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Total Phenolic, Flavonoid and Saponin Contents, and GC-MS Profile of the Methanolic Extract of Leaves of Crossopteryx Febrifuga

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ABSTRACT

The leaves of *Crossopteryx febrifuga* have been shown traditional to possess medicinal properties and are used for the treatment of malaria and inflammatory disorders. The objective of this study is to evaluate the phytochemical profile of the leaves of *Crossopteryx febrifuga* using gas chromatography-mass spectrometric technique. The leaves were extracted with n-hexane and methanol separately and subjected to phytochemical screening and quantification techniques. The methanol extract was partitioned using three solvents (n-hexane, ethyl acetate and n-butanol). The n-butanol fraction was further partitioned using column chromatography and gave four sub-fractions which were subjected to gas chromatography-mass spectrometric analysis. Phytochemical analysis of the extracts revealed presence of terpenoids, flavonoids, saponins and steroids. The quantification of three classes of phytochemicals identified gave total phenolic, flavonoid and saponin contents obtained were 44.40 ± 0.11 mg Gallic acid equivalent/g extract, 10.50 ±0.12 mg quercetin equivalent/g extract and 91.30 ±0.17 mg Ginsenoside Rb 1 equivalent/ g extract respectively. The gas chromatography-mass spectrometric analysis of the Sub-Fractions (SF) of n-butanol showed presence of 25, 32, 28 and 4 compounds in SF1, SF2, SF3 and SF4 respectively. Among the identified compounds the prominent ones were hexadecanoic acid methyl ester; diisooctyl phthalate; trans-13-octadecenoic acid methyl ester; 2,4-bis(1,2-dimethylethyl) phenol and [R-(Z)] 9-octadecenoic acid,12-hydroxymethyl ester. These compounds make up about 52% of the identified phytochemicals in the n-butanolic fraction. The presence of these phytochemicals could be responsible for its medicinal properties.

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Introduction

Phytochemicals in medicinal plants has been used as lead for drug designs and developments. These plants along the years have served as a critical part of human culture around the world and are sources of synthetic and traditional herbal medicine [1]. Curative pills and capsules we use as antibiotics, antimalarial and blood thinners in treatment of ailments are originally sourced from medicinal plants. To a very large extent, medicinal plants are used as alternative medicine for treatment of diseases of man and other animals since most of them do not have serious side effects when compared with synthetic drugs [2]. Compounds of interest can be obtained from the chemical modification or fermentation of some phytochemicals obtained from medicinal plants that are used as small-molecule drug precursors. Semi-synthetic approach has been used to create an alternative supply for active compounds with preventive properties from the plants due to their low yield of compounds and/or the high cost of total synthesis, although they are not necessarily pharmacologically active in their original naturally occurring forms [3]. Phytocomponents either as pure compounds or as standardized extracts containing a mixture of compounds provide unlimited opportunities for new drug leads.

Crossopteryx febrifuga is a monospecific African genus with a wide distribution. It is a twisted tree with conspicuous tubular flowers and it is widely distributed throughout the Savannah region of West and Tropical Africa. The generic epithet finds it root from Greek 'krossoi' and 'pteron' meaning fringed and wing respectively and is based on its seed shape. The specific epithet '*febrifuga*' from the word febrifuge relates to its medical use in fever treatment. *Crossopteryx febrifuga* is a deciduous savannah tree 1.8-15m tall, with a rounded crown and pendulous branchlets. The leaves are green, variable, elliptic-obovate or ovate-suborbicular; usually widest above the middle, abruptly acuminate or rounded at apex. The base is broadly cuneate to rounded, 6.5 to 11.5 cm broad; with five to six pairs prominently upcurving lateral nerves fading out near the margin. The veins form an irregular network; petiole 0.5 to 1.3 cm long.

The plant is used for treatment of pain and malaria in Northern Nigeria. It is also used for treating coughs, gastro-intestinal pains, skin diseases, swellings, diarrhoea, dysentery, gonorrhoea, worms, colds, chicken pox, respiratory infections, scabies and constipation. Its uses are cited from many African countries – Cote d'Ivoire, Ghana, Sierra Leone, Nigeria, Sudan, Malawi, Tanzania and Rhodesia. It is also used as an ordeal poison [4,5]. Other uses include the treatment of trypanosomiasis, *Staphylococcus aureus* infection [5-7]. Reported that the extract has gastroprotective effect [5]. This is indicative of the presence of the active phytocomponents that are responsible for these activities. *Crossopteryx febrifuga* belongs to the family of Rubiaceae, It is commonly found in all parts of Nigeria and the Hausa people of North Western Nigeria call it *Kasfiya, Kashin Awaki* or *Giyayyata* while it is known as *Ayeye* among the Yorubas in western Nigeria.

Methods

Sample Collection and Preparation

Leaves of *Crossopteryx febrifuga* were collected from a traditional health practitioneer from Omu-aran, Kwara State, Nigeria. The plant was then identified by a plant taxonomist, Prof. A. T. J. Ogunkunle of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. A voucher specimen was deposition in the department's herbarium and a specimen number LHO 566 attached to it. The leaves were later pulverized into powder using a sterilized food grinder. The powder was stored in a clean container until further use.

Extraction

Extraction was carried out on the air-dried and pulverized leaves of *Crossopteryx febrifuga*. 250 g of pulverized leaves was soaked with 1liter of n-hexane for two days and the solvent changed every twenty-four hours interval. The mixture was filtered to obtain the n-hexane extract. The residue was evaporated to dryness and soaked in 2.50 liters of methanol for two days and the solvent was changed every twenty-four hours. The two separate solvent extracts were concentrated using a rotary evaporator and finally evaporated to dryness.

Phytochemical Screening of Secondary Metabolites

Detection of phytochemical constituents was carried out for all the extracts using the standard procedures as described by [8].

Determination of Total Saponin Content

Crude methanolic extract of 1ml was placed in a conical flask and 10 cm3 of 20% aqueous ethanol added to it and heated over a hot water bath for 4hrs with continuous stirring at about 55^{o}C. The mixture was filtered and extracted three times with 10 ml of 20% aqueous ethanol. The extracts were combined and the volume reduced to 4 ml using a water bath at about 90^{o}C. The concentrated extract was transferred into a 250 ml separating funnel, and then 20 ml diethyl ether added and shaken vigorously. The aqueous layer was recovered and the process repeated. 10ml of n-butanol was added to the aqueous layer. The butanol fraction was recovered and washed twice with 10 ml of 5% NaCl. The solution was heated in a water bath to evaporate off the butanol. After evaporation, the sample was dried in an oven to constant weight to recover saponin. To prepare the recovered saponin for UV measurement, a fresh solution of vanillin-acetic acid (5% w/v, 0.2 ml) solution was prepared and perchloric acid (0.8 ml) was added and kept at 70oC for 15 minutes. The solution was cooled on ice for 20 seconds before adding glacial acetic acid (5 ml). The solution was scanned at 550 nm using a Cecil CE7200 UV spectrophotometer. The standard used was Ginsenoside Rb 1 as predetermined graphically by [9].

Determination of Total Flavonoid Content

Aluminium colorimetric method was used to determine the total flavonoid content of the methanolic extract of *Crossopteryx febrifuga* leaves. 1 ml of the crude methanolic leaf extract was dissolved in 5 ml of 50% methanol and to 1 ml of this solution was added 0.7 ml of 5% (w/w) NaNO2 and 10 ml of 30% (v/v) ethanol. This mixture was stirred for 5 mins and 0.7 ml of 10% AlCl3 (w/w) added to it and stirred for 6 mins. 5 ml of 1mol/l NaOH was added to the mixture and diluted to 25 ml with 30% (v/v) ethanol. The mixture was allowed to stand for 10 mins and the absorbance measured at 500 nm using a UV spectrophotometer. Quercetin was used as the standard and the total flavonoid content was expressed as quercetin equivalent in mg/g extract [10].

Determination of Total Phenolic Content

The total phenolic content of the crude methanolic extract of *Crossopteryx febrifuga* was determined using the Folin-Ciocalteau method. 5 mg of the methanolic leaf extract was weighed and dissolved with 5 ml of 50% methanol using a vortex mixer. 0.5 ml of this solution was pipette into a test tube and 3.5 ml of distilled water, 0.25 ml Folin-Ciocalteau reagent added to it. It was left to incubate for 8 minutes at room temperature. Then 1 ml of 20% Na2CO3 was added and left to incubate for 2 hrs. The absorbance was measured at a wavelength of 765 nm against a blank using a UV spectrophotometer. Gallic acid was used as the standard and the total phenolic content of the extract expressed in mg Gallic acid equivalents/mg extract [11].

Solvent-Solvent Extraction of Crude Methanolic Extract

Solvent-solvent extraction was also carried out. The crude methanolic extract was suspended in distilled water then sequentially extracted with 200 ml each of n-hexane, chloroform, ethyl acetate and n-butanol. The mixtures were shaken vigorously and were made to stand for some time to ensure proper extraction and separation. All the fractions obtained (i.e. n-hexane, chloroform, ethyl acetate and n-butanol fractions) were labelled properly and concentrated by evaporation using a rotary evaporator. After concentration, the fractions were screened for the presence of phytochemicals.

Fractionation of the Extracts by Column Chromatography

Column chromatography of butanolic fraction was carried out on a column using silica gel as the stationary phase using a gradient elution of methanol and ethylacetate. The gradient elution started with 50 ml methanol followed by methanol:ethylacetate in the following ratios; 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and ending with 50 ml of ethylacetate.

Thin layer chromatography was also performed on the column fractions using silica gel coated glass plate and a solvent mixture of chloroform and methanol in the ratio 9:1 was used to develop the spot.

Gas Chromatography-Mass Spectrometric Analysis

Gas Chromatography–Mass Spectrometry (GC–MS) analysis was performed using Agilent 7890A/5975C GC–MSD instrument and split (50:1) injection system. The GC was fitted with an Agilent 19091S- 33HP-5MS capillary column (30.00 m × 0.25 mm inner diameter, 0.25μm phase thickness). The GC oven was programmed from 100 °C held for 4 min to final temperature of 300 °C at the rate of 4 °C/min and held isothermally at final temperature of 240 °C for 10 min. Helium at a constant flow rate of 1.5 mL/min was used as carrier gas and running time of 49 min. A total of 1 μL aliquot of sample was injected automatically. The samples were analyzed in the full scan mode. The electron ionization energy of 70 eV, source temperature of 250 °C and solvent delay of 5 min were employed. These compounds were identified based on their mass spectrum, molecular weight, and fragment ions obtained from the mass spectrum. These parameters were matched with those of reference compounds obtained from National Institute of Standards and Technology 2011 database which were incorporated into the computer system of the equipment.

Results and Discussion

Results

Phytochemical Screening

The leaf extracts were screened to establish the class of phytochemicals in the extracts. The identified classes of

phytochemicals are presented in Table 1. It revealed the presence of steroid, flavonoid, saponin and absence of terpenoid, alkaloid and tannin in the methanolic extract.

Table 1: Phytochemicals in n-Hexane and Methanolic Extracts

Extracts	Gly	Tan	Flav	Sap	Ste	Alk	Terp
Methanol	-	-	+	+	+	-	-
n-hexane	-	-	-	-	-	-	+

KEY: Gly – glycosides; Tan – tannins; flav – flavonoids; Ste – steroids; Alk – Alkaloids; Terp – Terpenoids; + = present; - = absent

Quantification of Phytochemicals in Methanolic Extract

Some of the identified phytochemicals were quantified as presented in Table 2. The total phenolic, flavonoid and saponin contents were expressed in mg Gallic acid equivalent/g extract; quercetin equivalent/g extract and gensinoside Rb 1 equivalent/g extract respectively.

Table 2: Quantities of some Selected Phytochemicals in the Methanolic Extract

Methanolic extract	Total Phenolic content (mg Gallic acid equivalent/g extract)	Total Flavonoid content (mg quercetin equivalent/g extract)	Total saponin content (mg gensinoside Rb 1 equivalent/g extract)
Leaves	44.40 ± 0.11	10.50 ± 0.12	91.30 ± 0.17

Phytochemicals Screening of Fractions Obtained from the Methanolic Extract

The result of the phytochemical screening of the fractions obtained from fractionation of the methanolic leaf extract is presented in Table 3.

Table 3: Phytochemicals in Fractions of the Methanolic Extract

	n-hexane	Ethylacetate Extract	Butanol	Aqueous
Steroid	+	-	-	-
Flavonoid	-	+	+	+
Terpenoid	-	-	-	-
Alkaloid	-	-	-	-
Saponin	+	-	+	-
Tannin	-	-	-	-

Key: + = present; - = absent

Chemical Components of Butanolic Fraction Detected by Use of GC/MS

The sixty-one (61) collected sub-fractions on grouping gave seven (7) combinations which were obtained using thin-layer chromatography on a silica gel-coated glass plate. The first three groupings ranging from sub-fractions 1-29 did not show the presence of any compound. Combined sub-fractions 30-38 showed the presence of 25 compounds as presented in Table 4. Combined sub-fractions 39-49 contained 32 compounds as shown in Table 5; combined sub-fractions 50-53 contained 28 compounds as shown in Table 6 while the combined sub-fractions 54-61 afforded only four compounds as shown in Table 7. Their total ion chromatograms are presented in Figures 1-4.

Table 4: GC-MS Data Compounds Identified in Combined Sub-Fractions (SF1) 30-38

S/N	Peak Number	Retention Time	Peak Height	Compound Name	Percentage
1	2	20.131	291305	Phenol,2,4-bis(1,1-dimethylethyl)	2.986
2	3	20.594	117623	Undecanoic acid, 10-methyl-, methylester	1.178
3	13	34.593	2688192	Hexadecanoic acid, methyl ester	25.094
4	14	35.607	778050	Dibutyl phthalate	7.791
5	16	37.721	325663	i-propyl-14-methylpentadecanoate	3.115
6	17	39.403	230893	Behenic alcohol	2.625
7	18	39.710	192714	9,12-octadecadienoic acid, methyl ester, (E, E)	1.755
8	19	39.923	511033	9-octadecenoic acid, methyl ester (E)	5.108
9	20	40.761	517953	Methyl stearate	5.149
10	22	42.481	195680	Hexadecanoic acid butyl ester	1.917
11	23	51.282	110754	Thiocarbamic acid, N, N-dimethyl, S-1, 3-diphenylbutenyl ester	1.078
12	24	51.658	171498	Thiocarbamic acid, N, N-dimethyl, S-1, 3-diphenylbutenyl ester	1.708
13	25	52.033	2240528	Diisooctyl phthtalate	23.554

Table 5: Chemical Composition of sub-Fractions (SF2) 39-49

S/N	Peak Number	Retention Time	Peak Height	Compound Name	Percentage
1	4	20.131	280417	Phenol, 2,4-bis(1,1-dimethylethyl)-	2.524
2	5	20.594	165373	Undecanoic acid, 10-methylmethyl ester	1.509
3	10	27.881	172566	13, 16-octadecadiynoic acid, methyl ester	1.677
4	12	30.158	87733	2-Hexadecanol	0.718
5	16	32.585	116037	Phthalic acid, isobutyl octadecyl ester	0.925
6	17	33.067	61101	2-hexadecanol	0.671
7	18	34.587	2005819	Hexadecanoic acid,methyl ester	18.509
8	19	35.613	378470	Phthalic acid, butyl hex-3-yl ester	3.798
9	24	39.923	1097554	Trans-13-octadecenoic acid, methyl ester	10.624
10	25	40.761	577164	Methyl stearate	5.633
11	27	42.475	70439	As-indacen-1(2H)-one, 3,6,7,8-tetrahydro-3,3,6,6-tetramethyl	0.612
12	30	52.033	1529639	Diisooctyl phthalate	15.445
13	25	52.033	2240528	Diisooctyl phthtalate	23.554

Table 6: Chemical Composition of Fractions (SF3) 50-53

Peak number	Retention time	Peak height	Compound name	Percentage
1	11.924	28470		
2	13.375	21086		1.611
3	15.508	52117		3.159
4	16.165	11515		0.582
5	18.085	33272		1.481
6	19.887	25352		0.971
7	20.137	306048	Phenol, 2,4-bis(1, 1-dimethylethyl)-	12.341
8	20.594	54376	Undecanoic acid, 10-methyl-, methyl ester	2.539
9	22.301	39340		1.667
10	22.527	34156		2.210
11	23.065	76923	2-hexadecanol	3.043
12	25.367	14679		0.580
13	26.136	18516		0.721
14	26.924	13479		0.743
15	27.869	42777	Cyclopropanebutanoic acid, 2-[[2-2[(2-pentylcyclopropyl)methyl]cyclopropyl] methyl]cyclopropyl]methyl]-, methyl ester	1.938
16	30.158	47826	2-hexadecanol	1.765
17	30.308	16594	9, 10-secocholesta-5,7,10(19)-triene-3,24,25,triol, (3β, 5Z, 7E)-	0.560
18	31.684	15908		0.704
19	33.755	15249		0.735
20	34.599	343056	Hexadecanoic acid, methyl ester	17.300
21	35.006	58641	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	2.601
22	36.676	24754	2-hexadecanol	1.033
23	39.429	20554	9-octadecene. 1,1'-[1,2-ethanediylbis(oxy)bis-(Z,Z)-	0.807
24	39.735	16049	Z, Z-3,15-Octadecadien-1-ol acetate	0.666
25	39.935	173873	11-octadecenoic acid, methyl ester	9.193
26	40.767	140288	Heptadecanoic acid, 16-methyl-, methyl ester	7.700

27	42.162	202280	Phenol-4,4'-(1-methylethylidene)bis-	10.001
28	52.033	249105		11.536

Table 7: Chemical Composition of Sub-Fractions (SF4)54-61

Peak number	Retention Time	Peak Height	Compound Name	Percentage
1	34.593	563999	Hexadecanoic acid, methyl ester	2.912
2	39.716	1099267	9,12-octadecadienoic acid(Z, Z)-, methyl ester	5.472
3	39.923	1114087	9-octadecenoic acid, methyl ester, (E)-	5.982
4	45.365	13816757	9-octadecenoic acid, 12-hydroxy-, methyl ester [R-(Z)]-	85.643

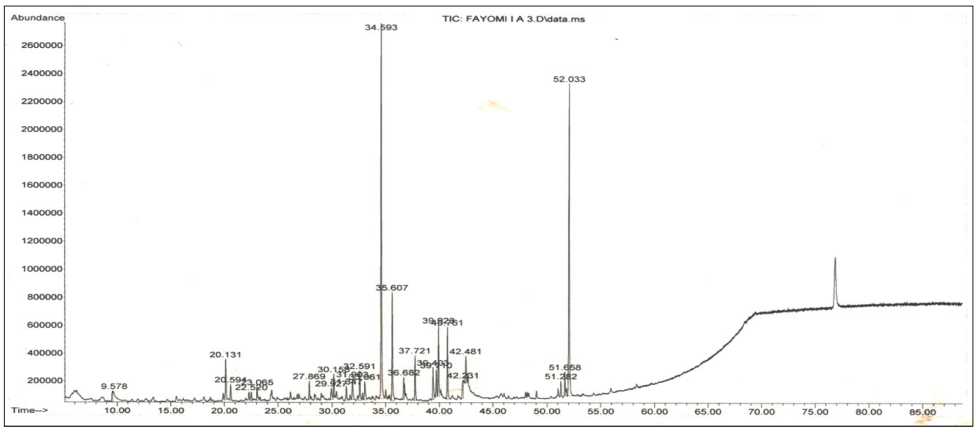

Figure 1: Chromatogram of Butanolic Column Fractions (SF1) 30-38

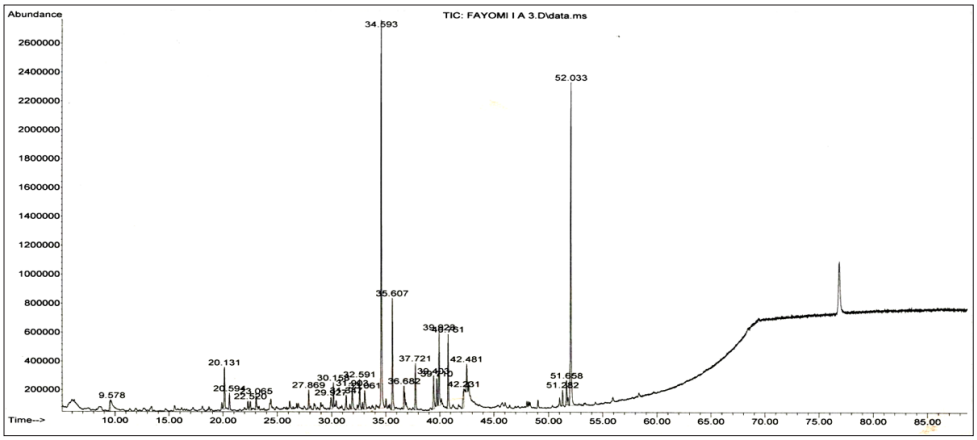

Figure 2: Chromatogram of Butanolic Fractions (SF2) 39-49

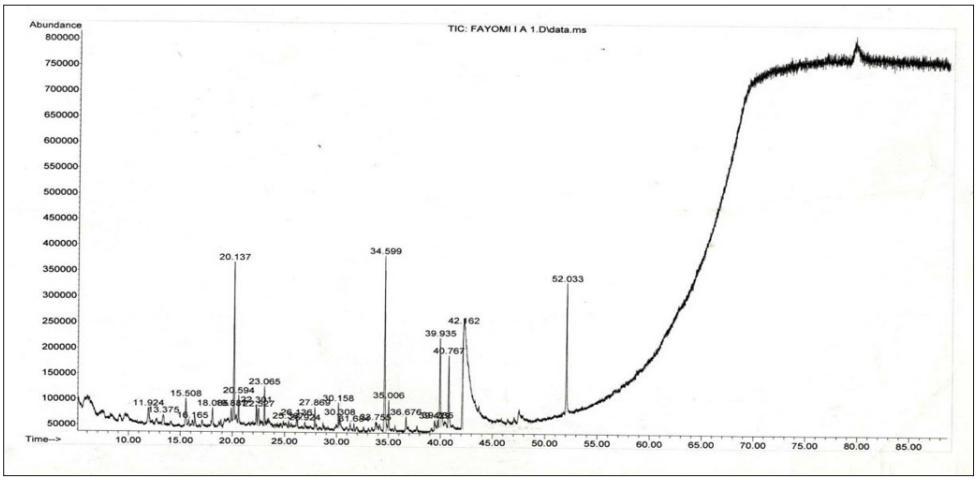

Figure 3: Chromatogram of Butanolic Column Fractions (SF3) 50-53

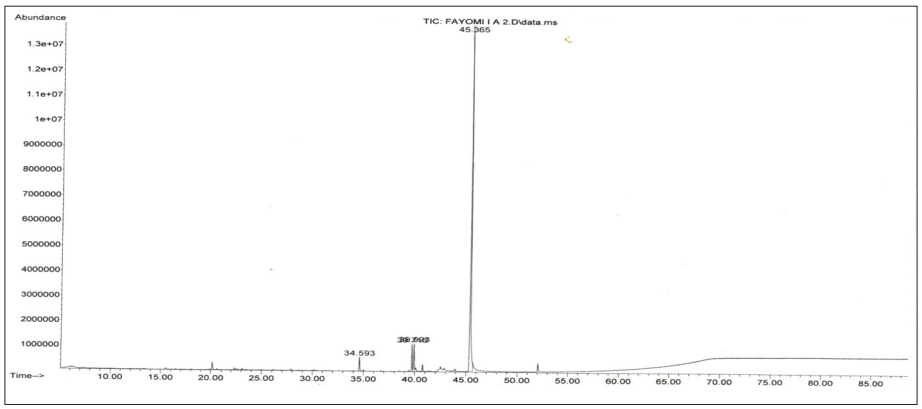

Figure 4: Chromatogram of Butanolic Column Fractions (SF4) 54-61

Discussion

The knowledge of the chemical constituents of medicinal plants is helpful in the discovery of therapeutic agent for some ailments as well as sources of new drug candidates. Medicinal plants are also of interest in biotechnology, as most of the drug industries depend in part on plants for the production of pharmaceutical compounds which are leads or precursors for certain drugs. Qualitative and quantitative phytochemical analysis is a way of determining the quantity and specific phytochemicals present in plant samples.

The phytochemical screening of the methanolic leaf extract indicated the presence of flavonoids, saponins and steroids; alkaloids, terpenoids, tannins and glycosides were absent. While the n-hexane extract indicates the presence of terpenoids only. The presence of this class of compounds in the solvent uses can be attributed to the polarity of the solvents. Methanol is a polar solvent due to the presence of the hydroxyl functional group and the extent of the polarity can be said to be ascribed to the short hydrocarbon chain present in the compound. n-hexane is a non-polar solvent and its ability to extract terpenoids is indicative of the presence of the non-polar terpenoids which are mainly hydrocarbons without heteroatoms. Phytochemicals are plant chemicals that have protective or disease preventive properties non-nutritive [12]. Different phytochemicals have been found to possess a wide range of activities such as antimicrobial [13]. Phytochemicals of various kinds have different antimicrobial activities depending on the class of compounds to which it belongs ranging from glycosides, alkaloids, terpenoids and saponins to various categories of flavonoids.

Flavonoids are free radical scavengers and antioxidants which show strong anticancer activity, prevent oxidative cell damage, and protect cells against all stages of carcinogenesis [14]. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities 2002) [15].

In the determination of the total phenolic, total flavonoid and total saponin content, spectrophotometric method was used to quantify the total phenolic, total flavonoid and total saponin content of the crude methanolic extract. The methanolic extract of the leaves of Crossopteryx febrifuga showed a high content of saponin (91.30 ± 0.17 mg gensinoside Rb 1 equivalent/g extract), the total flavonoid content (10.50 ± 0.12 mg quercetin equivalent/g extract) and total phenolic content (44.40 ± 0.11 mg Gallic acid equivalent/g extract).

Saponin has been reported to have a wide range of pharmacological and medicinal activities. Interestingly, it has been reported that it has low oral toxicity in humans [16]. The presence of saponin in plants have been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs [17]. It has been revealed that saponin have both hypertensive and cardiac depressant properties [18]. They have been found to be potentially useful for the treatment of hypercholesterolemia which suggested that saponin might be acting by interfering with intestinal absorption of cholesterol, thus have antidiabetic effects 2009) [19]. In addition, they have been reported to have antinematicidal, molluscicidal, insecticidal and antioxidant properties anti-cancer agents aphrodisiac properties anti-protozoal effects antibiotic, antifungal, antiviral, hepatoprotective, anti-inflammatory and anti-ulcer effects [20-24]. The use of the use of the leaves of Crossopteryx febrifuga in ethnomedicine as drugs is thus suggested; due to the high concentration of saponin in them and the several health beneficial effects reported to be associated with saponin.

Solvent – solvent partitions of the crude methanolic extract with n-hexane, ethylacetate and n-butanol leaving a residue of the aqueous fraction were screened for phytochemicals. The n-hexane fraction showed the presence of steroid and saponin, flavonoids and saponins were identified in the ethyl acetate and n-butanol fractions while the aqueous fraction revealed the presence of flavonoids only. Finding has revealed that steroids possess the ability to lower body cholesterol. It competes with cholesterol for micelles which transport lipids and cholesterol into the intestinal mucosa, hence; reducing cholesterol absorption into gastrointestinal tract [25]. The butanolic fraction gave 61fractions on separation using column chromatography. These fractions were later subjected to thin layer chromatography in order to group the fractions according to their retention factors. The groupings gave different colours on concentration and are oily. These oil colours are as a result of the presence of fatty acids in the separated fractions.

On screening, fractions 1-29 did not reveal the presence of any of the screened phytochemicals. All other fractions showed presence of phenolics.

Gas chromatography- Mass spectrometry is an analytical tool of great importance that can separate and identify compounds in complex combination of plant extracts. The instrument can ascertain the compounds present in each extract of the plant. Combined fractions 30-38 showed presence of 25 compounds. 13 compounds were identified from the NIST library coupled with the GC-MS. The first compound to be identified was phenol, 2, 4-bis(1,1-dimethylethyl) with retention time of 9.578 minutes and the last being Diisooctyl phthalate with retention time of 52.033minutes. Among the compounds that were identified, hexadecanoic acid, methyl ester showed the largest percentage composition of 25.094% and retention time of 34.593 minutes,

thereafter Diisooctyl phthalate with percentage composition of 23.554% and retention time of 52.033minutes. Other compounds identified are Undecanoic acid, 10-methyl-, methylester, i-propyl-14-methylpentadecanoate, Behenic alcohol, 9,12-octadecadienoic acid, methyl ester, (E, E), 9-octadecenoic acid,methyl ester (E), Methyl stearate, Hexadecanoic acid butyl ester, Thiocarbamic acid, N, N-dimethyl, S-1, 3-diphenylbutenyl ester. The classes of compounds identified are majorly alcohols, fatty esters and phthalates. The identified compounds possess many biological activities. Hexadecanoic acid methyl ester has been reported to have the ability to decrease blood cholesterol [26] and also inhibits the cyclooxygenase (II) enzymes and, thus, produces a selective anti-inflammatory action [27]. Diisooctyl phthalate also known as bis (2-ethylhexyl) phthalate (DEHP), bis (6-methylheptyl) phthalate alongside dibutyl phthalate have been reported among phthalates that are used as plasticizer and are also known to be antimicrobial and antifouling [26]. Behenic alcohol also called n-docosanol has also been reported to be antiviral by its ability to resist infection by a variety of lipid-enveloped viruses including herpes viruses [28].

GC-MS revealed the presence of 32 compounds in fraction 39-49. Of these 32 compounds, 14 were identified from the NIST library coupled with the Gas Chromatograph-Mass Spectrometer. The first compound to be identified by the NIST library was phenol-2,4-bis(1,1-dimethylethyl)- with retention time of 20.131 minutes while the last to be identified was Diisooctyl phthalate with retention time of 53.002 minutes. The compounds with the highest percentage composition were hexadecanoic acid methyl ester and diisooctyl phthalate which constitutes 18.509 and 15.445% respectively. Other compounds present are Phenol, 2,4-bis(1,1-dimethylethyl)-, Undecanoic acid 10-methylmethyl ester; 13, 16-octadecadiynoic acid methyl ester, 2-Hexadecanol, isobutyl octadecyl ester, 2-hexadecanol, Phthalic acid, isobutyl octadecyl ester, 2-hexadecanol, Phthalic acid, butyl hex-3-yl ester, Trans-13-octadecenoic acid methyl ester, Methyl stearate, As-indacen-1(2H)-one, 3,6,7,8-tetrahydro-3,3,6,6-tetramethyl. The class of compounds identified are alcohol, phthalates, carbonyl and fatty esters.

Gas chromatography-Mass spectrometery analysis of fractions 39-49 also reveals the presence of hexadecanoic acid methyl ester (18.509%) and diisooctyl phthalate (15.445%). Following these compounds in order of percentage abundance is Trans-13-octadecenoic acid, methyl ester (10.624%) which has been reported to be anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, antileukotriene—D4, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge and flavoring [29].

Fractions 50-53 revealed the presence of 28 compounds, 14 were identified from the NIST library coupled with the Gas Chromatograph-mass spectrometer. The first compound to be identified was Phenol, 2,4-bis(1, 1-dimethylethyl)-, with retention time of 20.137 while the last was phenol-4,4'-(1-methylethylidene) bis- with retention time 42.162 minutes. The compounds with the highest percentage composition were hexadecanoic acid methyl ester, Phenol, 2,4-bis(1, 1-dimethylethyl)-, Phenol-4,4'-(1-methylethylidene)bis- and 11-octadecenoic acid, methyl ester which constitutes 17.300%, 12.341%, 10.001% and 9.193% respectively. Other compounds present in the composite fraction are Undecanoic acid, 10-methyl-, methyl ester, 2-hexadecanol, Cyclopropanebutanoic acid, 2-[[2-2[(2-pentylcyclopropyl) methyl] cyclopropyl]methyl] cyclopropyl]methyl]-, methyl ester, 2-hexadecanol, 9, 10-secocholesta-5,7,10(19)-triene-3,24,25,triol, (3β, 5Z, 7E)-, Hexadecanoic acid, methyl ester, Benzene propanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, 2-hexadecanol, 9-octadecene. 1,1'-[1,2-ethanediylbis(oxy)bis-(Z,Z)- Z,Z-3,15-Octadecadien-1-ol acetate, Heptadecanoic acid, 16-methyl-, methyl ester. The classes of compounds identified in this fraction are more of alcohols and fatty esters. Others are acetate, alkene, phenols and steroids.

Phenol, 2,4-bis(1,1-dimethylethyl) has been reported to be antimicrobial antifungal antitumor [30-34].

Fraction 54-61 revealed the presence of four compounds which were identified as fatty acid esters. 9-octadecenoic acid, 12-hydroxy-, methyl ester [R-(Z)] - takes the largest percentage with 85.643% of the component present in the combined fractions. Other compounds present are Hexadecanoic acid, methyl ester, 9, 12-octadecadienoic acid (Z, Z)-, methyl ester and 9-octadecenoic acid, methyl ester, (E)- with percentage composition of 2.912%, 5.472% and 5.982% respectively.

In terms of the identified phytochemicals, the GC-MS analysis of the methanolic leaf extract shows that the leaves of Crossopteryx febrifuga contain fatty acids, fatty acid esters, carbonyl compounds, phenolics, steroids and alcohols. These fatty acids content of the leaves of Crossopteryx febrifuga are majorly responsible for the antibacterial and antifungal activity of the leaves.

Conclusion

The research work carried out shows that Crossopteryx febrifuga contains phytochemicals such as flavonoids, steroids and saponins. The methanolic extract of the leaves contain a total phenolic content of 44.40 ± 0.11 mg Gallic acid equivalent/g extract, total flavonoid content of 10.50 ± 0.12 mg Quercetin equivalent/g extract and total saponin content of 91.30 ± 0.17 mg Ginsenoside Rb 1 equivalent/g extract. The high saponin and phenolic content indicates that the plant can be used as a natural source for antioxidant and can be used to explore new drug leads.

The GC-MS analysis revealed the presence of a total of 89 compounds in all fractions out of which only 43 of these compounds were identified. Among the compounds identified, the prominent ones are hexadecanoic acid methyl ester, diisooctyl phthalate, trans-13-octadecenoic acid methyl ester, phenol-2, 4-bis(1,2-dimethylethyl)-, and 9-octadecenoic acid, 12-hydroxy-, methyl ester [R-(Z)]- which are mainly fatty acid methyl esters and phthalate and alcohol. The presence of these compounds which makes up averagely 51.86% of the total composition of the butanolic fraction of the methanolic extract complement the knowledge of the biological activities of the plant leaves which are majorly antibacterial and antifungal and also its uses in treatment of ailments such as cough, skin diseases, worms and scabies because these fatty acid ethyl esters also possess antioxidant capacity.

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