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Gas Chromatography-Mass Spectrometry (GC-MS) Analysis and Phytochemical Screening of Polyherbal Aqueous Leaves Extract (PALE)

M. Idu[1], M. O. Aihiokhai[2*], C. A. Imoni[2], C. E. Akokigho[3] and N. C. Olali[3]

[1]Department of Plant Biology and Biotechnology, University of Benin, PMB 1154, Benin City, Nigeria.
[2]Department of Biological Sciences, Faculty of Natural and Applied Sciences, Michael and Cecilia Ibru University, Agbarha-Otor, PMB 100, Ughelli, Delta State, Nigeria.
[3]Department of Biotechnology, Faculty of Natural and Applied Sciences, Michael and Cecilia Ibru University, Agbarha-Otor, PMB 100, Ughelli, Delta State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author MI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MOA and CAI managed the analyses of the study. Authors CEA and NCO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Polyherbal plant extracts which usually comprise of two or more plant parts often contain a wide array of key phytoactive constituents relevant in attaining greater therapeutic efficacy. The active constituents derived from individual plants are insufficient to provide attractive pharmacological action when compared to a combination of multiple herbs.
Objective: To conduct phytochemical screening of polyherbal aqueous leaf extracts (PALE) and analysis of compounds present in it by gas chromatography-mass spectrometry (GC-MS).
Materials and Methods: The polyherbal extract was prepared from the combined aqueous extracts of leaves of *Alchornea cordifolia, Sorghum bicolor* and *Pennisetum glaucum* using ratio

**Corresponding author: E-mail: markaihiokhai@yahoo.com;*

1:1:1 w/v. Phytochemical screening was done via standard analytical methods. The identification and characterization of compounds by GC-MS analysis was performed on gas chromatography system coupled with mass spectrometry.

Results: The phytochemical analysis of PALE revealed the presence of phenols, saponins, flavonoids, alkaloids and tannins in varying quantities. GC-MS analysis of the extract depicts the presence of key bioactive compounds. Thirty-two bioactive compounds were identified with various retention time and % peaks. The major compounds identified in terms of % peak area are n-Hexadecanoic acid (6.72), Hexadecanoic acid, ethyl ester (7.28), 9,12-Octadecadienoic acid (16.54) and 9-Octadecenoic acid ethyl ester (12.92). Disulfide, dimethyl (0.04), 2-Methoxy-4-vinylphenol (0.28), 1-Dodecanol (0.85), 10-Phenyldecanoic acid (0.12), 1-Hexadecanol (0.75), Methoxyacetic acid, pentadecyl ester (0.27), 9-Octadecenoic acid (Z)-, phenylmethyl ester (0.16), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) (1.09), were among the minor compounds identified in the extract. From the study, 9,12-Octadecadienoic acid indicated the highest peak with a retention time of 20.556 minutes and % peak area of 16.54%.

Conclusion: The presence of the revealed bioactive constituents in PALE may suggest its nutraceutical, pharmacological and therapeutic relevance. Therefore, in view of the medicinal importance associated with the observed bioactive constituents, further studies on the toxicity level of the extract is advised subsequently.

Keywords: Polyherbal aqueous leaves extracts (PALE); phytochemical screening; gas chromatography; mass spectrometry.

1. INTRODUCTION

The role of medicinal plants and their parts in medicine is immeasurable. At the moment, most of the present day medicines have plant origin as it has been implicated that modern medicine has evolved from folk medicine and traditional systems on the basis of pharmaceutical and chemical screenings carried out [1]. The knowledge of the active principle and medicinal compounds in plants is of immense importance, though being continuously updated through various biochemical evaluations [2]. There are a number of methods such as Thin Layer Chromatography, GC-MS, HPLC, HPTLC, NMR, XRD, FTIR etc. which are used in analysing various biochemical and chemical parameters of plants. In Nigeria, the use of herbs in traditional medicine is widely accepted as a significant instrument of health care. The interest in medicinal plants is primarily due to the inaccessibility of pharmaceutical drugs by poor people due to high cost. Most people in rural areas utilize these plants to bring about cure and relief from disease conditions with little or no knowledge about the safety and toxicity of such plants [3]. However, literature has documented several results showing toxicity resulting from the use of herbs on many occasions [4,5]. Herbal drugs are widely prescribed, even when their biological components are not known, as a result of their effectiveness, fewer side effects and relatively low cost [6].

By using herbal combinations, nature provides a balance of ingredients that may act as buffers, synergists or counterbalances, which work in harmony to rid the body of diseases and infirmities [7]. Some polyherbal extracts have been scientifically proven to be efficacious in the treatment of diseases, while many others are yet to be investigated [8]. The present work was undertaken to conduct phytochemical screening and GC-MS analysis of compounds present in the polyherbal aqueous leaf extracts (PALE) derived from *Alchornea cordifolia, Pennisetum glaucum* and *Sorghum bicolor* since not many reports are there on these plants combination.

Alchornea cordifolia (Schum and Thonn) Muell. Arg. commonly known as Christmas bush belongs to the family Euphorbiaceae. *A. cordifolia* is a plant widely used in Africa alone or in association with other plants to solve many health problems [2]. The leaves are simple and alternate, and are heart shaped at the base with long petiole. The inflorescence consists of auxiliary panicles and the flowers are greenish white [2]. *A. cordifolia* grows very well in tropical environment and it is available in Nigeria all year round without irrigation. *A. cordifolia* possesses the ability to provide large quantities of high quality forage matter all-year-round as well as the ability to maintain a sustainable environment through foliage droppings thus replenishing the soil. It has been reported that vegetables contain vitamins, essential amino acids, minerals,

antioxidants and proteins [9]. However, *A. cordifolia* leaves constitute a good source of several alkaloids, antioxidants, antitumor and antibacterial compounds [10].

Pennisetum glaucum (L) R. Br. are small-seeded grains with different varieties belonging to the Poaceae family. Pearl millet as it is commonly known is one of the most drought-resistant grains grown in over 40 countries predominantly in Africa and Asia as a staple food grain and source of feed and fodder, fuel and construction material [11]. The plant has been implicated to have high antioxidant properties and also commonly used as food for human consumption [12].

Sorghum bicolor (L) Moench (Poaceae family) commonly called sorghum, great millet or guinea corn is mostly grown in northern Nigeria for food, forage, syrup and sugar. The leaves are broad and coarse, similar in shape to those of corn, but shorter and wider, while the seeds are white, red or brown [13]. However, in herbal medicine, *S. bicolor* has been reported for its antiabortive, anticarcinogenic, stomachic, diuretic activities and a remedy for epilepsy [14]. Often, people drink a decoction of the leaves for measles [15]. Although, the metabolites produced by the plants to protect themselves against biotic and abiotic stresses have turned into medicines that people can use to treat various diseases [16].

Pharmacological studies of various plant parts of *S. bicolor, P. glaucum* and *A. cordifolia* have shown that they contain inherent active ingredients used to cure diseases and relieve pains [17]. PALE as a formulation is claimed to have antianemic property which is presently under investigation, however, this study was conducted to, identify the core active constituents present in it by phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analytical methods.

2. MATERIALS AND METHODS

2.1 Collection of Polyherbal Plant Materials

The polyherbal plant material comprising *Alchornea cordifolia, Sorghum bicolor, Pennisetum glaucum* were collected from the Local Government Area of Ikpoba Okha, Benin City, Edo State, Nigeria. They were identified and authenticated by a Botanist in the Herbarium Unit of Plant Biology and Biotechnology Department, Faculty of Life Sciences, University of Benin, Edo State, Nigeria.

2.2 Preparation of Polyherbal Aqueous Leaves Extracts

The leaves were rinsed and shade dried separately for 21 days and oven dried at 40^{o}c for 24 hours. The dried leaves were pulverized into powder using the British milling machine. The weights of the powdered leaves were separately taken using ratio 1:1:1 across the three samples and the extraction process commenced such that pulverized leaves weighing 4000 g each were extracted using maceration technique in aqueous solvent. Thereafter, the extract was stored in a sterile container and kept in a refrigerator at 4^{o}C. The extract is henceforth termed PALE (Polyherbal Aqueous Leaf Extract).

2.3 Phytochemical Analysis

The qualitative and quantitative phytochemical screening of PALE was carried out using standard methods [18,19]. The extract was screened for the presence of specific amount of alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins and glycosides constituents. All determinations were done in triplicates.

2.4 Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The phytocomponents of PALE was identified using gas chromatography-mass spectroscopy (GC-MS) detection system. The GC-MS analysis was accompanied using an Agilent 19091s GC system. The capillary column used was 933HP-1MS (30 x 250 µm; film thickness of 0.25µm; J & W Scientific, USA). The temperature program was set at as follows: initial temperature 60^{o}C held for 1.5297 min, 30^{o}C /min to 150^{o}C for 5 min, 30^{o}C /min to 250^{o}C held for 8 min. The total run time was 21.333 min, while the flow rate of Helium as a carrier gas was 0.79653mL/min. The MS system was performed in electron ionization (EI) mode with Selected Ion Monitoring (SIM). The ion source temperature and quadruple temperature were set at 230^{o}C and 150^{o}C respectively.

Identification and interpretation of compounds on Mass-Spectrum GCMS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000

patterns. The spectrum of individual unknown compounds was compared with the spectrum of known components stored in the NIST library. The name, molecular formula, weight and chemical structure of the components of the test materials were ascertained.

3. RESULTS AND DISCUSSION

The qualitative and quantitative phytochemical screening of PALE revealed the presence of secondary metabolites such as alkaloids, flavonoids, cardiac glycosides, saponins, steroids, terpenoids, phenols and tannins in varying quantities (Tables 1 and 2). Significantly high phenolic (1445.00 ± 1.17 mg/100g), flavonoid (917.00 ± 0.93 mg/100g) and tannin (267.00 ± 0.01) contents were detected, while alkaloid (6.00 ± 0.00 mg/100g) and saponins (7.00 ± 1.00 mg/100g) contents were considerably low (Table 2). Plants with high phenolic compounds are essential for growth, reproduction, protecting and preventing agents against pathogens and chronic illnesses such as cardiovascular disease, certain type of cancers, neurodegenerative diseases and diabetes [20]. Also, phenolic compounds have been implicated as antioxidant agents which act as free radical terminators. Flavonoids on the other hand containing hydroxyls, are very relevant in free radical scavenging effects of most plants and also antihyperglycaemic activity [21]. However, the presence of these phytochemicals in PALE is a significant finding in this present study.

GC-MS analysis of PALE revealed the presence of thirty-two compounds with specific retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (% peak area) as shown in Table 3. The GC-MS chromatogram of PALE showing distinct peaks of identified compounds is depicted in Fig. 1. In terms of % peak area, n-Hexadecanoic acid (6.72), Hexadecanoic acid, ethyl ester (7.28), 9,12-Octadecadienoic acid (16.54), 9-Octadecenoic acid ethyl ester (12.92), Octadecanoic acid, ethyl ester (6.70) were found to be the major compounds, whereas Disulfide, dimethyl (0.04), 2-Methoxy-4-vinylphenol (0.28), 1-Dodecanol (0.85), 10-Phenyldecanoic acid (0.12), 1-Hexadecanol (0.75), Methoxyacetic acid, pentadecyl ester (0.27), 9-Octadecenoic acid (Z)-, phenylmethyl ester (0.16), 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) (3.57), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) (1.09), 15-Hydroxypentadecanoic acid (1.32), 8,11,14-Eicosatrienoic acid (2.61), 8,11,14-Eicosatrienoic acid (0.51) are among the minor compounds. From the study, 9,12-Octadecadienoic acid indicated the highest peak value in the extract with RT: 20.556 mins and % peak area: 16.54 % (Table 3). However, the compounds pertaining to the peaks were identified by comparing the NIST library data of the peaks and mass spectra of the peaks with those reported in literature. This study is the first of its kind to analyse the chemical constituents of PALE using GC-MS.

Table 1. Qualitative phytochemicals screening of polyherbal aqueous extracts

Phytochemicals	Polyherbal aqueous extracts
Flavonoids	+
Saponins	+
Phenols	+
Alkaloids	+
Tannins	+

Key: + = present, - = not detected

Table 2. Quantitative phytochemicals screening of polyherbal aqueous leaves extract

Phytochemicals	Concentrations (mg/100 g)
Alkaloids	6.00±0.00
Saponins	7.00±1.00
Tannins	267.00±0.01
Flavonoids	917.00±0.93
Phenol	1445.00±1.17

Data presented as Mean ± SD; n = 3

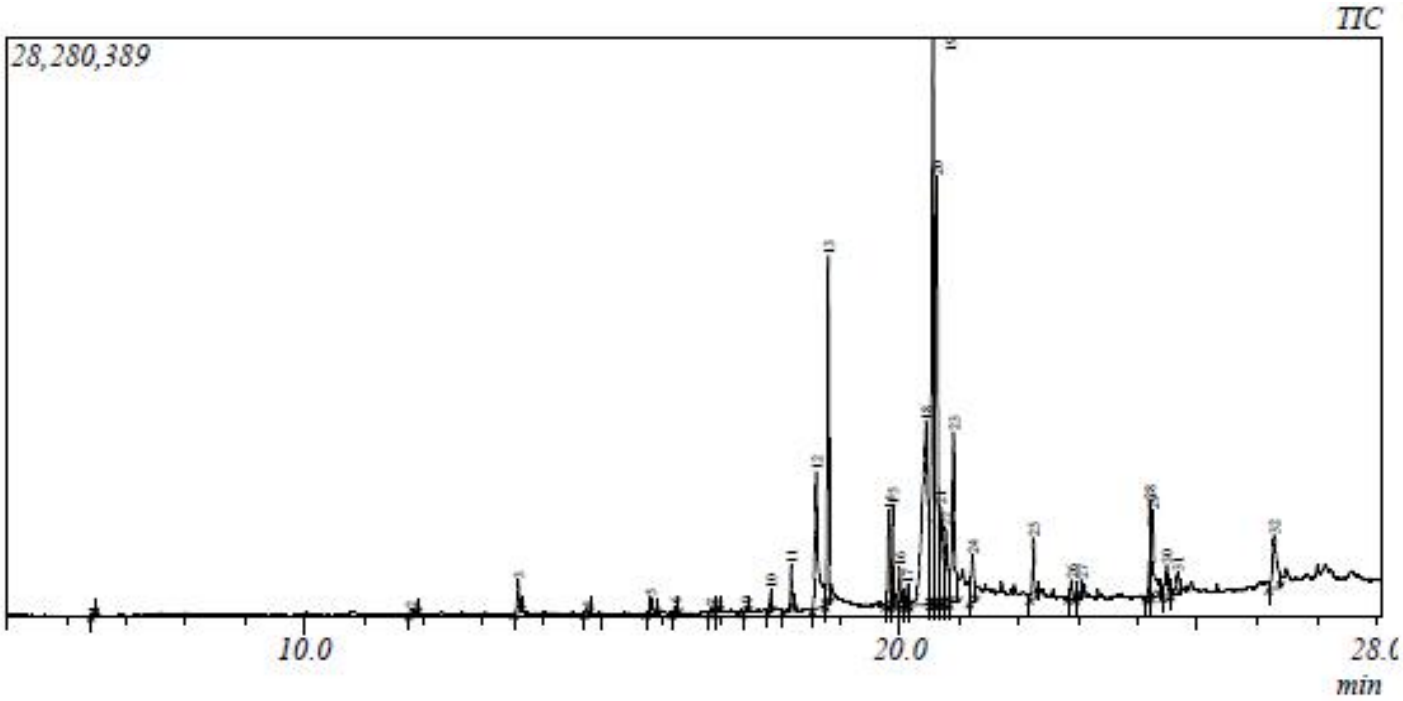
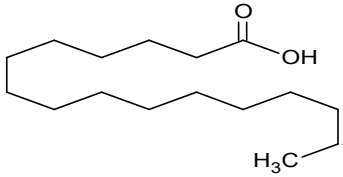
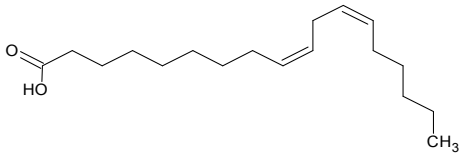
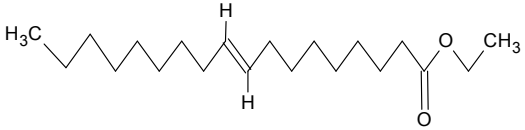


Fig. 1. GC-MS chromatogram of PALE indicating different peaks

Table 3. Phytoconstituents identified in PALE by GC-MS study

S. No.	Retention time (Min)	Phytoconstituents	Molecular formula	Molecular weight	%Peak area
1.	6.445	Disulfide, dimethyl	$C_2H_6S_2$	94	0.04
2.	11.796	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.28
3.	13.592	1-Dodecanol	$C_{12}H_{26}O$	186	0.85
4.	14.733	10-Phenyldecanoic acid	$C_{16}H_{24}O_2$	248	0.12
5.	15.811	1-Hexadecanol	$C_{16}H_{34}O$	242	0.75
6.	16.225	Methoxyacetic acid, pentadecyl ester	$C_{18}H_{36}O_3$	300	0.27
7.	16.842	9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	372	0.16
8.	16.926	Ethyl 14-methyl-hexadecanoate	$C_{19}H_{38}O_2$	298	0.10
9.	17.418	Cyclopentadecanone	$C_{15}H_{28}O$	224	0.19
10.	17.833	8-Phenyloctanoic acid	$C_{14}H_{20}O_2$	220	0.47
11.	18.178	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.92
12.	18.598	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.72
13.	18.797	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	7.28
14	19.815	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	2.16
15.	19.875	9-Octadecenoic acid (Z)-, methyl ester,	$C_{19}H_{36}O_2$	296	2.31
16.	19.996	Phytol	$C_{20}H_{40}O$	296	1.07
17.	20.132	Cyclopentanetridecanoic acid, methyl ester	$C_{19}H_{36}O_2$	296	0.51
18.	20.442	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	15.17
19.	20.556	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	16.54
20.	20.621	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	12.92
21.	20.682	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	4.19
22.	20.772	6,9,12-Octadecatrien-1-ol	$C_{18}H_{32}O$	264	3.56
23.	20.900	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	6.70
24.	21.224	Phenacyl 11-octadecenoate	$C_{26}H_{40}O_3$	400	2.11
25.	22.243	15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258	1.88
26.	22.884	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	0.51
27.	23.051	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.52
28.	24.202	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	2.61
29.	24.247	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	3.10
30.	24.486	15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258	1.32
31.	24.662	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)	$C_{19}H_{38}O_4$	330	1.09
32.	26.292	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)	$C_{21}H_{40}O_4$	356	3.57

Table 4. Important peaks of GCMS found in PALE

Peak No	Name	Structure	Biological activities
12	n-Hexadecanoic acid		Haemolytic anaemia, antioxidant
13	Hexadecanoic acid, ethyl ester		Antioxidant, anaemia, hypocholesterol
18-19	9,12-Octadecadienoic acid (Z,Z)-		Anti-inflammatory, antiarthritic,hepatoprotective, anticoronary, anti-histamic
20	E)-9-Octadecenoic acid ethyl ester		Steroids, fertility
23	Octadecanoic acid, ethyl ester		Antifungi, antiturmor, antibacteria/

4. CONCLUSION

This study has revealed that the polyherbal aqueous leaves extract possess metabolites that suggests its therapeutic properties. Therefore, ethanol is recommended for the extraction of the plant materials for subsequent study and evaluation. GCMS analysis of PALE revealed the presence of medicinally valued bioactive compounds such as Hexadecanoic acid which is used as an antioxidant and in the treatment anaemia as well as Octadecanoic acid implicated for antifungal and antitumor properties. In view of all the medicinal importance associated with the phytocompounds found in the extract, further investigation should be carried out in order to isolate, identify, characterize and elucidate the structures of these bioactive principles and enhance their potentials for industrial and pharmaceutical utilization. Also, the toxicity level and biological activity of the extract can as well be determined subsequently especially in the event of consideration for drug formulation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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