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

Njoku Ugochi Olivia*, Umeh Chinenyenwa Goodness and Ogugofor Martins Obinna

**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
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 identification**

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, ethyl acetate, 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]-, Methyl ester pentanoic acid, Cycloheptasiloxane, tetradecamethyl-, 3-Methyl-4-phenyl-1H-pyrrole, Hexadecamethyl cyclooctasiloxane, 5,6-Dimethoxybenzimidazole, Cyclohexasiloxane, 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 *Hibiscus 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...
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, hypcholesterolemic 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 anti ulcer, 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, hypcholesterolemic, nematicide, 5-alpha-reductase inhibitor, antihistaminic, anticonvulsive, insectigfuge, and anticancer properties [31]. Methyl palmitate reported as antioxidant, hypcholesterolemic, nematicide, flavoring agents, hemolytic, and 5-alpha-reductase inhibitor [32]. Cycloheptasiloxane, tetradecamethyl- has antimicrobial, anti-septic, 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

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

| No. | RT (min) | Name of compound | Molecular formula | MW (g/mol) | Peak area % | Structures of Compounds |
|-----|----------|------------------|------------------|------------|-------------|-------------------------|
| 1   | 15.7     | Rosmaric acid    | C$_{12}$H$_{18}$O$_6$ | 258.12     | 1.73        | ![Structure 1](image1.png) |
| 2   | 12.3     | Luteolin, 5,7,3',4'-tetrahydroxyflavone | C$_{15}$H$_{12}$O$_7$ | 304.16     | 0.93        | ![Structure 2](image2.png) |
| 3   | 8.2      | Luteolin, 5,7,3',4'-tetrahydroxyflavone | C$_{15}$H$_{12}$O$_7$ | 304.16     | 1.29        | ![Structure 3](image3.png) |
| 4   | 18.5     | Luteolin, 5,7,3',4'-tetrahydroxyflavone | C$_{15}$H$_{12}$O$_7$ | 304.16     | 1.29        | ![Structure 4](image4.png) |
| 5   | 21.0     | 1- Piperazinecarboxylic acid | C$_{6}$H$_{8}$O$_2$ | 114.15     | 2.58        | ![Structure 5](image5.png) |
| 6   | 13.9     | Phenol | C$_{6}$H$_{5}$ | 120.12     | 0.72        | ![Structure 6](image6.png) |
| 7   | 20.7     | Rosmaric acid | C$_{12}$H$_{18}$O$_6$ | 258.12     | 0.00        | ![Structure 7](image7.png) |
| 8   | 21.8     | Rosmaric acid | C$_{12}$H$_{18}$O$_6$ | 258.12     | 1.33        | ![Structure 8](image8.png) |
| 9   | 22.9     | Rosmaric acid | C$_{12}$H$_{18}$O$_6$ | 258.12     | 1.07        | ![Structure 9](image9.png) |
| 10  | 24.0     | Methylamine | C$_{3}$H$_{7}$N | 58.14      | 2.97        | ![Structure 10](image10.png) |
| 11  | 25.9     | Cyclohexanol | C$_{6}$H$_{12}$O$_2$ | 110.17     | 5.51        | ![Structure 11](image11.png) |
| 12  | 28.8     | 3-Methyl-1,1-dicyclohexylpropane | C$_{15}$H$_{24}$N$_2$ | 226.39     | 1.13        | ![Structure 12](image12.png) |
| 13  | 31.7     | Hexadecane | C$_{16}$H$_{34}$ | 226.41     | 1.95        | ![Structure 13](image13.png) |
| 14  | 33.8     | 5,7-Dimethylbenzyl alcohol | C$_{15}$H$_{22}$O | 226.39     | 1.98        | ![Structure 14](image14.png) |
| 15  | 35.9     | Cyclohexane | C$_{6}$H$_{12}$ | 84.14      | 2.79        | ![Structure 15](image15.png) |
| 16  | 36.9     | Methyl palmitate | C$_{15}$H$_{31}$O$_2$ | 270.56     | 6.29        | ![Structure 16](image16.png) |
| 17  | 37.2     | n-Hexadecanoic acid | C$_{16}$H$_{32}$O$_2$ | 256.45     | 11.65       | ![Structure 17](image17.png) |
| 18  | 37.4     | Palmitoleic acid | C$_{16}$H$_{30}$O$_2$ | 256.45     | 2.27        | ![Structure 18](image18.png) |
| 19  | 38.2     | Octadecenol | C$_{18}$H$_{34}$O | 274.52     | 2.19        | ![Structure 19](image19.png) |
| 20  | 38.2     | Octadecenol | C$_{18}$H$_{34}$O | 274.52     | 8.92        | ![Structure 20](image20.png) |
| 21  | 38.2     | Phytol | C$_{10}$H$_{18}$ | 140.28     | 9.19        | ![Structure 21](image21.png) |
| 22  | 28.4     | 11-Octadecen-9-ol | C$_{18}$H$_{34}$ | 264.58     | 4.37        | ![Structure 22](image22.png) |
| 23  | 30.2     | Anisole | C$_{9}$H$_{8}$O | 132.16     | 1.55        | ![Structure 23](image23.png) |
Acknowledgements
The authors wish to acknowledge Mr. Felix Nwafor who authenticated the plant material, and Mr. Mbaoji of Department of Pure and Industrial Chemistry, University of Nigeria Nsukka, for their assistance.

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.

Funding
No funding received.

Availability of data and materials
All data and material are available upon request.

Competing interest
The authors declare no competing interest.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Received: 6 November 2020 Accepted: 8 February 2021
Published online: 04 March 2021

References
1. Gopalakrishnan K, Udayakumar R (2014) GC-MS analysis of phytocomponents of leaf and stem of marsilea quadrifolia (L). Int J Biochem Res Rev 4(6):517–526
2. Patel DK (2015) Plant as a source of medicine. Med Aromat Plants S 3:1
3. Mahomoodally MF (2013) Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. Evid Based Complementary Altern Med 1: 1–14
4. Nisha K, Darshana M, Madhu G, Bhupendra MK (2011) GC-MS analysis and anti-microbial activity of Pidium guajava (leaves) grown in Malwa region of India. Int J Drug Dev Res 3(4):237–245
5. Starlin T, Prabha PS, Thayakumar BKA, Gopalakrishnan VK (2019) Screening and GC-MS profiling of ethanolic extract of Tylophora scucloriflora. Biomed Inform 15(6):425–429
6. Vasudeva N, Sharma SK (2008) Biologically active compounds from the genus Hibiscus. Pharm Biol 46:145–153
7. Schippers RR, Bosch CH (2004) Hibiscus asper hook F. In: Grubben GJH, Denton OA (eds) PROTA (plant resources of tropical Africa/Ressources végétales de l’Afrique tropicale), vol 2004, Wageningen, pp 314–315
8. Foyet HS, Abdou BA, Ponka R, Asongalem AO, Ogugfoor MO (2017) Cardioprotective potential of methanol extract of Costus al lira leaf on carbon tetrachloride-induced cardiotoxicity in albino rats. Asian J Pharm Res Health Care 9(2):1–58
9. Sorek L, Shapira D, Bar-On I (2010) Determination of total flavonoid contents of whole plant extracts of Gentiana lutea and Lycopus europaeus. J Food Sci 75:42–46
10. Sharath SS, Preeathy J, Kumar GS (2015) Screening for anti-ulcer activity of Convolvulus pluricaulis using pyloric ligation method in Wistar rats. Int J Pharm Sci 6(1):89–99
11. Abakew M, Mishra B, Gelaye DA (2017) Evaluation of anti-ulcer activity of the leaf extract of Oyisys quadrapiants Decne (Santalaceae) in rats. J Exp Pharmacol 91:1–11
12. Stoclet JC, Schini-Kerth V (2011) Dietary flavonoids and human health. Ann Pharmacother 45:78–90
13. Cao J, Zhang Y, Chen W, Zhao X (2010) The relationship between fasting plasma concentrations of selected flavonoids and their ordinary dietary intake. Br J Nutr 103(2):249–255
14. Njoku JO, Nwodo OFC, Ogugfoor MO (2017) Cardioprotective potential of methanol extract of Costus al lira leaf on carbon tetrachloride-induced cardiotoxicity in albino rats. Asian J Pharm Res Health Care 9(2):1–58
15. Leoum S, Lamont KT (2011) Natural polyphenols and cardioprotection. Mini-Rev Med Chem 11(14):1191–1199
16. Abdelhak R, Soraya B (2018) Phytochemical characterization, anti-inflammatory and anti-ulcer activity of a spontaneous succulent Delosperma resili. Univers J Agr Res 6(3):113–117
17. Sreeja PS, Arunachalam K, SaiKumar S, KasiPandi M, Dhiya S, Murugan R, Parimalahagran T (2018) Gastroprotective effect and mode of action of methanol extract of Sphenodesme involucrata var. paniculata (C.B. Clarke) Munir (Lamiaceae) leaves on experimental gastric ulcer models. Biomed Pharmacother 97:1109–1118
18. Alves-Silva JM, Zuzarte M, Marques C, Ligua S, Grao H (2016) Protective effects of terpenes on the cardiovascular system: current advances and future perspectives. Curr Med Chem 23(40):1–42
19. Kumar PP, Kumaravel P, Lalitha C (2010) Screening of antioxidant activity, total phenolics and GC-MS study of Vitis negundo. Afr J Biomed Res 47(10):191–195
20. Banajee J, Salunke M, Indapuruk K, Ghati U, Bhalerao S (2017) Estimation of serum malondialdehyde as a marker of lipid peroxidation in medical students undergoing examination-induced psychological stress. J Sci Soc 44:137–139
21. Nishantini A, Mohan VR, Jeeva S (2014) Phytochemical, FT-IR, and GC-MS analysis of stem and leaf of Tiliacora acuminate (tan) hook f and Thomas (mersisperrameae). Int J Pharm Sci Res 5(9):3977–3986
22. Rajeswari G, Murugan M, Mohan VR (2013) GC-MS analysis of bioactive components of Hugonia mystrix L. bark (Linaceae). J Pharm Biomed Sci 29: 818–824
23. Mary APF, Giri RS (2018) GC-MS analysis of bioactive compounds of Achnasynthes aspera. World J Pharm Res 7(1):1045–1056

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.