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Research Article

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GC-MS Analysis of Bioactive Compounds in the Ethyl Acetate Fraction of *Crossopteryx febrifuga* Leaves

Nma NY[1], Mann A[2*] and Muhammad BM[3]

[1]*Department of Chemical Sciences, Federal Polytechnic Bida, Bida, Nigeria*
[2]*Department of Chemistry, Federal University of Technology Minna, Minna, Nigeria*
[3]*Department of Chemical Sciences, Niger State Polytechnic Zungeru, Zungeru, Nigeria*

ABSTRACT

Background: Crossopteryx febrifuga is monospecific plant from Rubiaceae family that is widely distributed in savannah and tropical regions of Africa particularly in Nigeria. Ethnomedicinally, the leaves of this plant has been used for treatment of skin infections in Nupeland, Nigeria. However, there were no reports on the methanol extract of Crossopteryx febrifuga leaves against Multi-resistant Staphylococcus aureus (MRSA) and its Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The present study was aimed to determine the bioactive constituents in the methanol extract of Crossopteryx febrifuga leaves against Multi-resistant Staphylococcus aureus skin infections by GC-MS analysis. The methanol extract of Crossopteryx febrifuga leaves was characterized for its phytochemical composition by phytochemical screening and GC-MS analysis using standard methods. The phytochemical screening of the crude extract and the ethyl acetate fraction revealed the presence of flavonoids, tannins, saponins, steroids and anthraquinones while the GC-MS chromatogram revealed some peaks that represent fourteen compounds identified by their retention time and peak height. These compounds are: 6-methylenebicyclo [3, 2, 0] hept-3-en-2-one (C_8H_8O), phenol (C_6H_5OH) and phenol, 2, 6-dimethoxy- ($C_8H_{10}O_3$) with 31.56%, 29.507% and 8.417% percentage areas respectively. Others are 2-methoxy-4-vinylphenol ($C_7H_{10}O_2$) at 7.533%; phenol, 2-methoxy ($C_7H_8O_2$) at 6.308%; benzylmethanol (C_7H_8O)at 4.559%; 2H-1-benzopyran-3, 4-diol, 2-(3,4-dimethoxyphenyl)-3, 4- dihydro-6-methyl-, (2α, 3α, 4α)- ($C_{18}H_{16}O_5$)at 2.995%; 10, 11-dihydro -10- dydroxy-2, 3-dimethoxy-dibenz (b, f) oxepin ($C_{16}H_{16}O_4$) at 2.169%; Cyclopenta [1,3] cyclopropa [1,2] cyclohepten-3 (3aH)-one, 1, 2, 3b, 6, 7, 8–hexahydro-6, 6-dimethyl ($C_{13}H_{18}O$)at 1.453%; 2-furancarboxaldehyde,5-methyl ($C_6H_6O_2$) at 1.353%; levoglucosenone ($C_6H_6O_3$) at 1.248%; ethanone, 1-(1-cyclohexane-1-yl)- ($C_8H_{12}O$) at 0.976%; 6-oxa-bicyclo[3,1,0] hexane-3-one ($C_5H_6O_2$) at 0.962% and benzofuran, 3-methyl (C_9H_7O) at 0.956%. The presence of these chemical principles is an indication that the methanol extract of C. febrifuga leaves may yield compounds of pharmaceutical importance against MRSA skin infections if purely isolated.

Keywords: *Crossopteryx febrifuga*; Methanol extract leaves; Ethyl acetate fraction; Gas Chromatography-Mass Spectrometry; Phytochemical; Methicillin-resistant *Staphylococcus aureus;* Skin infections

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INTRODUCTION

Over a decade, there has been accelerated rise in skin infections caused by Methicillin-Resistant *Staphylococcus aureus* (MRSA) to the extent that available antibiotics that once destroyed them become inactive, hence the need to exploit the medicinal potentials of plant derived constituents as alternative.

Crossopteryx febrifuga is a plant from *Rubiaceae* family found in savannah regions of Africa. It is ethnobotanically used for treating skin and wound infections in Nupeland [1], as well asfor management of dry cough, fever, skin infections, diabetes, inflammation and pain by other ethnic groups in Nigeria [2,3]. It is known with several local names such: Nambisunsun (Nupe); Ayeye (Yoruba); Kasfiya (Hausa) and Rimajogoyi (Fulani); and commonly

called crystal bark in English [1-4]. The ethnobotanical uses are supported by scientific investigation of the leaf extract of this plant exhibiting antimicrobial, anti-diabetic, anti-plasmodial, anti-inflammatory and antioxidant effects in rats [5]. These activities are due to the natural products isolated from various parts of this plant [6]; which includes: quercetins, arabinosides, saponins; indole alkaloids (crossopterine), vitexcins, bisdesmodic and desmodicsaponins [7].
Literature survey has shown that no work has been reported on the GC-MS analysis of the methanolic extract of *C. febrifuga* leaves.

MATERIALS AND METHODS

Collection of *Crossopteryx febrifuga* Leaves

C. febrifuga leaves were collected from a hill side along Bida-Wuya road in Niger State, Nigeria. It was identified by a Botanist in NARICT, Zaria, Nigeria. The leaves were air-dried at laboratory temperature for 7 days; pulverized and sieved.

Preparation of Crude Extract

500 g of the pulverized sample was cold macerated in 70% methanol (v/v) for 72 h in three consecutive times, filtered using Whatman No. 1 filter paper and concentrated *in vacuo* with a rotary evaporator (Perkin Elmer 6000, China) at 35°C until needed for bioassay.

Partitioning of the Crude Extract

The air-dried crude methanolic extract (150 g) was partitioned by dissolving it in 250cm^3 methanol and extracting with 250 cm^3 each of *n*-hexane, dichloromethane, ethyl acetate and water respectively in a 500 cm^3 separatory funnel. The solvent soluble fractions obtained were dried, weighed and tested for bioassay to ascertain the most bioactive fraction [8].

Phytochemical Analysis

Methanol crude extract and ethyl acetate fraction being the most bioactive were screened for their phytochemical contents which include: flavonoids, tannins, saponins, glycosides, steroids, triterpenes and anthraquinones according to the methods described [9,10].

Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

The GC-MS analysis of the ethyl acetate fraction of the *Crossopteryx febrifuga* was performed using GC-MS QP-2010, Shimadzu, India. The machine is equipped with a fused silica capillary column of 25 m length, 0.25 mm diameter and 0.15 µm film thickness. The machine was operated at a temperature programme of 300°C for 45.75 minutes, at a signal plot of 50 Hz and noise interval of 5.276 to 5.576 minutes

Identification of the Components

The GC-MS interpretation was carried out using the data base of National Institute of Standard and Technology (NIST) since it has more than 62, 000 patterns. The fragmentation patterns of the spectra of the unknown components are stored in the NIST library of the machine. The name, molecular weight, retention time and the structure of each bioactive component of the ethyl acetate fraction were revealed by the machine and their suggested fragmentation pattern worked out in accordance with standard procedure [11].

RESULTS

The presence of flavonoids, saponins, tannins, steroids, triterpenes, glycosides and anthraquinones were found on phytochemical screening of methanol extract and ethyl acetate fraction of *Crossopteryx febrifuga* leaves (Table 1).
The GC-MS chromatogram of ethyl acetate fraction of C. *febrifuga* leaves revealed14 peaks (Figure 1) that represent fourteen compounds identified by their retention time (RT) and % peak area as presented in Table 2 while their chemical structures are shown in Figure 2.

Table 1: Phytochemical screening of the crude and the ethylacetate fraction

Secondary Metabolite	Test	Inference
Glycosides	Fehling's Test	+
Cardiac Glycosides	Keller-Killiani's	+
Saponins	Froth Test	+
Tannins	Lead Acetate	+
	Ferric Chloride	+
Flavonoids	Shinoda's	+
	Sodium hydroxide	+
Carbohydrates	Molisch's	-
	Fehling's	-
Steroids / Triterpenes	Lieberman-Burchard's	+
	Salkowski's	+
Alkaloids	Mayer's	-
	Wagner's	-
	Dragendorff's	-
Anthraquinones	Burntrager's	+

Key: + = Present; ─ = Absent

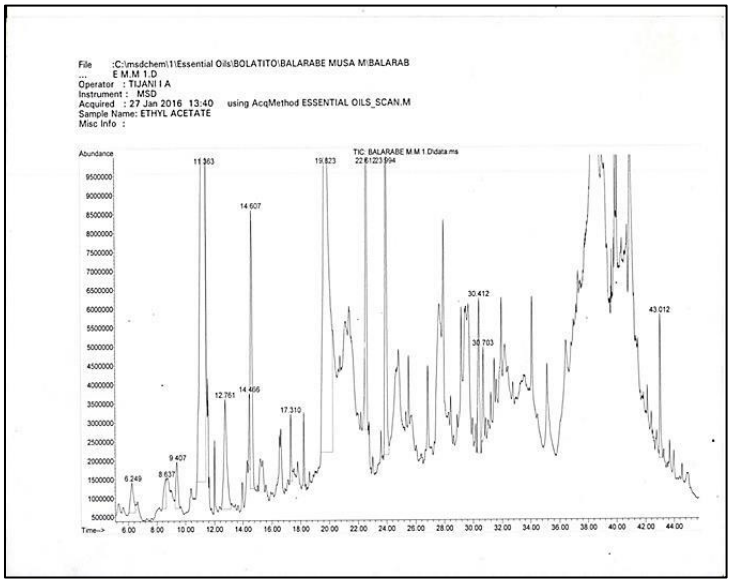

Figure 1: Gas chromatography- mass spectrometry chromatogram of ethyl acetate fraction of C. *febrifuga* leaves

Table 2: Bioactive compounds identified in the ethyl acetate fraction of *C. febrifuga* leaves using GC-MS

Peak No.	Compound	MW (g/mol)	Pea Area (%)	MF	RT (mins)
1	Levoglucosenone	126	1.248	$C_6H_6O_3$	6.249
2	6-oxa-bicyclo[3, 1, 0] hexane-3-one	98	0.962	$C_5H_6O_2$	8.367
3	2-Furancarboxaldehyde, 5-methyl	110	1.353	$C_6H_6O_2$	9.409
4	Phenol	94	29.5	C_6H_6O	11.363
5	Benzylmethanol	108	4.559	C_7H_8O	12.761
6	Ethanone, 1-(1-cyclohexane-1-yl)-	124	0.976	$C_8H_{12}O$	14.466
7	Phenol, 2-methoxy-	124	6.308	$C_7H_8O_2$	14.607
8	Benzofuran, 3-methyl	131	0.956	C_9H_7O	17.31
9	6-methylenebicyclo[3,2,0] hept-3-en-2-one	120	31.56	C_8H_8O	19.823
10	2-methoxy-4-vinylphenol	150	7.533	$C_7H_{10}O_2$	22.612
11	Phenol, 2,6-dimethoxy-	154	8.417	$C_8H_{10}O_3$	23.994
12	2H-1-Benzopyran-3, 4-diol, 2-(3,4-dimethoxyphenyl)-3, 4- dihydro-6-methyl-, (2α, 3α, 4α)-	312	2.995	$C_{18}H_{16}O_5$	30.412
13	Cyclopenta[1,3]cyclopropa[1,2]cyclohepten-3 (3aH)-one, 1, 2, 3b, 6, 7, 8– hexahydro-6, 6-dimethyl	190	1.453	$C_{13}H_{18}O$	3.703
14	10, 11-dihydro -10- dihydroxy-2, 3-dimethoxy-dibenz (b, f) Oxepin	272	2.169	$C_{16}H_{16}O_4$	43.012

The chemical structures of the bioactive compounds revealed by the GC/MS are shown in Figure 2.

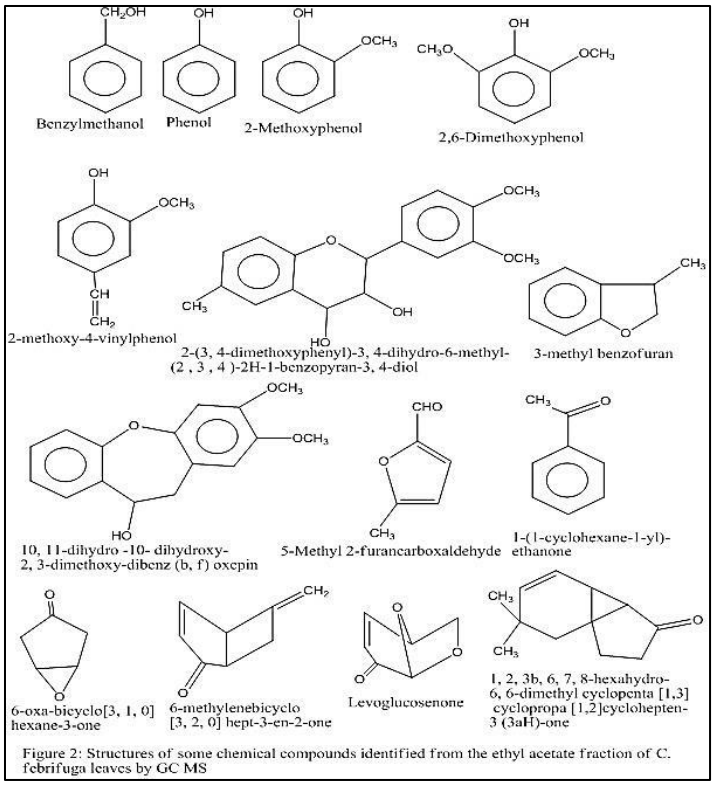

Figure 2: Structures of some chemical compounds identified from the ethyl acetate fraction of C. febrifuga leaves by GC MS

Figure 2: Structure of some chemical compounds identified from the ethyl acetate fraction of *C. febrifuga* leaves by GC MS

DISCUSSION

When the mass spectra of the chromatogram from the ethyl acetate fraction of *C. febrifuga* leaves were compared with NIST library, a total of 14 different compounds were identified and characterized according to their retention time and percentage area. The three prominent compounds are: 6-methylenebicyclo [3, 2, 0] hept-3-en-2-one (C_8H_8O), phenol (C_6H_5OH) and phenol, 2, 6-dimethoxy- ($C_8H_{10}O_3$) with 31.56%, 29.507% and 8.417% percentage areas respectively. Others are 2-methoxy-4-vinylphenol ($C_7H_{10}O_2$) at 7.533%; phenol, 2-methoxy ($C_7H_8O_2$) at 6.308%; benzylmethanol (C_7H_8O)at 4.559%; 2H-1-benzopyran-3, 4-diol, 2-(3,4-dimethoxyphenyl)-3, 4- dihydro-6-methyl-, (2α, 3α, 4α)- ($C_{18}H_{16}O_5$)at 2.995%; 10, 11-dihydro -10- dydroxy-2, 3-dimethoxy-dibenz (b, f) oxepin ($C_{16}H_{16}O_4$) at 2.169%; cyclopenta [1,3] cyclopropa [1,2] cyclohepten-3 (3aH)-one, 1, 2, 3b, 6, 7, 8–hexahydro-6, 6-dimethyl ($C_{13}H_{18}O$)at 1.453%; 2-Furancarboxaldehyde,5-methyl ($C_6H_6O_2$) at 1.353%; levoglucosenone ($C_6H_6O_3$) at 1.248%; ethanone, 1-(1-cyclohexane-1-yl)- ($C_8H_{12}O$) at 0.976%; 6-oxa-bicyclo[3,1,0] hexane-3-one ($C_5H_6O_2$) at 0.962% and benzofuran, 3-methyl (C_9H_7O) at 0.956%. Their retention times are 6.249, 8.367, 9.409, 11.363, 12.761, 14.466, 14.607, 17.310, 19.823, 22.612, 23.994, 30.412, 3.703 and 43.012minutes respectively.

As a result of low toxicity, availability, high efficacy, affordability and moderate or no microbial resistant, plant derived constituents accessed through herbal approach has become the available alternative in recent years, hence this has engaged the researchers in continuous search for phytoconstituents that could serve as a source of remedy for treating stubborn skin infections caused by MRSA and other prevailing human diseases [12].

Bioactive compounds from plants exhibit different pharmacological and biological effects when administered *in vivo* [13]. The present study revealed the presence of some important phytochemicals in the ethyl acetate fraction such as cardiac glycoside (Table 1) which exhibits some biological effect [14]. The GC/MS Chromatogram of the ethyl acetate fraction of *C. febrifuga* leaves shows 14 peaks (Figure 1).

Among the compounds identified are flavonoids e. g: 2-(3,4-dimethoxyphenyl)-3, 4-dihydro-6-methyl-, (2α, 3α, 4α)-2H-1-benzopyran-3, 4-diol (Table 2); they played significant role in preventing oxidative stress in biological systems by scavenging free radicals. Tannins that are not toxic compounds e.g. 10, 11-dihydro -10- dydroxy-2, 3-dimethoxy-dibenz (b, f) Oxepin are known for their antibacterial, antifungal, antidiarrheal, antioxidant and anti-inflammatory activities [15] while saponins are also known for their anti-inflammatory, cardiac depression and

hypercholesterolemic properties and steroids and triterpenes e.g. 6-oxa-bicyclo [3,1,0] hexane-3-one are complex hydrocarbons with strong antioxidant and anti-inflammatory activities [16]. However [17] reported that some similar compounds with similar fragmentation peaks have been isolated from *Foeniculum vulgare* plant and have shown anti – MRSA activity.

CONCLUSION

This research work has identified the presence of 14 chemical compounds that may account for the antimicrobial activity of *C. febrifuga* leaves extract against MRSA skin infections. The presence of these chemical compounds is an indication that the ethyl acetate fraction of *C febrifuga* may serve as a source of alternative remedy for curing MRSA skin infections. However, isolation of these compounds at individual molecular level and their biological activities will be of medical importance.

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