# *Original Research Article*

## Phytochemical profiling and GC-MS analysis of the Methanol extract of *Anthocleista grandiflora* Wood Bark


### ABSTRACT

The plant, *Anthocleista grandiflora* is a member of the family Gentianaceae and commonly known as the "forest fever tree" used traditionally for the treatment of various ailments. Its methanol bark extract was subjected to preliminary phytochemical analysis using standard methods and the presence of bioactive compounds was determined using Gas chromatography mass spectroscopy (GC-MS). Results showed the presence of flavonoids in high amount, saponins and cardiac glycosides in moderate amount while tannins, phenols, terpenoids and steroids were present in low amounts. Test for alkaloids, glycosides, anthroquinones, pholobatannins and anthrocyannins showed that they were however absent. GC-MS analysis showed plenty bioactive compounds in which Hexadecanoic acid, methyl ester (45%) and 9-Octadecenoic acid (Z)-, methyl ester (43%) were most prevalent. These findings supports its current use by some locals to treat stomach ulcer and further portrays it as having great potential in the treatment of myriads of diseases.



**Keywords:** Gas chromatography mass spectroscopy, phytochemical analysis, medicinal plants




### INTRODUCTION

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].

Figure 1: Images of *A. grandiflora* trees showing the bark

The plant, *Anthocleista grandiflora* Gilg is commonly known as the "forest fever" tree is a large tree of moist forests in the eastern and south-eastern African tropics and the comores. The flowers are in cymes these are often grouped into thyrses; sometimes umbel-like, scorpioid or reduced to single flower bracts usually small. The flowers are usually bisexualand cream coloured. It is not edible as food but possesses root, stems, bark, leaves and flowers which are claimed to have medicinal properties[6]. Regionally, preparations of the bark has also found use as an anthelmintic specifically for roundworms[7] antidiarrhoeal[8,9] and to treat diabetes, high blood pressure and venereal diseases[9]. Furthermore, in the northern continent, epilepsy is remedied with the aid of the stem bark decoction[10]. In Nigeria, some persons use the wood bark of the plant for the treatment of ulcers, hence the study was conceived with the aim of identifying the

bioactive components in the plant's wood bark by conducting phytochemical analysis of its aqueous extract and further also subjecting it to GC-MS analysis.

## MATERIALS AND METHODS

### Collection and identification of the Plant

Samples of fresh *Anthocleista grandiflora* stem bark were collected in February 2022, at Keana Local Government Area of Nasarawa State, Nigeria. The plant specimen was identified at the Department of Plant Science and Biotechnology Department, Nasarawa State University, Keffi. After due identification, the plant stem bark was sorted to eliminate any dead matter and other unwanted particles and stored for subsequent use. All the chemicals and reagents used were of analytical and GC-grade purity and products of Sigma Aldrich.

### Preparation of the methanol extract of *A. grandiflora* bark

The fresh bark was sliced into tiny pieces and air dried at room temperature for two (2) weeks. The dried bark was milled into fine powder using a mechanical blender. Three hundred grams of the fine powdered plant bark was suspended in 300ml of methanol and the suspension was allowed to extract for 48 hours, the suspension was filtered and concentrated to dryness in a rotary evaporator at 45-50 $^{o}$C.

Qualitative and quantitative Phytochemical analyses of *A. grandiflora* stem bark methanol extract. The preliminary phytochemical screening of the methanol extract of *A. grandiflora* was carried out according to the methods of Harborne[11], Trease and Evans[12] and Sofowara[13.]

### Identification of compounds

In order to identify the various bioactive components, the principle of "retention indices" was adopted to achieve the process and interpretation of mass

spectrum was conducted using the database of National Institute of Standards and Technology (NSIT). The data-base which is universal and consists of over 62,000 different patterns of already identified and known bio active compounds. The spectra of the unknown components of *A. grandiflora* methanol extract samples obtained were compared with the standard existing mass spectra of known components already stored in NIST library (NISTII)/database.

Statistical analysis. The data obtained for quantitative phytochemical analysis were analyzed using descriptive statistics to get the mean and standard deviations.

## RESULTS AND DISCUSSION

### Results

The results obtained in the study were presented in tables and interpreted for easy comprehension.

Phytochemical composition of methanol extract of *A. grandiflora* bark extract

As presented in table 1, the methanol extract of *A. grandiflora* stem bark was found to contain flavonoids in high amount (384.83±13.82 g/100mg), saponins (119.54±1.98 g/100mg) and cardiac glycosides (152.66±9.88 g/100mg) in moderate amount while tannins (7.48±0.49 g/100mg), phenols (31.40±1.80 g/100mg), terpenoids (28.88±0.68 g/100mg) and steroids (47.17±1.27 g/100mg) were present in low amounts. Alkaloids, glycosides, anthroquinones, pholobatannins and anthrocyannins were however absent.

Table 1: Phytochemical composition of methanol extract of *A. grandiflora* bark extract

| Phytochemicals | Qualitative composition | Quantitative Composition (g/100mg) |
|---|---|---|

| | | |
|---|---|---|
| Tannins | + | 7.48±0.49 |
| Saponins | ++ | 119.54±1.98 |
| Flavonoids | ++ + | 384.83±13.82 |
| Alkaloids | - | - |
| Glycosides | - | - |
| Phenols | + | 31.40±1.80 |
| Teroenoids | + | 28.88±0.68 |
| Cardiac glycosides | ++ | 152.66±9.88 |
| Anthroquinones | - | - |
| Steroids | + | 47.17±1.27 |
| Pholobatannins | - | - |
| Anthrocyannins | - | - |

Results are expressed in mean ± SD (n = 3) + = Present in low amount, ++ Present in moderate amount, +++ = Present in high amount, - = Absent

**Constituent compounds from GC-MS analysis of methanol extract of *A. grandiflora* bark extract**

Table 2 is a presentation of the major compounds identified in *A. grandiflora* bark extract through GC-MS analysis. The captured features in the table include CAS, name of the compound, molecular formula, molecular weight and peak area (%). The major compounds identified include 9-Oxabicyclo[6.1.0]nonane, Ledol, 13,16-Octadecadiynoic acid methyl ester, Decanoic acid methyl ester, Dodecanoic acid methyl ester, Spiro[cyclopropane-1,6'-[3]oxatricyclo[3.2.1.0(2,4)]octane] Methyl tetradecanoate, Pentadecanoic acid methyl ester, 9-Hexadecenoic acid, methyl ester, (Z)-, Hexadecanoic acid methyl ester, cis-Vaccenic acid,

Heptadecanoic acid methyl ester, 9,12-Octadecadienoic acid methyl ester, 9-Octadecenoic acid (Z)- methyl ester, Methyl stearate, 9,12-Octadecadienoic acid methyl ester, Methyl 10-trans 12-cis- octadecadienoate, Oleic acid, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester, 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl.

Table 2: GC-MS analysis of methanol extract of *A. grandiflora* bark extract

| S/No. | CAS | Name of the compound | Molecular formula | Molecular weight | Peak area (%) |
|---|---|---|---|---|---|
| 1 | 004925-71-7 | 9-Oxabicyclo[6.1.0]nonane | $C_8H_{14}O$ | 126.20 | 0.18 |
| 2 | 000577-27-5 | Ledol | $C_{15}H_{26}O$ | 222.3663 | 0.1518 |
| 3 | 056846-98-1 | 13,16-Octadecadiynoic acid, methyl ester | $C_{19}H_{30}O_2$ | 290.4 | 0.0846 |
| 4 | 000110-42-9 | Decanoic acid, methyl ester | $C_{11}H_{22}O_2$ | 186.29 | 0.1181 |
| 5 | 000111-82-0 | Dodecanoic acid, methyl ester | $C_{13}H_{26}O$ | 214.3443 | 0.8096 |
| 6 | 107079-37-8 | Spiro[cyclopropane-1,6'-[3]oxatricyclo[3.2.1.0(2,4)]octane] | $C_{10}H_{14}$ | 134.2182 | 0.5616 |
| 7 | 000124-10-7 | Methyl tetradecanoate | $C_{15}H_{30}O$ | 242.3975 | 1.0333 |
| 8 | 007132-64-1 | Pentadecanoic acid, methyl ester | $C_{16}H_{32}O$ | 256.4241 | 0.1438 |
| 9 | 001120-25-8 | 9-Hexadecenoic acid, methyl ester, (Z)- | $C_{16}H_{30}O_2$ | 254.41 | 0.3847 |
| 10 | 000112-39-0 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O$ | 270.4507 | 45.9432 |
| 11 | 000506-17-2 | cis-Vaccenic acid | $C_{18}H_{34}O_2$ | 282.46 | 0.4647 |
| 12 | 001731-92-6 | Heptadecanoic acid, methyl ester | $C_{18}H_{36}O$ | 284.4772 | 0.1642 |
| 13 | 002462-85-3 | 9,12-Octadecadienoic acid, methyl ester | $C_{19}H_{34}O_2$ | 294.5 | 3.0296 |
| 14 | 000112-62-9 | 9-Octadecenoic acid (Z)-, methyl ester | $C_{19}H_{36}O$ | 296.4879 | 43.0856 |
| 15 | 000112-61-8 | Methyl stearate | $C_{19}H_{38}O$ | 298.5038 | 3.0894 |
| 16 | 002462-85-3 | 9,12-Octadecadienoic acid, methyl ester | $C_{19}H_{34}O_2$ | 294.5 | 0.4647 |
| 17 | 1000336-44-2 | Methyl 10-trans,12-cis- octadecadienoate | $C_{19}H_{34}O_2$ | 294.47 | 0.5478 |
| 18 | 000112-80-1 | Oleic Acid | $C_{18}H_{34}O_2$ | 282.47 | 0.1683 |
| 19 | 000112-80-1 | Oleic Acid | $C_{18}H_{34}O_2$ | 282.47 | 0.0997 |
| 20 | 000111-03-5 | 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester | $C_{21}H_{40}O_4$ | 356.5399 | 0.2129 |
| 21 | 004602-84-0 | 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- | $C_{15}H_{26}O$ | 222.37 | -0.7326 |

The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in Figure2. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The GC-MS study of the methanol extract of the bark of *A. grandiflora* had shown the presence of lots of phytochemicals which strength contribute to the medicinal bioactive of that plant.

MULTI-USER SC. RES. LAB. ABUZ Library Search Report

Data Path : D:\MassHunter\GCMS\1\5977\Abdul\

Data File : HASSAN SANI1.D

Acq On : 15 Feb 2022 12:20

Operator : Multi-User Science Research Laboratory

Sample : A.G METHANOL

ALS Vial : 2 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex

Integration Events: ChemStation Integrator - autoint1.e

Phytochemical analysis 2021.M Tue Feb 15 18:07:37 2022.

Figure 2: GC-MS analysis of *A. grandiflora*

**Discussion**

In the current study, the phytochemical composition and GC-MS analysis of the methanol extract of *A. grandiflora* was done because the plant bark is currently been used for the relief and management of stomach ulcer hence the need to explore the bioactive component of the plant bark with the view to providing a scientific back up for its pharmacological potency.

It is scientifically believed that for a plant material to be pharmacologically active, it must possess some bioactive compounds which by working together, may be responsible for some pharmacological activities. The identified compounds may also be used to develop drugs. One way to be able to identify such compounds is by conducting phytochemical analysis on the plant material, further subjecting it to instrumentations such as GC-MS analysis makes it easier by bringing out more features of the present compounds. Many researchers such as (Velmurugan and Anand[14] have used GC-MS method to identify bioactive compounds in plant.

A number of phytochemicals and other compounds were identified in this study which include flavonoids, saponins cardiac glycosides, tannins, phenols, terpenoids and steroids. These bioactive phytoconstituents could be responsible for the therapeutic ability of *A. grandiflora* methanol extract. All these phytochemicals are known to confer one medicinal role or the other. For instance Flavonoids have been reported to possess antioxidant, anticarcinogenic, antimicrobial antitumor, allergenic, anti-inflammatory and antidiarrheal properties [15]. Flavonoids are also essential for the treatment of ulcer[16].

Naturally occurring terpenoids often exhibit a variety of biological activities such as anti-inflammatory, anti-HIV, anti-tumour-promoting, ichthyotoxic and antimycobacterial activities[17]. Reports have confirmed the antiproliferative and apoptotic effects of cardiac glycosides in several cancer cell lines, including breast[18], prostate[19], melanoma[20] pancreatic[21], lung[22], leukaemia[23] and renal adenocarcinoma[24]. Cardiac glycosides have been a cornerstone of the treatment of heart diseases[25]. Phenols have been reported to possess antioxidant[26], antibacterial and antifungal[27]. All of these established findings are most likely to be linked to the biological activities of *A. grandiflora.*

The GC-MS which is one of the well-known best techniques used to identify the constituents of volatile bioactive matter what could be either long chain, branched chain hydrocarbons, alcohols acids, esters and other organic matter was used in this study and the result further proved that the methanol extract of *A. grandiflora* is rich in plenty bioactive compounds which include 9-Oxabicyclo[6.1.0]nonane, Ledol, 13,16-Octadecadiynoic acid methyl ester, Decanoic acid methyl ester, Dodecanoic acid methyl ester, Spiro[cyclopropane-1,6'-[3]oxatricyclo[3.2.1.0(2,4)]octane] Methyl tetradecanoate, Pentadecanoic acid methyl ester, 9-Hexadecenoic acid, methyl ester, (Z)-, Hexadecanoic acid methyl ester, cis-Vaccenic acid, Heptadecanoic acid methyl ester, 9,12-Octadecadienoic acid methyl ester, 9-Octadecenoic acid (Z)- methyl ester, Methyl stearate, 9,12-Octadecadienoic acid methyl ester, Methyl 10-trans 12-cis- octadecadienoate, Oleic acid, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester, 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl, among which Hexadecanoic acid, methyl ester (45 %) and 9-Octadecenoic acid (Z)-, methyl ester (43 %) showed the highest peak. The pharmacological and biological properties of these compounds have previously been elucidated. For example

Hexadecaionic acid, methyl ester is reported as antioxidant, anti-inflamatory and hypochlosterolemic agent [28].

Going by these outcomes, it can be said that *A. grandiflora* bark consists of enormous potential of pharmacological constituents, therapeutic phytocompounds responsible for various pharmacological actions like antimicrobial, antioxidant anti-inflammatory, antidiabetic, analgesic, antiaging, anticancer, hepatoprotective, hypercholesterolemic, antihistaminic, antiandrogenic, antifibrinolytic, diuretic, antiasthma activities, preservative etc. These major chemical compounds identified are considered to play a role in plants defense mechanisms and they may be grouped as protective compounds found in the plant, referred generally as "phytoanticipins" and 'phytoprotectants[29]. Thus, the identification of a various phytochemical compounds from methanol extracts of *A. grandiflora* bark display significant medicinal potential of the plant.

## CONCLUSION

The wood bark of *A. grandiflora* contains many important phytochemical components such as flavonoids, saponins cardiac glycosides, tannins, phenols, terpenoids and steroids. It is true that the presence of these phytochemicals in this plant bark suggests that it has vast pharmacological potentials. The GC-MS analysis of methanol extract showed a number of pharmacologically active components in which Hexadecanoic acid, methyl ester (45 %) and 9-Octadecenoic acid (Z)-, methyl ester (43 %) were the highest. However, further studies are needed to isolate the bioactive components in the plant bark. This study may be useful to explore the pharmacological and medicinal applications of the plant even beyond the current use.

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