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STUDIES ON PHYTOCHEMICAL SCREENING OF *Parkia biglobossa* ETHANOL AND HEXANE EXTRACT USING GC-MS ANALYTICAL TECHNIQUE

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ABSTRACT
Plants are rich source of secondary metabolites with potential biological activities against insects and pathogens. The objective of the present study is to characterize possible bioactive phytochemical constituents in the extract of stem bark and pod part of *P. biglobossa* using ethanol and Hexane as solvent using Gas Chromatography-Mass Spectrometry (GC-MS) analytical technique. The result of the study revealed the presence of various compounds like n-Hexadecanoic acid, Olean-12-en-28-al, 1,2,3-Benzenetriol, Octadecanoic acid, Phenol, 2,4-bis(1,1-dimethylethyl)-, 9,12-Octadecadienoic acid methyl ester and Vitamin E (2H-1-Benzopyran-6-ol) in ethanolic and hexane extract of pod. The result further showed that n-Hexadecanoic acid, Oleic Acid, 9,12-Octadecadienoic acid (Z,Z)-, 1,2,3-Benzenetriol, 9,12-Octadecadienoic acid metal esther, 9-Octadecenoic acid and Glycerin are present in ethanol and Hexane extracts of stem bark. These compounds have potential anti-microbial, anti-oxidant, pesticide and anticancer activity among others.

Key words: Ethanol extracts, G.C-MS analysis, Hexane Extract *P. biglobossa*, Phytochemical.

INTRODUCTION
Parkia biglobossa is found in a wide range of environments in Africa and is primarily grown for its pods that contain both a sweet pulp and valuable seeds. Various parts of the locust bean tree are used for medicinal purposes {[3]}. Indigenous healers in Africa use different parts of the locust bean tree for health benefits {[13]}. The tree was also one of two plants "listed as having real wound-healing properties in South-Western Nigeria, influencing the proliferation of dermal fibroblasts significantly {[3]}. Pods of Locust bean (*P. biglobossa*) contain the highest chemical called alkaloid parkine that occurs in pods and bark which may be responsible for the phytochemical action againt insect and pathogens {[14]}. Fayinminu{[9]} reported that, aqueous extract of the pod husk of Locust bean contain quantum of chemicals with the highest phytochemicals which formed the basis of using it as a bio-pesticide. Indigenous healers in Africa use different parts of the locust bean tree for health benefits. *P. biglobosa* was one of the highest cited plants used for treating hypertension {[13]}. The extracts from *P. biglobosa* have been used as natural pesticides against different pests and diseases due to the phytochemicals present in different parts of the plant. Phytochemicals are certain non-nutritive, biologically active plant chemicals which have some disease and insect preventive properties. They provide plants with colour, flavour and natural protection against pests {[16]}. Fawole{[10]} discovered that, *Fusarium* wilt of cowpea could be managed with *Parkia* husk ethanolic extract. The effectiveness of *P.biglobosa* ethanolic seed extract in controlling *Meloidogyne incognita* infestation in tomato plants was reported {[5]}. Also {[18]} discovered the antifeedant effect of petroleum ether and ethanolic seed extracts of *Parkia biglobosa* against cowpea bean storage pest (*Callosobruchus maculatus*). {[6]} determined the phytochemical composition

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of aqueous extracts of leaves and pods of *P. biglobosa* and found out that aqueous extracts of the pods had the highest phytochemical composition. This study therefore aims in determining the metabolite present in stem bark and pod of *P. biglobodssa* using GC-MS analytical technique.

Materials and Method

Collection and Preparation of Locust Bean Pod and Stem Bark

The locust bean pod and stem bark was collected from the locust bean trees from Gubi campus of Abubakar Tafawa Balewa University Bauchi. The plant materials were washed with water to remove dirty material and it was dried under the shade to prevent the denaturation of chemical substance for about two weeks. It was grounded, sieved and it was store in a polythene bag in the laboratory.

Extraction Method

The stem bark and pod powder was extracted using ethanol and hexane solvent separately. The extraction was performed by weighing 80 g of each grounded samples, mixing them with 250 mL of hexane and ethanol in 500 mL conical flask and subjected to soaking for 24 hours. The solvent suspension was filtered and concentrated using rotary evaporator to yield the crude extract. The crude extracts were stored in an amber bottle at 4°C until further analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Soxhlet Extracted Oils

The extract oil were analysed separately, one after another by subjecting them to chemical profiling via GC-MS technique as described by {[17]}. The analysis was perform using a QP2010SE SHIMADZU, Japan equipped with GC-2010 capillary column with viscosity comp time: 0.2 sec pumping time: 1 sec. Injecting volume 1uL, Injection Mode: Splitless, Purge Flow: 3.0 mL/min, Pumping Times: 3, port dwell time: 0.3 sec. Washing volume: 8 UL, column oven temperature 60.0°C, injection temperature: 250.00°C, flow control mode: linear velocity, pressure, 108. kPa, total flow: 9.8 mL/min, column flow; 3.22 mL/min, linear velocity: 46.3 cm/sec, purge flow: 3.0mL/min, split ratio: 1.1. The oven temperature was set between 80.0°C to 280.0°C, hold time between 1.00-5.00min at a rate of 10°C/min. They were Equilibrium Time: 1.0 min, ion source temperature: 230-00°C, interface temperature: 250.00°C, solvent cut time: 3.50 min, threshold: 1000 start time: 4.50 min, end time: 21.80 min, event time: 0.50sec, Start m/z 45.00, End m/z: 700.00 scan speed: 1428, sample inlet unit: GC. The chemical compounds in the oil were identified based on GC retention time on GC-2010 capillary column matched with EL MS library of the NIST/EPA/NIH mass spectral library according to NIST (2005). The analysis was carried out BOB GLOBAL RESOURCES LIMITED www.bobglobalresources.com

Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology NIST. The spectrum of the known components stored in the NIST library. The name, molecular weight retention time, peak area

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percentage and chemical structure of the components of the test materials were ascertained using GC- 17A version Programming software.

RESULTS AND DISCUSSION

The result of GC-MS analysis revealed the presence of 69, 24, 53 and 22 bio-active compounds in Pod ethanol, Pod Hexane, Stem bark ethanol and Stem bark Hexane extract of *P. biglobossa* respectively. Ten compounds with the highest peak area percentage were reported on the basis of their peak area percentage and biological activity. The compounds with the highest peak area percentage in ethanol extract of pod were cis-9-Hexadecenal (7.81%), follow by 9-Octadecenamide (Z)- (6.71%), Alpha.-D-Glucopyranoside (4.30%), n-Hexadecanoic acid (4.17%), 9-Heptadecanone (3.35%), Olean-12-en-28-al (3.19%), 1,2-Cyclopentanedimethanol (3.13%), 3-hydroxy-4,4-dimethyl (2.97%), 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dion, 1,2,3-Benzenetriol and 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl (2.33%) (Table 1). More so, the compound identified in hexane extract of pod were 1,2,3-Benzenetriol (16.82%), cis-9-Hexadecenal (13.86%), Octadecanoic acid (13.63%), 7-Hexadecenoic acid (6.42%) methyl ester, (Z)-, Phenol, 2,4-bis(1,1-dimethylethyl)-(5.34), 9,10-Secocholesta-5,7,10(19)-triene-1,3-diol (5.25%), Vitamin E (2H-1-Benzopyran-6-ol) (4.91%), (R)-(-)-14-Methyl-8-hexadecyn-1-ol (4.41%), cis-5,8,11-Eicosatrienoic acid (4.11%) and 9,12-Octadecadienoic acid (3.01%) (Table 2). In addition, the predominant compounds in stem bark extract were, n-Hexadecanoic acid (16.94%), 9-Octadecenamide(Z)- (11.31%), Oleic Acid (10.86%), 1,2,3-Benzenetriol (3.83%), 2H-Pyran2-(2-heptadecynyloxy)tetrahydro (3.67%), cis-11-Eicosenamide (3.61%), 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dion (2.92%), Bis(tridecyl) phthalate (1.91%), cis-Z-.alpha.-Bisabolene epoxide (1.88%) and 2-Naphthalenol, 1,2-dihydro- acetate (1.80%) in that order (Table 2). However, the compounds identified in Stem Bark hexane Extract were 9,12-Octadecadienoic acid (Z,Z)- (49.34%), n-Hexadecanoic acid (18.43%), Octadecanoic acid (13.82%), 9-Octadecenoic acid (Z)- (2.69%), 9,12-Octadecadienoic acid (2.24%), 1,2,4-Benzenetriol, 1,2,3- (2.13%) Benzenetriol (2.04%), Hexadecanoic acid methyl ester (1.43%), Glycerin (1.47%) and 9-t-Butyltricyclo [4.2.1.1(2, 5)] decane-9, 10-diol (1.08%) in that order (Table 2).

Furthermore, molecular weight, molecular formula and chemical formula of bio-compound identified with GC-MS analysis were presented (Table 3-6). From the result alpha.-D-Glucopyranoside recorded the highest (504 g/mol) molecular weight followed by 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl (452 g/mol.) and 1,2,3-Benzenetriol recorded the lowest (126 g/mol.) molecular weight in ethanol extract of pod (Table 3). The result further revealed that, 9,10-Secocholesta-5,7,10(19)-triene-1,3-diol have the highest (488 g/mol) molecular weight followed by Vitamin E (2H-1-Benzopyran-6-ol) (430 g/mol.) and 1,2,3-Benzenetriol have the lowest (126 g/mol.) molecular weight in hexane extract of pod (Table 4). The result also revealed that, Bis(tridecyl) phthalate have the highest (530 g/mol.) molecular weight followed by 2H-Pyran, 2-(2-heptadecynyloxy)tetrahydro (336 g/mol.) and 1,2,3-Benzenetriol (126 g/mol.) in ethanol extract of stem bark (Table 5). In addition 9-Octadecenoic acid (Z)- having the highest (296 g/mol.) molecular weight and

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was followed by 9,12-Octadecadienoic acid (294 g/mol.) and Glycerin (96 g/mol.) have the lowest molecular weight in hexane extract of stem bark (Table 6.).

In the present study, the GC-MS analysis of both ethanol and hexane extracts of *P. biglobossa* led to the identification of different type of chemical compounds which include hexadecanoic acid methyl ester, 9-octadecenoic acid methyl ester), n-hexadecanoic acid methyl ester, Vitamin E (2H-1-Benzopyran-6-ol), 1,2,3-Benzenetriol, Olean-12-en-28-al, 9,12-Octadecadienoic acid (Z,Z)-, Phenol, 2,4-bis(1,1-dimethylethyl)-, Oleic acid and Glycerin. These compounds possess several bioactivities including antifungal, antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic flavour, haemolytic, 5-alpha reductase inhibitor, potent antimicrobial activity as reported by{[4]}{[5]}{[11]}{[14]}{[20-22]}{[26]}{[28]}{[29]}. Phytochemical compound of 9-octadecenoic acid, methyl esters have also been reported as Antibacterial and antifungal {[8]}.

Table 1: Brief Description of Bio-activity of Chemical Compounds Identified in Ethanol and Hexane Extract of Pod of *Parkia biglobossa* Using GC-MS analytical Techniques

Solvent	Compound Name	Retention Time (sec.)	Peak Area percentage (%)	Bio-activity
Ethanol	cis-9-Hexadecenal	17.28	7.81	No activity reported.
	9-Octadecenamide, (Z)-	18.62	6.71	No activity reported.
	Alpha.-D-Glucopyranoside	14.46	4.30	No activity reported.
	n-Hexadecanoic acid	16.13	4.17	Antioxidant, Nematicide, Pesticide, Lubricant, Haemolytic {[11]}{[28]}
	9-Heptadecanone	18.68	3.35	No activity reported.
	Olean-12-en-28-al	21.31	3.19	Anti-microbial, therapeutic
	1,2-Cyclopentanedimethanol, 3-hydroxy-4,4-dimethyl	19.75	3.13	No activity reported.
	1,2,3-Benzenetriol	11.73	2.48	Antioxidant, Antiseptic, Antibacterial, Antidermatitic, Fungicide, Pesticide,Candidicide {[26]}
	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl	17.80	2.33	No activity reported.
	Total		40.43%	.
Hexane	1,2,3-Benzenetriol	11.70	16.82	Pesticide, Antioxidant, Antiseptic, Antibacterial,

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				Antidermatitic, {[26]}
	cis-9-Hexadecenal	17.28	13.86	No activity reported.
	Octadecanoic acid	16.13	13.68	Antifungal, Antibacterial{[28]}
	7-Hexadecenoic acid, methyl ester, (Z)-	16.98	6.42	No activity reported.
	Phenol, 2,4-bis(1,1-dimethylethyl)-	12.53	5.34	Antioxidant, anti-inflammatory, Fungicide Insecticide{[11]}
	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol	20.56	5.25	No activity reported.
	Vitamin E (2H-1-Benzopyran-6-ol)	18.66	4.91	antidiabetic, Antidermatitic, Antileukemia, Anticancer, Vasodilator, Hepatoprotective, Hypocholesterolemic, Antibronchitic, Anticoronary{[20]}
	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	17.52	4.41	No activity reported.
	cis-5,8,11-Eicosatrienoic acid	21.55	4.11	No activity reported.
	9,12-Octadecadienoic acid methyl ester	16.95	3.01	Hepatoprotective, Anti-histaminic, Antieczemic , Hypocholesterolemic{[20]}
	Total		77.81	

Table 2: Brief Description of activity of Chemical Compounds Identified in Ethanol and Hexane Extract of Stem bark of *Parkia biglobossa* Using GC-MS analytical Techniques

Solvent	Compound Name	Retention Time(min.)	Peak Area Percentage (%)	Bio-activity
Ethanol	n-Hexadecanoic acid	16.13	16.94	Anticancer, anti-imflamatory{[14]}{[28]}
	9-Octadecenamide	18.62	11.31	No activity reported.
	Oleic Acid	17.29	10.86	Cancer preventive, Anemiagenic, Insectifuge, Antiandrogenic, Dermatitigenicpreventive, Anemiagenic, Insectifuge, Antiandrogenic, Dermatitigenic{[14]}{[26]}{[28]}
	1,2,3-Benzenetriol	11.63	3.83	Antioxidant, Antiseptic, Antibacterial, Antidermatitic Fungicide, Pesticide, Antimutaginic Dye

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				Candidicide{[26]}
	2H-Pyran, 2-(2-heptadecynyloxy)tetrahydro	20.47	3.67	No activity reported.
	cis-11-Eicosenamide	19.75	3.61	No activity reported.
	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dion	17.41	2.92	No activity reported.
	Bis(tridecyl) phthalate	19.49	1.91	
	2-Naphthalenol, 1,2-dihydro-, acetate	8.74	1.80	No activity reported
Hexane	9,12-Octadecadienoic acid (Z,Z)-	16.98	49.34	Anti-cancer, Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Antiandrogenic, Hypocholesterolemic 5-Alpha reductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic,Antiacne {[20]}{[4]}
	n-Hexadecanoic acid	16.18	18.43	Antioxidant, Pesticide, Flavor, 5Alpha Reductase inhibitor, Antifibrinolytic, Hemolytic, Lubricant, Nematicide, Antialopecic{[14]}{[8]} {[28]}
	Octadecanoic acid	17.46	13.82	Antioxidant, Antimicrobial activity {[28]}
	9-Octadecenoic acid	16.98	2.69	Antihypertensive, increase HDL and decrease LDL{[20]}
	9,12-Octadecadienoic acid metal esther	16.95	2.24	Hepatoprotective, Anti-histaminic, Antieczemic , Hypocholesterolemic{[20]}
	1,2,4-Benzenetriol	12.76	2.13	No activity reported.
	1,2,3-Benzenetriol	11.63	2.04	Antioxidant, Antiseptic, Antibacterial, Antidermatitic Fungicide, Pesticide, Antimutaginic Dye Candidicide{[26]}
	Hexadecanoic acid, methyl ester	15.82	1.43	Antioxidant, Pesticide, Flavor, Antifibrinolytic, Hemolytic, Lubricant, Nematicide,

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				Antialopecic {[20]}{[11]}
	Glycerin	6.43	1.47	Reduce inflammation, anti-microbial {[21]}
	9-t-Butyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	19.49	1.08	No activity reported.

Table 3: Chemical Composition, Molecular Weight, Molecular formula and Chemical Structure of Ethanol Pod Extract of *Pakia biglobossa* Revealed by GC-MS Analysis

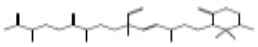
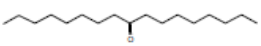
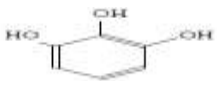
S/N	Compound Name	Molecular weight (g/mol.)	Molecular formula	Chemical structure
1	alpha.-D-Glucopyranoside	504	$C_{18}H_{32}O_{16}$	
2	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl	452	$C_{33}H_{56}$	
3	Olean-12-en-28-al	424	$C_{30}H_{48}O$	
4	9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	
5	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	
6	9-Heptadecanone	254	$C_{17}H_{34}O$	
7	cis-9-Hexadecenal	238	$C_{16}H_{30}O$	
8	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dion	212	$C_{11}H_{16}O_4$	
9	1,2-Cyclopentanedimethanol, 3-hydroxy-4,4-dimethyl-	174	$C_9H_{18}O_3$	
10	1,2,3-Benzenetriol	126	$C_6H_6O_3$	

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Table: 4 Chemical Composition, Molecular Weight, Molecular formula and Chemical Structure of Pod Hexane Extract of *Pakia biglobossa* Revealed by GC-MS Analysis

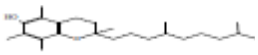
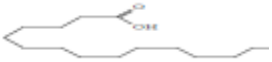
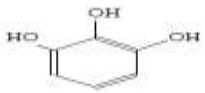
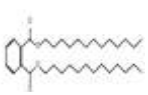
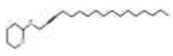
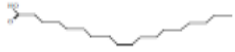
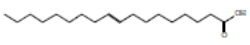
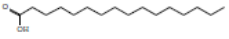
S/N	Compound Name	Molecular weight (g/mol.)	Molecular formula	Chemical Structure
1	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol	488	$C_{30}H_{52}O_3Si$	
2	Vitamin E (2H-1-Benzopyran-6-ol)	430	$C_{29}H_{50}O_2$	
3	cis-5,8,11-Eicosatrienoic acid	378	$C_{23}H_{42}O_2Si$	
4	Octadecanoic acid	284	$C_{18}H_{36}O_2$	
5	9,12-Octadecadienoic acid	280	$C_{18}H_{32}O_2$	
6	7-Hexadecenoic acid, methyl ester, (Z)-	268	$C_{17}H_{32}O_2$	
7	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	252	$C_{17}H_{32}O$	
8	cis-9-Hexadecenal	238	$C_{16}H_{30}O$	
9	Phenol, 2,4-bis(1,1-dimethylethyl)-	206	$C_{14}H_{22}O$	
10	1,2,3-Benzenetriol	126	$C_6H_6O_3$	

Table 5: Chemical Composition, Molecular Weight, Molecular formula and Chemical Structure of Stem Bark Ethanol Extract of *Pakia biglobossa* Revealed by GC-MS Analysis

S/N	Compound Name	Molecular weight (g/mol.)	Molecular formula	Chemical structure
1	Bis(tridecyl) phthalate	530	$C_{34}H_{58}O_4$	
2	2H-Pyran, 2-(2-heptadecynyloxy)tetrahydro	336	$C_{22}H_{40}O_2$	
3	cis-11-Eicosenamide	309	$C_{20}H_{39}NO$	
4	Oleic Acid	282	$C_{18}H_{34}O_2$	
5	9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	
6	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	

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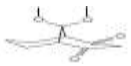
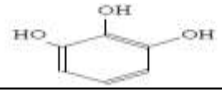
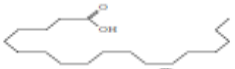
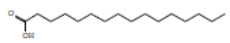
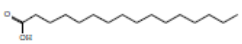
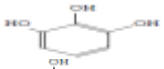
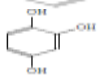
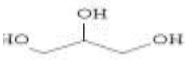
7	cis-Z-.alpha.-Bisabolene epoxide	220	$C_{15}H_{24}O$	
8	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dion	212	$C_{11}H_{16}O_4$	
9	2-Naphthalenol, 1,2-dihydro-, acetate	188	$C_{12}H_{12}O_2$	
10	1,2,3-Benzenetriol	126	$C_6H_6O_3$	

Table: 6 Chemical Composition, Molecular Weight, Molecular formula and Chemical Structure of Stem Bark Hexane Extract of *Pakia biglobossa* Revealed by GC-MS Analysis

S/N	Compound Name	Molecular weight (g/mol.)	Molecular formula	Chemical Structure
1	9-Octadecenoic acid (Z)-	296	$C_{19}H_{36}O_2$	
2	9,12-Octadecadienoic acid	294	$C_{19}H_{34}O_2$	
3	Octadecanoic acid	284	$C_{18}H_{36}O_2$	
4	9,12-Octadecadienoic acid (Z,Z)-	280	$C_{18}H_{32}O_2$	
5	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	
6	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	
7	1,2,3-Benzenetriol	126	$C_6H_6O_3$	
8	1,2,4-Benzenetriol	126	$C_6H_6O_3$	
9	9-t-Butyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	224	$C_{14}H_{24}O_2$	
10	Glycerin	92	$C_3H_8O_3$	

CONCLUSION

The presence of several bio-active compounds in plant ascertains their use to remedy several diseases and insect pest by the local farmers. It could be concluded that stem bark and pod of *P. biglobossa* contain bio-active compound that could be used to treat several diseases and pests.

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