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Phytochemical Evaluation, GC-MS Profiling and Antimicrobial Activity of Two Herbal Mixtures Marketed in Anambra State

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The pharmacological properties of herbal medicinal products have been attributed to different phytocompounds present in plants. Phytocompounds have constituted an alternative source of antimicrobial agents. This study was designed to investigate the bioactive constituents of two herbal mixtures marketed in Anambra State used in the treatment of Staphylococcus infection and typhoid and malaria. Qualitative phytochemical screening was carried out on the two herbal mixtures following documented procedures. Antimicrobial activity was assessed using modified disc diffusion method. The chemical constituents of the herbal drugs were assessed using gas chromatography-

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mass spectrophotometry (GCMS) and the mass spectra of the identified compounds compared with those of the National Institute of Standards and Technology database library. The preliminary phytochemical screening revealed the presence of alkaloids, phenols, saponins, tannins, steroids, glycosides, anthraquinones and absence of flavonoids in Sample A and presence of alkaloids, saponins, tannins, steroids, glycosides, anthraquinones and absence of phenols in Sample B. The GC-MS analysis revealed the following major bioactive compounds: 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (59.41%), Oleic Acid (7.98%), 1-Cyclohexylnonene (6.49%), 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (5.31%), cis-9-Octadecenoic acid, propyl ester (2.62%), 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (1.83%) and others at different concentration ranging from 0.02-1.77% in sample A; Acetamide, N-(aminocarbonyl) (31.14%), n-Hexadecanoic acid (15.33%), Tetradecanoic acid (11.97%), Hexadecanoic acid, ethyl ester (11.58%), Hexadecanoic acid, propyl ester (10.55%) and others at different concentrations ranging from 0.07-6.11% in sample B. The antimicrobial activity test showed that herbal mixture A inhibited the growth of four out of six clinical isolates used with mean inhibition range of 12.15±0.49 to 18.50±0.51 while herbal mixture B inhibited the growth of three out of six clinical isolates used with mean inhibition range of 12.55±0.35 to 18.80±0.28mm. The herbal mixtures contain diverse bioactive compounds and the presence of these multifunctional bioactive compounds in the two herbal mixtures explains the various pharmacological activities of these herbal drugs.

Keywords: Phytocompounds; GC-MS profiling; multifunctional; pharmacological properties; staphylococcus infection.

1. INTRODUCTION

Plants are rich source of bioactive phytochemicals [1]. Phytochemical comes from the Greek word "phyto" for plant [1]. It refers to every naturally occurring chemical compound present in plants [1]. These chemical compounds are also known as secondary metabolites and they include; alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, glycosides, tannins, resins, volatile oils etc [2]. There are more than a thousand known phytochemicals [3]. They accumulate in different parts of the plants such as the roots, stems, leaves, flowers, fruits or seeds [2]. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases [3]. Diversity of secondary metabolites isolated from different plants have been proven to exhibit anticancer, antibacterial, analgesic, antifungal, antioxidant, anti-inflammatory, antitumor, antiviral and other biological activities [2]. Tannins, alkaloids, saponins, flavonoids and Phenols have been reported to exhibit antibacterial activity against Salmonella Typhi [4]. Several studies have also shown that alkaloids, flavonoids and terpenoids have antiplasmodial activity [5]. These biological activities suggest why plants and herbs have been part of healthcare of many countries and constitute alternative sources of therapeutic substances [6]. It is estimated that approximately one quarter drug contains plant extract or active ingredients obtained from plant substances [1]. Many drugs used in conventional medicine were also originally derived from plants. Salicylic acid is a precursor of aspirin that was originally derived from white willow bark and the meadowsweet plant (*Filipendula ulmaria* (L.) Maxim.) [7]. Quinine and Artemesinin are antimalarial drugs derived from *Cinchona pubescens* Vahl bark and *Artemisia annua* L. plant, respectively [6,7]. Morphine and codeine derived from the opium poppy (*Papaver somniferum* L.), are used in the treatment of diarrhea and pain relief [7]. In the recent years, medicinal plants are gaining increasing popularity globally as drugs, complementary and alternative medicines, food supplements, cosmetics [8]. Herbal drugs are increasingly used worldwide during the last few decades as seen in the rapidly growing global and national markets of herbal drugs [6]. The workhorse of contemporary plant metabolite profiling is no doubt gas chromatography coupled to mass spectrometry (GC-MS) [9]. Therefore, in this study the preliminary phytochemical screening, GCMS analyses and antimicrobial activity of two herbal mixtures was carried out to profile the chemical constituents in them that are responsible for their antimicrobial activity and other medicinal properties.

2. MATERIALS AND METHODS

2.1 Sample Collection

The two liquid popular herbal mixtures were obtained from the manufacturer in Anambra

State. They were selected on the strength of their popularity, affordability and claim of efficacy. The samples were labeled A and B as indicated in Table 1.

2.2 Phytochemical Analysis

All the herbal mixtures were screened qualitatively for the presence of phytochemicals using the standard methods as described by Abubakar and Mainul, [10], Akande et al., [11] Pandey and Triphati [12], Sorescu et al., [13].

2.2.1 Test for tannins

2 ml of each sample was taken and placed in a test tube and a few drops of 5% ferric chloride added. Formation of blue-black coloration indicated presence of tannins.

2.2.2 Test for phenols

5ml of each test sample was added 3ml of 10% lead acetate solution. A bulky white precipitate indicated presence of phenolic compounds.

2.2.3 Test for alkaloids

1ml of Wagner's reagent was added to 1 ml of each sample in a test tube and allowed to stand for some time. The development of reddish-brown precipitate indicated the presence of alkaloids.

2.2.4 Test for flavonoids

5 ml of 10% ammonium hydroxide solution was added to 2ml of each sample in a test tube. An intense yellow coloration was observed in the samples but on addition of the dilute acid became colorless indicating the presence of flavonoids.

2.2.5 Test for glycosides

2mL of each sample was taken and diluted with equal volume of water. Then 0.5mL of lead acetate was added, shaken, and filtered. Again, the mixture was extracted with equal volume of chloroform, evaporated, and dissolved the residue in glacial acetic acid. Then few drops of ferric chloride were added. Again, the whole mixture was placed into a test tube containing 2mL of sulfuric acid. Emergence of reddish brown layer that turned bluish green indicated the presence of glycosides.

2.2.6 Test for steroids

2ml of each sample was taken and added in 2ml of chloroform, 3 drops of concentrated sulphuric acid carefully added to form a lower layer. Formation of reddish-brown color at the interface will indicate the presence of steroids.

2.2.7 Test for saponins

5mL of distilled water was mixed with 10ml of each sample and shaken vigorously. Formation of froth which persisted for 15minutes indicated the presence of saponins.

2.2.8 Test for anthraquinones

1 ml of each test sample was taken and few drops of 10% ammonia solution added. Formation of a pink precipitate indicated the presence of anthraquinones.

Table 1. Herbal mixtures and their indications

Product Code	Product Name	Form	Compositions	Indications	NAFDAC Reg.no
A	Alokings S.T.D mixture	Liquid	*Picnizinthus zingolane, Zinigiber officinale, Allium sativum, Aloe barbadensis, Carica papaya,* Afafara	*Staphylococcus aureus* infection	Absent
B	Alokings Quit notice	Liquid	*Zinigiber officinale, Allium sativum,* Vitamin B6, C, E, iron, zinc, calcium*, Carica papaya,* Afafara	Malaria and Typhoid	Absent

2.3 Gas Chromatography Mass Spectrometry Analysis

GCMS analysis was carried out using Agilent Mass Hunter Model 5977 and Agilent 19091S-433µl HP-5MS Ultra Inert: 30m x 250µm x 0.25µm analytical column. Helium was used as the carrier gas at a flow rate of 1.0ml/min. 1.0µl injection volume, Injector temperature was 250^0C, Ion source temperature was 230^0C. Interface temperature was 250^0C. Oven temperature was 50^0C held for 2mins with an increase of 5^0c/min to 180^0C ending with 270^0C (20^0C/min). Mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70ev as acquisition mass range from 45-700 a.m.u. Further identification was made by comparison of their mass spectra with spectrum of known compounds stored in the library.

2.4 Microbial Assay

2.4.1 Collection of test organisms

Five bacteria namely *Salmonella enterica, Escherichia coli, Staphylococcus aureus, klebsiella pneumoniae,* and *Pseudomonas aeruginosa* and one fungus *Candida albicans* were obtained from the Reference Laboratory Section of Conig-Simonne Laboratories, Awka, Anambra State, Nigeria. The organisms were further identified and confirmed using standard protocols for cultural and morphological identification, as well as biochemical characterization of isolates. The organisms were maintained on nutrient broth for bacteria and sabouraud dextrose broth for yeast (*Candida*) at 37^oC for 24 hours.

2.4.2 Preparation of media

All the media used for the research which were Nutrient agar, Sabouraud dextrose, Nutrient broth, Muller Hinton agar etc. were prepared according to the manufacturer's instructions.

2.4.3 Preparation of paper discs

Whatmann filter paper (6mm in diameter) was sterilized at 180^0C for one hour.

2.4.4 Standardization of Inoculum

The test organisms were standardized by using a sterile wire loop to pick 3–5 pure cultures of the test microorganism and emulsified in 3–4 ml of sterile physiological saline. The turbidity reading of the 0.5 McFarland Standard was recorded as absorbance in a Spectrophotometer at 625 nm and the turbidities of the test inoculums were adjusted to match the absorbance of the 0.5 McFarland standard at the same wavelength, using physiological saline.

2.4.5 Antimicrobial susceptibility test

The antibacterial activities of the herbal samples against the test bacteria were evaluated by modified disc diffusion method as described by Agbo and Mboto [1]. Exactly 0.10ml of 0.5 McFarland standardized suspension of test inoculums were cultured onto the Mueller-Hinton agar plates by pour plate method. It was swirled and allowed to diffuse. After solidification, 0.05ml of the prepared samples were used to impregnate the 6mm filter paper discs and placed on two portions of the agar plate using a sterile forceps. The commercially prepared antibiotic discs were also aseptically placed on the surface of seeded agar plates 24mm apart. The plates were allowed to stand for five minutes for effective diffusion before incubation at 37^oC for 24 hours. After incubation, the inhibition zone diameters of the various plates were measured and recorded in millimeters. All experiments were done in triplicates. Positive controls were set up using 50 µg/ml Ciprofloxacin for bacteria and Fluconazole for fungi. Negative controls were set up with sterile physiological saline.

3. RESULTS

3.1 Phytochemical Results

The preliminary phytochemical analysis result is shown in Table 2. It revealed the presence of alkaloids, phenols, saponins, tannins, steroids, glycosides, anthraquinones and absence of flavonoids in Sample A and presence of alkaloids, saponins, tannins, steroids, glycosides, anthraquinones and absence of phenols in Sample B.

3.2 Antimicrobial Susceptibility Results

The result of the antimicrobial activities of the two herbal samples is shown in Table 3. The result shows that zones of inhibition range from 12.15 to 18.80mm. Sample A had inhibition zone of 18.50mm against *S. aureus* and sample B had inhibition zone of 18.80mm against *S. enterica.*

Table 2. Qualitative phytochemical result

Sample	Alkaloids	Phenols	Saponins	Flavonoids	Steroids	Tannins	Glycosides	Anthraqu-inones
A	+	+	+	-	+	+	+	+
B	+	-	+	+	+	+	+	+

Table 3. Mean inhibition zones (mm)

Sample	*S. enterica*	*E. coli*	*K. pneumoniae*	*S. aureus*	*P. aeruginosa*	*C. albicans*
A	0.00±0.00	15.25±0.25	0.00±0.00	18.50±0.51	13.70±0.42	12.15±0.49
B	18.80±0.28	13.80±0.28	0.00±0.00	0.00±0.00	0.00±0.00	12.55±0.35
Ciprofloxacin	23.5	16.3	21.3	25.3	19	-
Fluconazole	-	-	-	-	-	26

Table 4. Compounds identified in sample A by GC-MS

Peak	Retention Time	Area%	Compounds	Molecular Formular	Molecular Weight	Pubchem CID
1	33.228	59.41	9-Octadecenoic acid (Z)-,2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.54	5283468
2	30.351	7.98	Oleic Acid	$C_{13}H_{34}O_2$	282.5	445639
3	35.023	6.49	1-Cyclohexylnonene	$C_{15}H_{28}$	208.38	5364533
4	33.676	5.31	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222.37	445070
5	34.827	2.62	cis-9-Octadecenoic acid, propyl ester	$C_{21}H_{40}O_2$	324.5	5356106
6	34.984	1.83	9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356.5	5319879
7	34.715	1.77	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{38}ClO$	298.89	9817754
8	32.863	1.71	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	$C_{16}H_{28}O_2$	252.39	110680
9	30.393	1.64	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5	5282761
10	33.134	1.48	9-Octadecenoic acid (Z)-2-hydroxyethyl ester	$C_{20}H_{38}O_3$	326.5	5364420
11	32.993	1.20	cis-11-Hexadecenal	$C_{16}H_{30}O$	238.41	5364495
12	33.308	1.18	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.5	5364412
13	33.054	1.17	Cyclopropaneoctanal,2-octyl-	$C_{19}H_{36}O$	280.5	550143
14	28.708	1.04	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	985
15	33.172	0.86	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268.39	5363633

Peak	Retention Time	Area%	Compounds	Molecular Formular	Molecular Weight	Pubchem CID
16	32.593	0.53	Propanamide, N-acetyl-	$C_5H_9NO_2$	115.13	536086
17	29.474	0.46	Isopropyl palmitate	$C_{19}H_{38}O_2$	298.5	8907
18	19.444	0.44	(3-Methyl-oxiran-2-yl)-methanol	$C_4H_8O_2$	88.11	234485
19	19.001	0.43	N-Methoxymethyl-N-methylformamide	$C_4H_9NO_2$	103.12	560292
20	28.299	0.40	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5	12366
21	30.118	0.37	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	965
22	30.794	0.36	n-Propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324.5	87131822
23	32.424	0.28	7,11-Hexadecadienal	$C_{16}H_{28}O$	236.39	543335
24	18.921	0.20	8-Azabicyclo[4.3.1]decan-10-one,8-methyl-	$C_{10}H_{17}NO$	167.25	541485
25	19.548	0.18	MDMAmethylene homolog	$C_{12}H_{17}NO_2$	207.27	82672270
26	20.086	0.15	Diallylmethylsilane	$C_7H_{14}Si$	126.27	249905732
27	19.624	0.13	dl-Allo-cystathionine	$C_7H_{14}N_2O_4S$	222.26	834
28	19.994	0.13	1-Octanamine	$C_8H_{19}N$	129.24	8143
29	20.206	0.10	Methyl7,9-tridecadienyl ether	$C_{14}H_{26}O$	210	5364214
30	19.585	0.09	N-Methoxymethyl-N-methylacetamide	$C_5H_{11}NO_2$	117.15	542250
31	19.766	0.06	Piperidine,3-(bromomethyl)-	$C_6H_{12}BrN$	178.07	21666817
32	20.155	0.04	Propane,1-(1-methylethoxy)-	$C_6H_{14}O$	102.17	12304
33	18.974	0.02	2-Propanol, 1-propoxy-	$C_6H_{14}O_2$	118.17	15286

Table 5. Compounds identified in sample B by GC-MS

Peak	Retention Time	Area%	Compounds	Molecular Formular	Molecular Weight	Pubchem CID
1	6.070	31.14	Acetamide,N-(aminocarbonyl)-	$C_3H_6N_2O_2$	102.09	68956
2	29.063	11.97	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	11005
3	28.606	11.58	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5	12366
4	29.160	15.33	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	985
5	29.680	10.55	Hexadecanoic acid, propyl ester	$C_{19}H_{38}O_2$	298.5	75232
6	5.699	6.11	3,7-Diacetamido-7H-s-triazolo[5,1-c]-s-triazole	$C_7H_9N_7O_2$	223.19	536650
7	27.982	3.38	1,3-Adamantanediacetamide	$C_{14}H_{22}N_2O_2$	250.34	541702
8	30.523	3.23	Oleic Acid	$C_{18}H_{34}O_2$	282.5	445639
9	30.295	2.72	n-Propyl 9-octadecenoate	$C_{21}H_{40}O_2$	324.5	5356106
10	26.686	1.53	Propanenitrile,3-amino-2,3-di(hydroxymino)-	$C_3H_4N_4O_2$	128.09	13625968
11	27.227	1.35	Terodiline	$C_{20}H_{27}N$	281.4	23480
12	30.948	0.43	n-Propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324.5	87131822
13	25.911	0.23	Propanamide,3-(3,4-dimethylphenylsulfonyl)-	$C_{11}H_{15}NO_3S$	241.31	541768
14	27.291	0.19	Benzenemethanol.alpha-[(methylamino)methyl]-	$C_{10}H_{15}NO$	165.23	913
15	24.830	0.19	Arginine	$C_6H_{14}N_4O_2$	174.20	6322
16	35.831	0.17	Z-8-Pentadecen-1-ol acetate	$C_{17}H_{32}O_2$	268.4	5363212
17	33.129	0.07	7,11-Hexadecadienal	$C_{16}H_{28}O$	236.39	543335

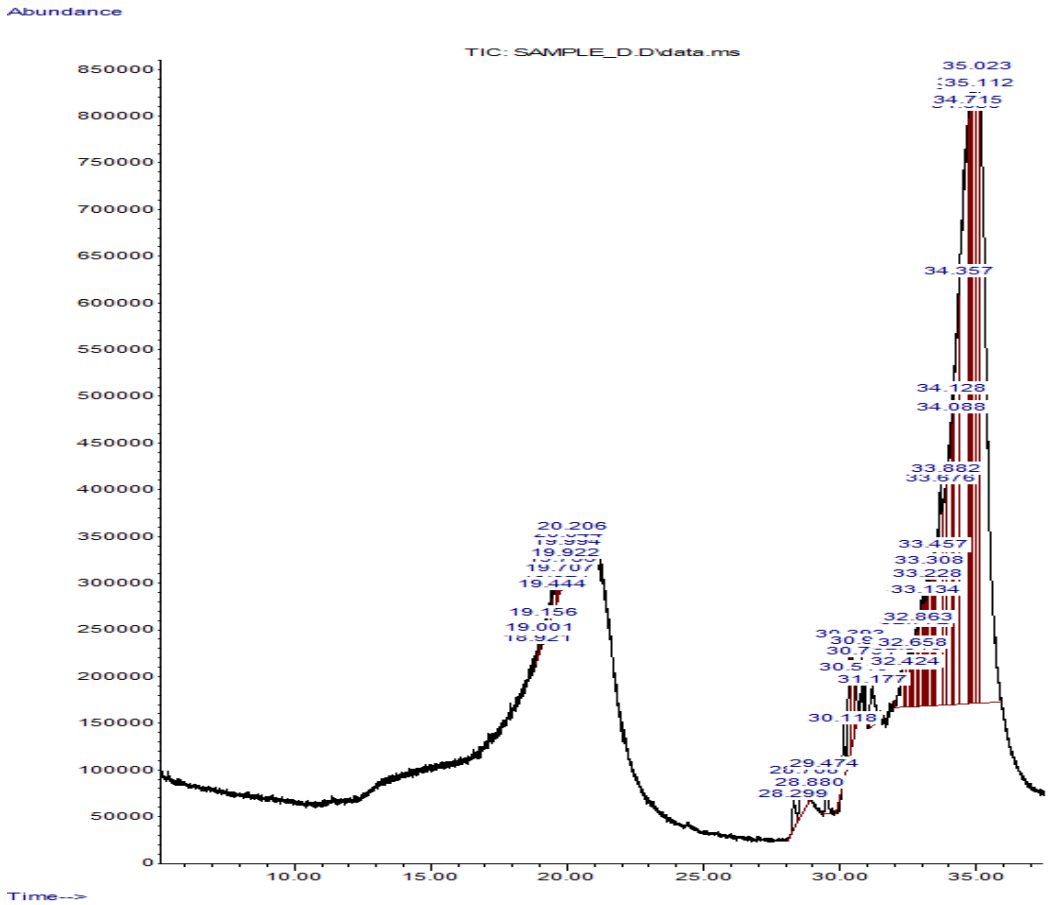

Fig. 1. GC-MS chromatogram of sample A

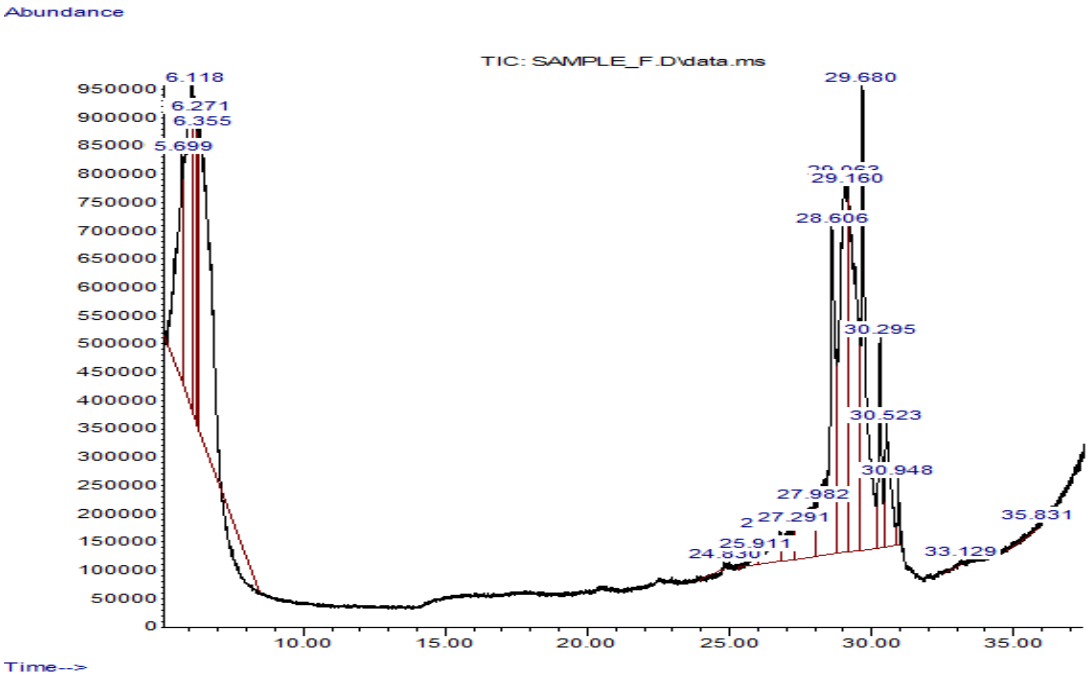

Fig. 2. GC-MS chromatogram of sample B

3.3 GC-MS Analysis Results

The GC-MS chromatograms of sample A and B are shown in Figs. 1 and 2 and the different bioactive compounds identified summarized in Tables 4 and 5.

4. DISUSSSION

The result of the phytochemical analysis which indicated the presence of alkaloids, saponins, tannins, steroids, glycosides, anthraquinones in both samples, is related to that of Shu et al*.* [14] who reported the presence of tannins, flavonoids, saponins, alkaloids, glycosides, terpenoids, steroids and absence of resins in ten herbal medicines marketed in Enugu and contrary to that Abdullahi et al*.* [15] who indicated the presence of alkaloids, cardiac glycosides, saponins, flavonoids, tannins and absence of anthraquinones in herbal concoctions marketed in Kaduna. Alkaloids are known to exhibit the following activities; antimicrobial, antidiarrheal, anti-inflammatory, analgesic, anticancer, antioxidant, antiasthma, antimalarial, antidepressant, anaesthetic, antidiabetic [9,16] and cardioprotective [17]. Phenols are antimicrobial, anti-inflammatory, antioxidant [9,18], disinfecticides, anticonvulsants, analgesic [19]. Tannins are known for their antimicrobial, antioxidant, antiparasitic, anti-inflammatory and immunomodulation [20], anti-diarrheal [21] antidiabetic activities [22]. Flavonoids are known for their antioxidant, anti-inflammatory, antimicrobial, anticancer [23], antitumor, blood cholesterol lowering [9], anti-allergic, cardioprotective [23], antidiabetic activities [24]. Saponins are also known to be fungicidal, antimicrobial, antioxidant, anticancer, immune boosters, anti-cancer, anti-inflammatory [9,25] cholesterol lowering [9]. Steroids are known as anti-inflammatory, antitumour, anticancer agents [26]. Anthraquinones are known as anticancer, anti-inflammatory, antimalarial, diuretic, antioxidant, laxatives, antibacterial, antifungal [27,28]. Glycosides are known for their cardiovascular. antibacterial, anticancer, antinflammatory [29], analgesic [30]. Zuo and Fang [31] reported that glycosides exhibited activity against *S.aureus*. Rhiham and Elshimy [32] showed that glycosides exhibited antibacterial activity against *Salmonella enterica*. Moreover, Musa et al*.,* [4] noted that tannins, alkaloids, saponins, flavonoids and phenols exhibit antibacterial activity against *Salmonella Typhi* and Taek et al*.,* [5] reported that alkaloids and flavonoids have antiplasmodial activity. The presence of these phytochemicals must have contributed to the efficacy of Sample A and B. Most of the phytocompounds identified in these herbal mixtures by GC-MS have also been identified by some scholars and their pharmacological activities noted. Oleic acid has been found to be antimicrobial, anti-cancer, anti-inflammatory, antiadrogenic, anti-oxidant, anti-convulsant, antidiabetic, antiobesity, immuno stimulant, antihypocholesterolemic, antidiarrheal, dermatitigenic and diuretic [33,34]. Adegoke et al., [35] reported anticancer, antitumour and edemagenic property of 9-Octadecenoic acid (Z)-,2,3-dihydroxypropyl ester. 1-Cyclohexylnonene has been reported to demonstrate strong antimicrobial activities [9]. 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- has been noted to exhibit antimicrobial, antitumor, antioxidant, analgesic and anti-inflammatory activities [36,37]. *Staphylococcus aureus* is an opportunistic Gram-positive pathogen that readily forms biofilm which enhances its drug-resistance [38]. Lopes et al., [39] and Tan et al*.,* [40] reported that 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- inhibits biofilm formation and disrupt established polymicrobial biofilms of *Staphylococcus aureus*. 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester was reported to be an antibacterial agent [9]. 9-octadecenoic acid exhibits antimicrobial, anti-cancer, anti-inflammatory and heptatoprotective activity [41]. Yamuna et al*.,* [42] reported 9-octadecenoic acid to be an immuno stimulant. Hexadecanoic acid ethyl ester has been found to exhibit anti-Inflammatory, antioxidant, antiandrogenic and hypocholesterolemic activities [35,43,44]. Shah et al*.,* [45] noted its hemolytic activity and Baba et al*.,* [9] noted its antimicrobial activity. Cis-vaccenic acid have been noted to be anti-Inflammatory, antimicrobial, antihypocholesterolemic, to improve insulin sensitivity and lower LDL cholesterol [33,41,45]. N-hexadecanoic acid was reported to be antioxidant, antiadrogenic, antibacterial, antifungal, anticancer, anti-inflammatory, haemolytic and anti-malarial [9,33,41,43]. Hexadecanoic acid ethyl ester has been found to be antimicrobial [9], anti-Inflammatory, antioxidant, antiandrogenic, hypocholesterolemic [35,43,44] and hemolytic [45]. Tetradecanoic acid exhibits antifungal, anticancer, antioxidant, hypocholesterolemic, anti-fibronogenic, haemolytic, lowers blood cholesterol level and reduces the risk of heart disease [41,43,46]. Acetamide, N-(aminocarbonyl)- has been reported to be an anticonvulsant and a sedative [47]. Arginine has been noted for modulation of immune function,

wound healing, insulin sensitivity, lowering blood pressure, hormone secretion [48], regulation of vascular function and endothelial function [49]. Malaria is caused by endothelial dysfunction and Arginine has been proposed as a potential adjunctive therapy for severe malaria because of its ability to increase nitric oxide production in endothelial and other cells [50]. The two herbal mixtures inhibited the growth of their respective test organisms; *S. aureus* and *S. enterica* with zones of inhibition of 18.50mm and 18.80mm respectively. According to CLSI, Ciprofloxacin exhibits intermediate and susceptible activity against clinical isolates at 16-20mm and ≥ 21mm zones of inhibition respectively. Hence from the result, both samples showed intermediate activity compared to Ciprofloxacin which implies that the herbal drugs may be more effective at a higher dosage or more frequent dosage. This is similar to the study of Onyewenjo et al., [51] who reported that some of the herbal remedies marketed in Owerri had intermittent activity against *staphylococcus aureus, Escherichia coli* and *pseudomonas aeruginosa* in range 8mm-18mm.

5. CONCLUSION

Phytochemical screening and GC-MS analysis of the two herbal mixtures revealed the presence of diverse multifunctional compounds which have been shown to exhibit various pharmacological activities and the herbal mixtures inhibited their respective test organisms but they have to be taken at a higher dosage for more effectiveness. The presence of these pharmacological active compounds must have enhanced the use of the herbal mixtures as traditional drugs to treat and manage Staphylococcus infection, typhoid and malaria supporting their claim of efficacy by the producers.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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