nwafor of the Pharmacognosy and Environmental Medicine Department, University of Nigeria Nsukka. The plant was deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, with the voucher number PCG/UNN/0350.

Preparation of plant material

Hibiscus asper leaves were air-dried at room temperature and pulverized into powder for extraction. The powder (1300 g) was macerated in 80% methanol and allowed to stand for 48 h at room temperature. The mixture was filtered with Whatman No. 1 filter paper and the filtrate was concentrated using a rotary evaporator to get a brownish black semi-solid extract.

Solvent partitioning of the crude methanol extract was done by using the protocol designed by Kupchan and Tsou [12] and modified version of Wagenen et al. [13]. Fractionation was carried out using n-hexane, ethylacetate, and 20% aqueous methanol (v/v). Crude extract (20 g) was weighed and dissolved in 250 ml of 20% aqueous methanol (v/v) to form a stock solution. Then, 250 ml of n hexane was added to the solution and poured into a separating funnel. The mixture was allowed to stand for 20 min for proper separation, and the upper part was collected in a beaker. The aqueous methanol part was washed repeatedly with n hexane, after which the different n hexane fractions were collected. The above procedure was repeated using ethyl acetate. At the end, ethylacetate fractions were collected and concentrated [14]. The aqueous methanol fraction was used for further studies after subjecting the different fractions to a preliminary study.

Preliminary phytochemical screening

Phytochemical profiling of crude extract and aqueous methanol fraction of *Hibiscus asper* leaves were carried out using the procedures as described by Harborne [15], Trease and Evans [16], Harborne [17], and Soni and Sosa [18].

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was carried out in a combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer, fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m × 250 μm, film thickness 0.25 μm), interfaced with 5675C Inert MSD with Triple-Axis detector. Helium gas was used as carrier gas and was adjusted to column velocity flow of 1.0 ml/min.

Other GC-MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 16.2 psi; out time, 1.8 mm; and 1 μl injector in split mode with split ratio 1:50 with injection temperature of 300 °C. The column temperature started at 36 °C for 5 min and changed to 150 V at the rate of 4 °C/min. The temperature was raised to 250 °C at the rate of 20 °C/min and held for 5 min. The total elution was 47.5 min. The relative percent amount of each component was calculated by comparing its average peak area to total areas. MS solution software provided by supplier was used to control the system and to acquire the data.

Identification of compounds

Identification of components was achieved based on their retention indices and interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NSIT). The database consists of more than 62,000 patterns of known compounds. The spectra of the unknown components of *Hibiscus asper* fraction obtained were compared with the standard mass spectra of known components stored in NIST library (NISTII).

Results

Phytochemical screening of aqueous methanol fraction of *Hibiscus asper* leaf revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, steroids, and terpenoids as shown in Table 1.

Table 1 Phytoconstituents of aqueous methanol fraction of *Hibiscus asper* leaf

Phytoconstituents	Relative abundance (mg/g)	
Total phenol	+	12.38 ± 2.42
Tannins	+	8.02 ± 3.21
Flavonoids	+	11.08 ± 2.03
Alkaloids	+	4.30 ± 0.03
Saponins	+	4.92 ± 2.42
Glycosides	+	0.64 ± 0.04
Terpenoids	+	2.03 ± 0.22

Values are present as mean ± SD. "+" indicates present

Gas chromatography-mass spectroscopy profiling of aqueous methanol fraction of *Hibiscus asper*

A total of 23 compounds were identified from the GC-MS analysis of methanol fraction of *Hibiscus asper* leaves exhibiting various phytochemical activities. The chromatogram is presented in Fig. 1, while the chemical constituents with their retention time (RT), molecular formula, molecular weight (MW), and concentration (%) in the MFHAL are presented in Table 2. The following bioactive compounds were present in the GC-MS analysis carried on methanol fraction of *Hibiscus asper* leaves: Benzeneacetaldehyde, Benzene, 1,2,3,5-tetramethyl-, Benzene, 1-ethyl-2,4-dimethyl-, Azulene, 1-Piperazinecarboxaldehyde, Phthalan, Benzene, 2-methoxy-1,3,4-trimethyl, Acetic acid, [bis[(trimethylsilyl)oxy]-, Pyrrolidine-5-one, 2-[3-hydroxypropyl]-, Methylester pentanoic acid, Cycloheptasiloxane, tetradecamethyl-, 3-Methyl-4-phenyl-1H-pyrrole, Hexadecamethyl cyclooctasiloxane, 5,6-Dimethoxybenzimidazole, Cyclononasiloxane, Methyl palmitate, Pentasiloxane, dodecamethyl-, 9,12-Octadecadienoic acid, methyl ester, 9, 12,15-Octadecatrienoic acid, methyl ester, Phytol, 9, 12,15-Octadecatrien-1-ol, (Z, Z, Z), and Amonafide.

Discussion

Phytochemical screening of aqueous methanol fraction of *Hibsicus asper* revealed the presence of phyto-compounds that have been documented to have antioxidant and other activities. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals [19] implicated in several diseases. Flavonoids have anti-oxidative and mucosal protective effect [20, 21]. Flavonoid-rich vegetables are widely used functional foods since they can be used to treat cardiovascular diseases [22]. They are characterized by their good bioavailability and, hence, constant dietary consumption of flavonoids has been reported to give pharmacologically relevant plasma concentrations in humans [23]. In addition, several studies have reported the possible cardioprotective effects of flavonoids against ischemia reperfusion [24, 25]. Saponins may activate mucous membrane protective factors, while tannins reduce the permeability of mucosa to chemical irritation. Consequently, they reduce inflammation, exert astringent and protective action on the stomach mucosa, and curb excess acidity. In addition, terpenoids and alkaloid compounds have also been reported to have potent activity against

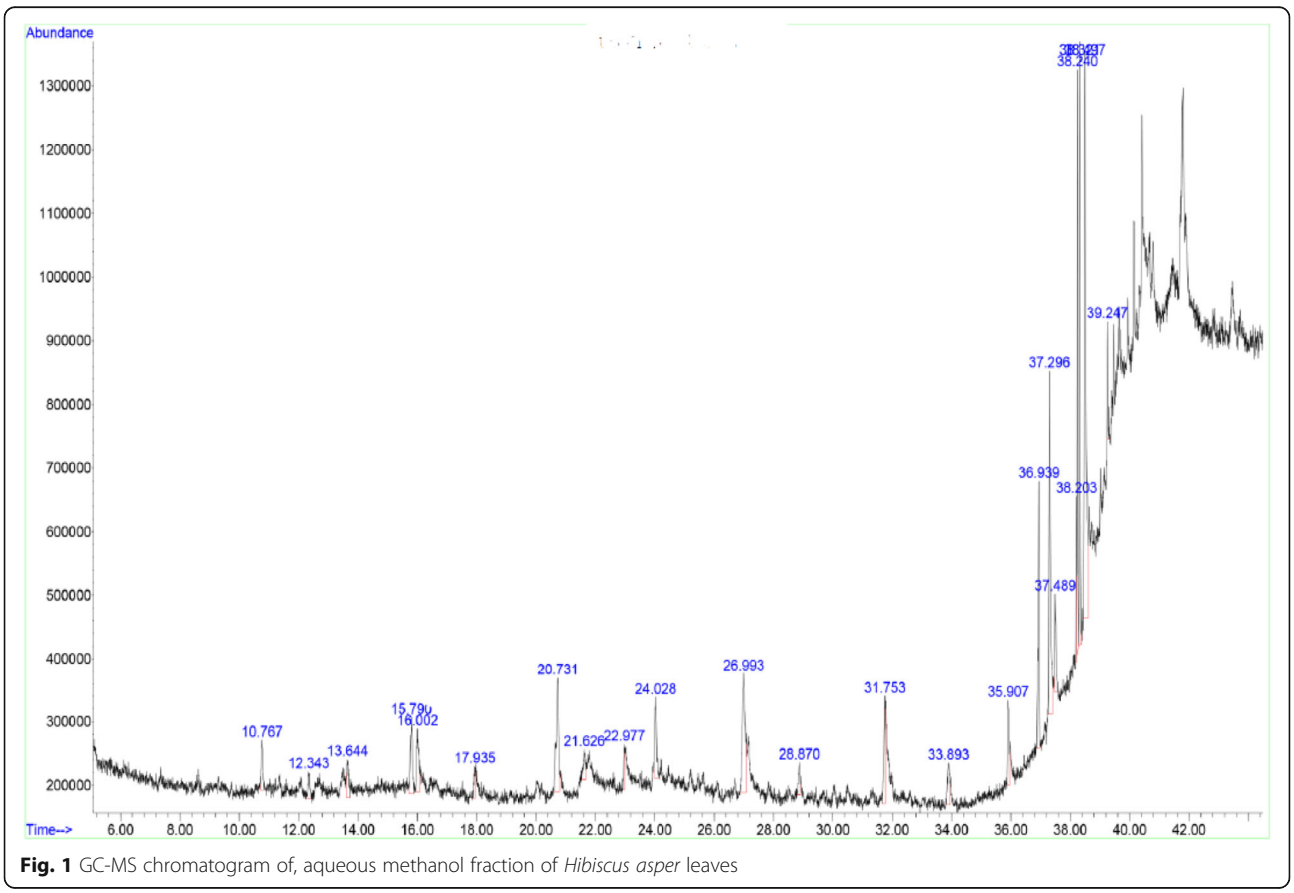

Fig. 1 GC-MS chromatogram of, aqueous methanol fraction of *Hibiscus asper* leaves

Table 2 Bioactive compounds found in aqueous methanol fraction of *H. asper*

No.	RT (min)	Name of the compound	Molecular formula	MW g/mol	Peak Area %	Structures of Compounds
1	10.767	Benzeneacetaldehyde	C_8H_8O	120.15	1.73	
2	12.343	Benzene, 1,2,3,5-tetramethyl-	$C_{10}H_{14}$	134.22	0.93	
3	13.644	Benzene, 1-ethyl-2,4-dimethyl-	$C_{10}H_{14}$	134.22	1.29	
4	15.790	Azulene	$C_{10}H_8$	128.17	3.24	
5	16.002	1-Piperazinecarboxaldehyde	$C_5H_{10}N_2O$	114.15	2.58	
6	17.935	Phthalan	C_8H_8O	120.15	0.72	
7	20.731	Benzene, 2-methoxy-1,3,4-trimethyl	$C_{10}H_{14}O$	150.22	6.00	
8	21.626	Acetic acid, [bis[(trimethylsilyl)oxy]-	$C_{11}H_{29}O_6PSi_3$	372.57	1.33	
9	22.977	Pyrrolidine-5-one, 2-[3-hydroxypropyl]-	$C_7H_{13}NO_2$	143.18	1.07	
10	24.028	Methylester pentanoic acid	$C_9H_{18}O_2$	158.24	2.97	
11	26.993	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	519.07	7.51	
12	28.870	3-Methyl-4-phenyl-1H-pyrrole	$C_{11}H_{11}N$	157.21	1.13	
13	31.753	Hexadecamethyl Cyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	593.2	2.95	
14	33.893	5,6-Dimethoxybenzimidazole	$C_9H_{10}N_2$	146.19	1.98	
15	35.907	Cyclononasiloxane	$H_{18}O_9Si_9$	414.9	2.79	
16	36.939	Methyl palmitate	$C_{17}H_{34}O_2$	270.5	6.29	
17	37.296	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	11.05	
18	37.489	Pentasiloxane, dodecamethyl-	$C_{12}H_{36}O_4Si_5$	384.84	2.27	
19	38.203	9,12-Octadecadienoic acid, methylester	$C_{19}H_{34}O_2$	294.47	2.19	
20	38.240	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.45	8.92	
21	38.321	Phytol	$C_{20}H_{40}O$	296.5	9.18	
22	38.497	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)	$C_{18}H_{32}O$	264.45	20.37	
23	39.247	Amonafide	$C_{16}H_{17}N_3O_2$	283.32	1.55	

gastric ulcers [26, 27]. Terpenoids have been reported to relax cardiovascular smooth muscle by inhibition of Ca^{2+} influx in vascular smooth muscle or via quenching of reactive oxygen species (ROS) and stimulation of nitric oxide (NO) synthesis [28]. The presence of these phytochemicals in methanol fraction of *H. asper* leaves possibly indicates its numerous medicinal properties such as anti-inflammatory, anti-ulcer, and anti-oxidative properties, among others.

Among the identified bioactive components, 9, 12, 15-Octadecatrien-1-ol (Z, Z, Z) has highest percent peak area. This compound has antioxidant and antibacterial properties [1]. n-Hexadecanoic acid has antioxidant, 5-alpha-reductase inhibitor, anti-fibrinolytic, hemolytic, antimicrobial activity, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, and hemolytic properties [5]. 9, 12, 15-Octadecatrienoic acid, methyl ester (Z, Z, Z) has anti-inflammatory, cancer preventive, hepatoprotective, antioxidant, and hypocholesterolemic properties [5]. Phenolic compounds, esters, alkanes, aldehydes, alkenes, and ketones are the other major volatile compounds present which have antiulcer, anti-inflammatory, anti-arthritic, antidiabetic, hypolipidemic, and cytotoxic activities [29]. Phytol was reported with antioxidant and neuroprotective, antimicrobial, anticancer, anti-inflammatory, and anti-diuretic activities [29, 30]. 9,12- Octadecadienoic acid, methyl ester has anti-inflammatory, anti-arthritic, hepatoprotective, antiandrogenic, hypocholesterolemic, nematicide, 5-alpha-reductase inhibitor, antihistaminic, anticoronary, insectifuge, antieczemic, and antiacne properties [31]. Methyl palmitate reported as antioxidant, hypocholesterolemic, nematicide, flavoring agents, hemolytic, and 5-alpha-reductase inhibitor [32]. Cycloheptasiloxane, tetradecamethyl- has antimicrobial, antiseptic, hair-conditioning agent, and skin-conditioning agent-emollient properties [33].

Conclusion

In the present study, *Hibiscus asper* leaves have shown to have various secondary metabolites which possess many pharmacological properties of which antioxidant activity is one. The GC-MS analysis showed the presence of 23 phytochemical constituents which contribute the activities like antimicrobial, antioxidant, anticancer, hypercholesterolemic, anti-inflammatory, and other activities. Hence, the presence of phytochemicals is responsible for their therapeutic effects. Further investigation is required for possible development of novel drugs using some of the bioactive compounds found in *H. asper.*

Abbreviation

H. asper: *Hibiscus asper*; GC-MS: Gas chromatography-mass spectroscopy; AMFHAL: Aqueous methanol fraction of *Hibiscus asper* leaves; Ca^{2+}: Calcium ion; RT: Retention time

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Plant authentication
The leaves were identified and authenticated by Mr. Felix Nwafor of Pharmacognosy and Environmental Medicine Department, University of Nigeria Nsukka. The plant was deposited in the herbarium of Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, with the voucher number PCG/UNN/0350.

Authors' contributions
All the authors contributed in the design of the study. NUO and OMO sourced the plant materials, while UCG dried and extracted the plant material. All the authors contributed in the fractionation of the plant extract. All the authors contributed in the phytochemical profiling of the plant fraction. NUO and OMO contributed in the GC-MS evaluation of the plant sample, and in the interpretation of the results. All the authors contributed in preparing the manuscript. All the authors read and approved the manuscript.

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