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Original Article

Chemical Characteristics and Biological Activities of *Annona squamosa* Fruit Pod and Seed Extracts

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Abstract

Background and objectives: *Annona squamosa* (*A. squamosa*) is a medicinal plant, used in ethnomedicinal treatment of various ailments. However, there is a dearth of information on the chemical constituents of this plant's fruit pod and chemical parameters of the seed oil. The objectives of this study were, therefore, to determine the chemical characteristics and biological activities of extracts of the fruit pod and seed oil of *A. squamosa.*

Methods: Crude methanol extract of the dried and pulverized fruit pod were partitioned using n-hexane and dichloromethane (DCM), the fractions concentrated *in-vacuo* to yield n-hexane and DCM fractions of the fruit pod. The n-hexane extract of the dried ground seed was concentrated *in vacuo* to afford the seed oil. The fractions and the seed oil were subjected to gas chromatography-mass spectroscopy (GC-MS) analysis. The seed oil was characterized for chemical properties using standard methods. The seed oil, crude methanol extract of seed pod and fractions were assayed for antibacterial properties using both Gram-positive and Gram-negative bacteria. The seed oil was also examined for antioxidant activity.

Results: The results from chemical analyses of the seed oil indicated that acid value, iodine value, saponification value and total phenol were 1.91 (as % oleic acid), 109.8 g I_2/kg, 204.8 g KOH/kg and 36.2 mg gallic acid equivalent (GAE)/kg, respectively. GC-MS analysis revealed the presence of 14, 8 and 15 compounds in n-hexane and DCM fractions of the fruit pod and seed oil, respectively. Of the compounds identified, octadec-9-enoic acid, 9,10-dehydroisolongifolene and androsterone were the most abundant. The extracts displayed broad spectrum antibacterial activity against the 13 bacterial strains tested, except for *Bacillus polymyxa*, *Enterococcus faecalis* and *Bacillus cereus*, which were resistant to the n-hexane and DCM fractions of the fruit pod.

Conclusions: The findings in this study indicated that the extracts and oil of *A. squamosa* contain bioactive compounds which have antibacterial and antioxidant properties, and the oil could be applied both as industrial and edible oil.

Keywords: *Annona squamosal*; Antibacterial activity; Antioxidant activity; Octadec-9-enoic acid; Iodine value; Saponification value.

Abbreviations: AV, acid value; AOAC, Association of Official Analytical Chemists' methods; DCM, dichloromethane; DPPH, 2, 2-diphenyl-1-picryl-hydrazil; FRP, Ferric ion reducing power; FFA, free fatty acid; GAE, gallic acid equivalent; GC-MS, gas chromatography-mass spectroscopy; IV, iodine value; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentrations; NIST, National Institute of Standard Technology; SV, saponification value; TP, total phenol..

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Introduction

The growing resistance of pathogenic bacterial isolates against antibiotics as well as resurgence of old disappeared diseases have lead researchers to focus on bioactive natural compounds that will be effective, with no side effect, in treatment of diseases.

Annona squamosa belongs to Annonaceae family, which comprises about 135 genera and over 2,300 species.[1,2] The most important genera having the largest number of species are *Annona*, with 166 species. *A. squamosa* is commonly known as custard apple, sweet sop and sugar apple and is cultivated in tropical areas

and sub-tropical regions worldwide.[3] The plant is an evergreen tree which reaches 3–8 m in height. The leaves are lanceolate, 6–17 cm in length and 3–5 cm in width, while its fruits are 5–10 cm in diameter, with many round protuberances, and can be either heart-shaped, conical, ovate, or round. The seeds of the plant are 1.3–1.6 cm long; they are smooth, shiny, blackish or dark brown in color.[4]

The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problem, worm infection, constipation, hemorrhage, dysentery, fever, and ulcer,[5] and also reported to possess antidiabetic activity.[6] Different parts of *A. squamosa* have been used in the treatment of various ailments and human diseases because the plant contains several bioactive compounds. The plant is said to possess biological activities, such as analgesic, anti-inflammatory, antimicrobial, cytotoxic, antioxidant, antilipidimic, antiulcer hepatoprotective, vasorelaxant, antitumor larvicidal insecticidal anthelmintic, molluscicidal properties, and genotoxic effect.[7] The fruit of *A. squamosa* has hematinic, sedative, stimulant and expectorant properties and are also useful in treating anemia and burning sensation.[8] The seeds are useful in treating lice infection in the hair.[9]

Hopp *et al.*[5] isolated three annonaceous acetogenins (9-hydroxy asimicinone, squamoxinone B and C) from bark of *A. squamosa*. In spite claims of the medicinal properties of the *A. squamosa* plant, there is dearth of empirical information on the chemical composition and biological activities of the plant's fruit pod and seed oil. Therefore, this study was designed to investigate the extracts of the fruit pod and seed for chemical constituents, antioxidant activity, and anti-microbial properties. The results of this study will provide empirical information that justifies the use of *A. squamosa* for medicinal purpose, and the possibility of harnessing its oil for nutritional and industrial purposes.

Materials and methods

Plant collection

A. squamosa fruit pod and seeds used for this study were collected in Ile-Ife, southwest of Nigeria, identified at the Herbarium in the Department of Botany, Obafemi Awolowo University, Ile-Ife (voucher number: IFE-17927).

Extraction of A. squamosa seed

The seed was removed from the capsule, dried, pulverized, packed in air-tight plastic containers and kept in the freezer until use. The pulverized sample of the seed was soaked in distilled n-hexane for 72 h, after which it was filtered and concentrated using a rotary evaporator at 40 °C. The extract thus obtained was labeled n-hexane extract and kept in a desiccator, and subsequently used for both biological assay and gas chromatography-mass spectroscopy (GC-MS) analysis. The extraction of the seed oil for chemical analysis was carried out using soxhlet extractor and n-hexane as the extracting solvent.

Extraction and partitioning of A. squamosa fruit pod

The dried and pulverized fruit pod (42 g) was soaked in distilled methanol for 48 h, after which it was filtered. The extraction process was repeated thrice, for optimum yield. The extracts were pooled and then concentrated using a rotary evaporator at 40 °C. The crude methanol extract thus obtained was partitioned with n-hexane and DCM to afford respective fractions, which were kept for further analysis.

GC-MS analysis of the samples

The n-hexane extract of the seed (seed oil), and n-hexane and DCM fractions of the fruit pod were taken for GC-MS analysis. The samples were analyzed using gas chromatography (19091J-413; Agilent, Santa Clara, CA, USA) coupled to a mass spectrometer (model 5975C) with triple-axis detector equipped with an auto injector (10 µL syringe). Helium gas was used as the carrier gas.

All chromatography was performed on a capillary column having specification length of 30 m, internal diameter of 0.2 µm, thickness of 320 µm, and treated with 5% phenyl methyl siloxane. Other GC-MS conditions were pressure of 3.2875 psi and a flow time of 1.5 mL/min. The column temperature started at 80 °C for 2 mins and increased to 280 °C at the rate of 3 °C/min for 20 mins. The total elusion time was 88.667 mins. Identification of the compounds was carried out by comparing the mass spectra obtained with those of the mass spectra from the National Institute of Standard Technology (NIST) library (NISTII).

Determination of chemical parameters of A. squamosa seed oil

The chemical parameters were determined as reported by the Association of Official Analytical Chemists' methods (AOAC 920.158; AOAC 936.15; AOAC 936.16; AOAC 933.08 for iodine, saponification, acid and peroxide values, respectively).[10]

Biological activity

Antibacterial sensitivity testing of the extracts

The antibacterial activity of n-hexane extract of the seed, and n-hexane and DCM fractions of the fruit pod were determined using the agar-well diffusion method described by Akinpelu *et al*.[11] The test organisms were reactivated in nutrient broth for 18 h before use. Exactly 0.1 mL of standardized test bacterial strains (10^6 cfu/mL of 0.5 McFarland standards) was transferred into Mueller-Hinton agar medium at 40 °C. This was thoroughly mixed together and later poured into pre-sterilized Petri dishes. The plates were allowed to set and wells were bored into the medium using a 6-mm sterile cork borer. These wells were then filled up with the prepared solutions of the extracts. Care was taken not to allow the solution to spill on the surface of the medium. The concentration of the extract used was 25 mg/mL, while the concentration of streptomycin used as positive control was 1 mg/mL. The plates were left on a laboratory bench for 1 h to allow proper in-flow of the solution into the medium before incubating them at 37 °C for 24 h. The plates were not stock-piled, to allow even distribution of temperature around the plates in order to avoid false results. The plates were later observed for zones of inhibition, which is an indication of susceptibility of the test organisms to the extracts.

Determination of minimum inhibitory concentrations (MIC) of the extracts

The minimum inhibitory concentration (MIC) of n-hexane seed extract, and n-hexane and DCM fractions of the fruit pod were

determined according to the method described by Akinpelu and Kolawole.[12] A 2 mL aliquot of different concentrations of the solution was added to 18 mL of pre-sterilized molten nutrient agar, to give final concentrations ranging between 0.39 and 12.5 mg/mL. The mixture was then poured into sterile Petri dishes and allowed to solidify. The plates were left on the laboratory bench overnight to ascertain their purity. The surfaces of the plates were allowed to dry well before striking with standardized inoculum of the test organisms and incubated aerobically at 37 °C for 48 h. The plates were later examined for the presence or absence of bacterial growth. The MIC was taken as the lowest concentration of the extracts that inhibited the growth of the test organisms.

Determination of minimum bactericidal concentration (MBC) of the extracts

The minimum bactericidal concentration (MBC) of the extracts were assessed by taking a sample from the streaked line of the MIC test and cultured on fresh sterile nutrient agar plates. The plates were incubated at 37 °C for 72 h. The MBC was taken as the concentration of the extracts that did not support the bacterial growth on the medium.

Antioxidant activity assay of the seed oil

The anti-oxidant activity of the seed oil was accessed through three parameters: the total phenol, ferric ion reducing power (FRP) and 2,2-diphenyl-1-picryl-hydrazil (DPPH) assay.

Determination of total phenol

Total phenol (TP) of the seed oil was measured as previously described by Moreno *et al*.[13] and estimated spectrophotometrically using Folin–Ciocalteu's phenol reagent assay with gallic acid as the standard.[14] The TP content was expressed as mg/kg gallic acid equivalent (GAE) and linearity range for the standard was between 0–40 mg/L GAE (R^2 = 0.9928).

Measurement of free radical scavenging activity

This was determined using the DPPH reagent, according to Brand-Williams *et al*.[15] The oil (0.5 mL) was put in screw cap test tubes, and 4 mL of methanol and 4 mL of 0.1 mmol L^{-1} methanol solution of DPPH were added and shaken. A blank probe was obtained by mixing 4 mL of 0.1 mmol L^{-1} methanol solution of DPPH and 0.5 mL of deionized distilled water (ddH_2O). After 30 mins of incubation in the dark at room temperature, the absorbance was read at 517 nm against the prepared blank. Various concentrations of standard catechin (0, 2, 4, 6, 8 and 10 mg/mL) were used to generate the standard curve, and the result was extrapolated from linear curve equation (y = 0.033x, R^2 = 0.995); the result was expressed as IC_{50} catechin equivalent.

Determination of FRP

The FRP assay was carried out according to Stratil *et al.*,[16] with slight modifications. FRP was measured using the potassium ferricyanide assay. The oil (1 mL) was added to 2.5 mL phosphate buffer (0.2M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%, w/v). The mixture was incubated at 50 °C for 20 mins. After adding trichloroacetic acid solution (2.5 mL, 10%, w/v), the mixture was separated into aliquots of 2.5 mL and diluted with 2.5 mL of water. To each diluted aliquot, 5 mL of ferric chloride solution was added. After 30 mins, absorbance was measured at 700 nm. Ascorbic acid was used as standard and the FRP value of extracts was expressed as the ascorbic acid equivalent (mg AAE/g), and the content was calculated from a linear equation of the standard y = 5.661x and R^2 = 0.988.

Statistical analysis

Results were expressed as mean and standard deviation of three determinations, and data were subjected to one-way analysis of variance to determine the levels of significant difference by performing a multiple comparison post-test (Tukey) and were considered significant at $p \leq 0.05$. GraphPad InStat version 3.06 for Windows 2003 was used for the analysis.

Results and discussion

GC-MS analysis: N-hexane fraction of A. squamosa fruit pod

The chromatogram of GC-MS analysis of the n-hexane fraction of the fruit pod and the chemical characteristics of compounds detected are presented in Figure 1 and Table 1, respectively.

This fraction contained a mixture of compounds, mainly monoterpenes, diterpenes, sesquiterpene and derivatives, fatty acids, and fatty acid esters. Fourteen compounds were identified, 9,10-dehydro-isolongifolene, a sesquiterpene is the main compound (20.90%) in this fraction (Table 1). Previously, 9,10-dehydro-isolongifolene was found in the wood oil of giant sequoia *(Sequoiadendron giganteum* (Lindl.) Buchh) by Jerković *et al.*[17] and reported to be one of the main constituents of the leaves essential oil of *Cedrelopsis grevei* which exhibited good anticancer, anti-inflammatory, antioxidant and antimalarial activities.[18]

DCM fraction of A. squamosa fruit pod

The gas chromatogram and list of chemical constituent of the DCM fraction of *A. squamosa* fruit pod are as shown in Figure 2 and Table 2, respectively.

Eight compounds were identified in the fraction, the major ones are androsterone (7.83%) and spathulenol (6.22%). Androsterone is a natural product which has been found in pine pollen and is well known in many animal species.[19] It is an inhibitory androstane neurosteroid,[20] acting as a positive allosteric modulator of the $GABA_A$ receptor[21] and exerts anticonvulsant effect.[22]

Spathulenol, a volatile oil, is a tricyclic sesquiterpene alcohol with basic skeleton similar to the azulenes. It occurs in waterwort distillery (*Artemisia vulgaris*) and tarragon (*Artemisia dracunculus*), among other plants.[23] It is an anesthetic and a vasodilator agent, possessing antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities.[24] Selene *et al.*[25] reported that spathulenol was identified as a major constituent in the essential oils of four Croton species, which displayed good antioxidant activity. According to them, spathulenol was active against the enzyme *Leishmania infantum* trypanothione reductase, showing

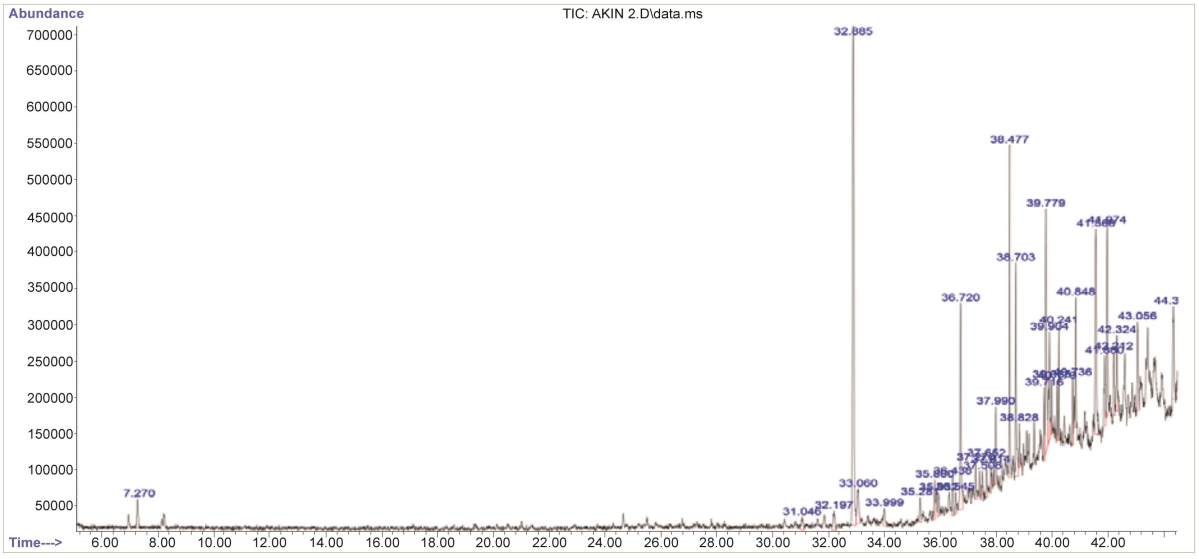


Fig. 1. Gas chromatogram of the n-hexane fraction of *A. squamosa* fruit pod.

Table 1. Chemical constituents of n-hexane fraction of *A. squamosa* fruit pod

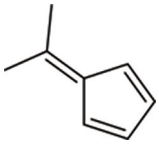
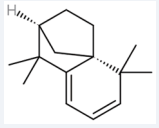
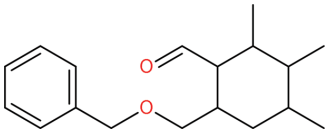
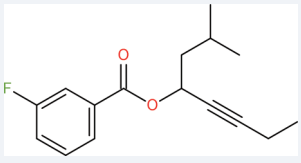
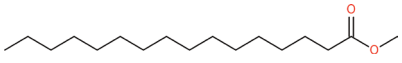
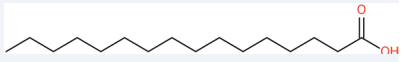
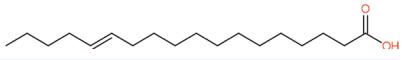
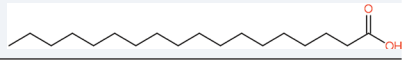
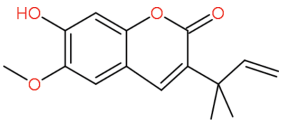
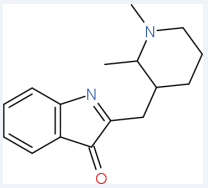
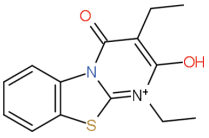
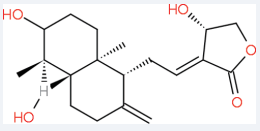
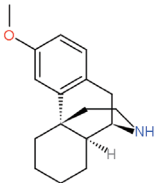
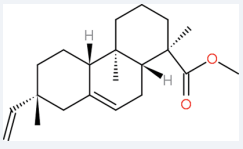
S/N	Compound	RT in m	PA, %	MF	MM in g/mol	Structural formula
1	5-(propan-2-ylidene)cyclopenta-1,3-diene	7.3	1.08	C_8H_{10}	106.16	
2	9,10-dehydro-isolongifolene	32.9	20.90	$C_{15}H_{24}$	204.35	
3	6-((benzyloxy)methyl-2,3,4-trimethylcyclohexyl) formaldehyde	33.1	1.91	$C_{18}H_{26}O_2$	274.40	
4	2-methyloct-5-yn-4-yl-3-fluorobenzoate	37.6	1.11	$C_{16}H_{19}FO_2$	262.14	
5	Methyl palmitate	38.5	6.20	$C_{17}H_{34}O_2$	270.45	
6	N-hexadecenoic acid	38.7	4.68	$C_{16}H_{32}O_2$	256.42	
7	Trans-13-octadecenoic acid	39.8	7.28	$C_{18}H_{34}O_2$	282.46	
8	Octadecanoic acid	39.9	3.11	$C_{18}H_{36}O_2$	284.48	

Table 1. Chemical constituents of n-hexane fraction of *A. squamosa* fruit pod *- (continued)*

S/N	Compound	RT in m	PA, %	MF	MM in g/mol	Structural formula
9	3-(1,1-dimethylallyl)- scopoletin	40.2	1.10	$C_{15}H_{16}O_4$	260.10	
10	2-[(1,2-dimethylpiperidin-3-yl) methyl]-3H-indol-3-one	40.2	2.08	$C_{16}H_{20}N_2O$	256.34	
11	1,3-diethyl-4-oxo-4H-benzo[4,5]thiazolo[3,2-a] pyrimidin-1-ium-2-olate	40.7	1.53	$C_{14}H_{15}N_2O_2S^+$	274.34	
12	Andrographolide	40.8	2.85	$C_{20}H_{30}O_5$	350.45	
13	Nordextromethorphan	41.6	7.12	$C_{17}H_{23}NO$	257.37	
14	(1R,4aR,4bS,7R,10aR)-methyl-1,4a,7-trimethyl-7-vinyl1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro phenanthrene-1-carboxylate	44.3	3.32	$C_{21}H_{32}O_2$	316.48	

MF, molecular formula; MM, molecular mass; PA, peak area; RT, retention time.

excellent interaction energies, making it a promising agent for leishmaniasis control.

N-hexane extract of A. squamosa seed

The chromatogram obtained from the GC-MS analysis of the n-hexane extract of the seed of *A. squamosa* is shown in Figure 3. The chemical compounds identified through comparison of the mass spectra, based on ≥50% matching, with the NIST library are listed with their retention time (RT) and peak area (PA%) in Table 3.

Fifteen compounds were identified from this extract, which constitutes 86.71% of the total detected compounds in the extract, and 9-octadecenoic acid is the main compound in this extract. 2,4-decadienal and 1-dodecanol are other compounds present in appreciable proportions. 9-octadecenoic is a monounsaturated fatty acid present in human diet in the form of its triglycerides and it is said to be responsible for the hypotensive effect of olive oil.[26] 2,4-decadienal was implicated in the nematicidal activity exhibited by *Ailanthus altissima* methanol extract against the root knot nematode *Meloidogyne javanica*.[27] Dodecanol or lauryl alcohol, is a fatty alcohol produced industrially from palm kernel oil or coconut oil, and it is used to make surfactants, lubricating oils, and pharmaceuticals. It is found to inhibit the activity of *Candida albicans*.[28] Anethole, a principal component of anise oil, has been found to prolong the transient antifungal effect of dodecanol.[29]

Antibacterial analysis

The crude methanol extract of the fruit pod (S1) and n-hexane extract of the seed inhibited the growth of all the bacterial strains tested. The other two fractions, that is n-hexane (S2) and DCM (S3) fractions of the fruit pod, inhibited 11 and 12 of the bacterial strains tested, respectively. Overall, both the extracts and fractions exhibited broad spectrum activities against the bacterial strains and compared favorably with the standard antibiotic-streptomycin

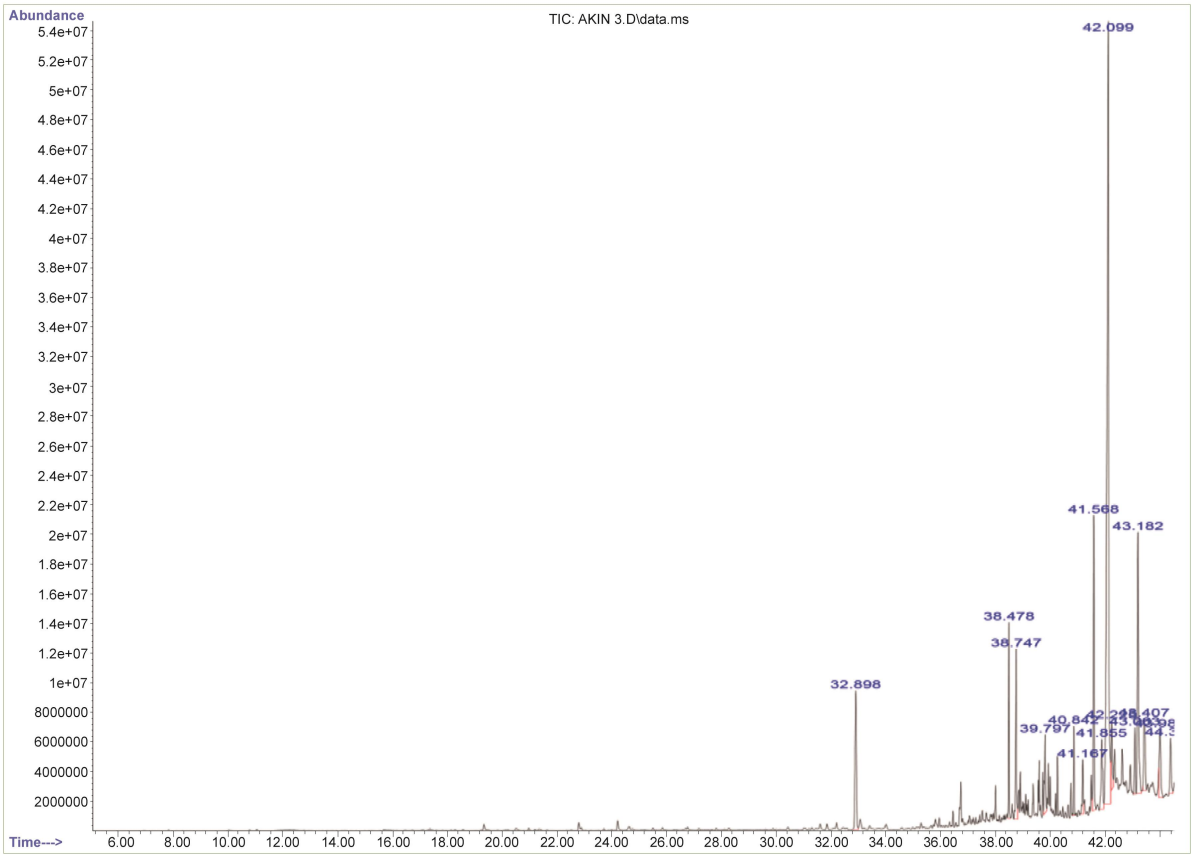


Fig. 2. Gas chromatogram of the DCM fraction of *A. squamosa* fruit pod.

Table 2. Chemical constituents of DCM fraction of *A. squamosa* fruit pod

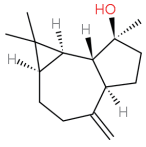
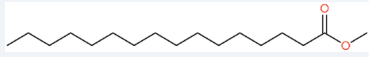
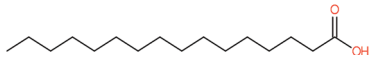
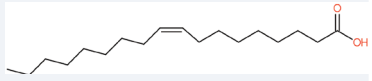
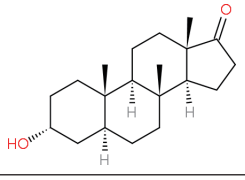
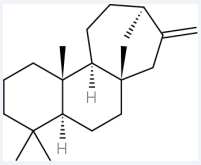
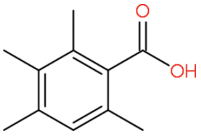
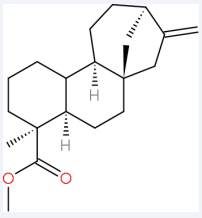
S/N	Compound	RT in m	PA, %	MF	MM	Structural formula
1	1,1,7-trimethyl-4-methylene decahydro-1H-cyclopropa[e]azulen-7-ol (spathulenol)	32.9	6.22	$C_{15}H_{24}O$	220.35	
2	Methyl palmitate	38.5	3.10	$C_{17}H_{34}O_2$	270.45	
3	N-hexadecanoic acid	38.7	4.72	$C_{16}H_{32}O_2$	256.42	
4	Oleic acid	39.8	2.63	$C_{18}H_{34}O_2$	282.46	
5	Androsterone	41.6	7.83	$C_{19}H_{30}O_2$	290.44	

Table 2. Chemical constituents of DCM fraction of *A. squamosa* fruit pod - *(continued)*

S/N	Compound	RT in m	PA, %	MF	MM	Structural formula
6	Kaur-16-ene	43.1	2.16	$C_{20}H_{32}$	272.47	
7	2,3,4,6-tetramethyl-benzoic acid	44.0	3.21	$C_{11}H_{14}O_2$	178.23	
8	Methyl-4,11-dimethyl-8-methylenetetradecahydro-6a,9-methanocyclohepta[a]napthalene-4-carboxylate	44.4	2.48	$C_{21}H_{32}O_2$	316.48	

MF, molecular formula; MM, molecular mass; PA, peak area; RT, retention time.

used as positive control (Table 4). The results obtained from the study support the usefulness of *A. squamosa* in folklore remedies to treat infections caused by pathogens in humans. This serves as a pointer towards the development of antimicrobial agents of natural origin for treatment of superbugs that have developed resistance against the available antibiotics.

Among the bacterial strains that were susceptible to the extracts from *A. squamosa* are *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus cereus* and *B. anthracis*, which are all known to cause infections in humans.[30] These pathogens are now gradually developing resistance against the available antibiotics used as therapy against infections caused by these pathogens. There is an urgent need to source potent antimicrobials, especially of natural origin, to combat infections caused by these pathogens. Thus, antimicrobials produced from *A. squamosa* may go a long way in healthcare delivery to take care of the menace of these pathogens.

MIC and MBC exhibited by extracts against bacterial strains

The results obtained from the MIC and MBC analyses of the ex-

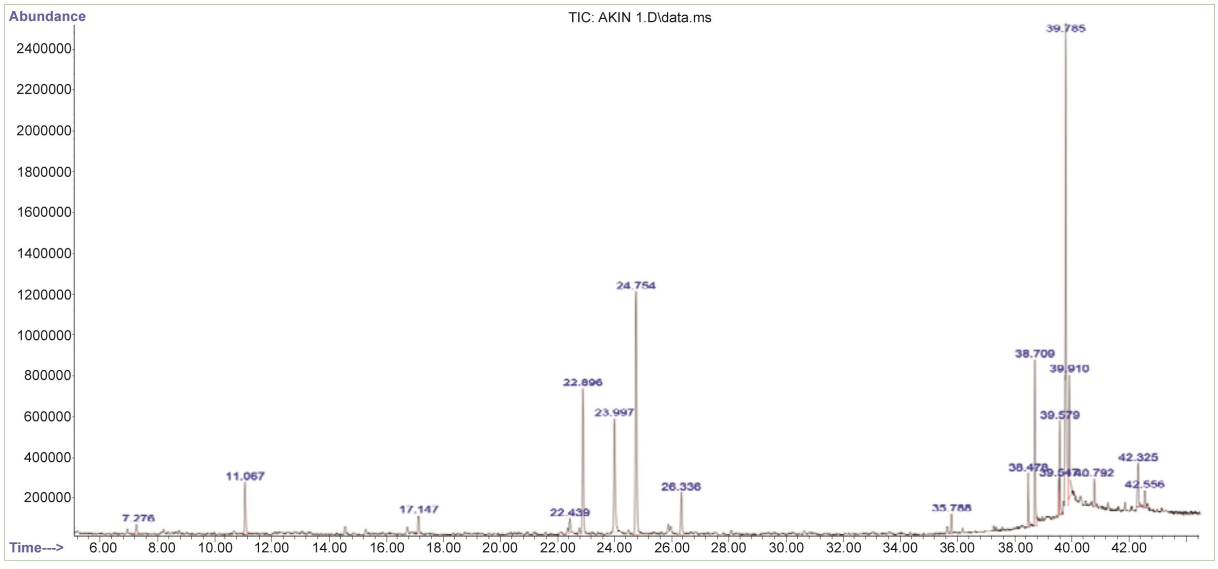
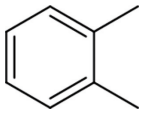
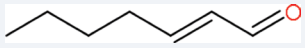
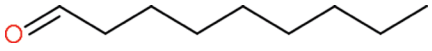
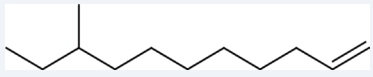
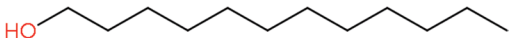
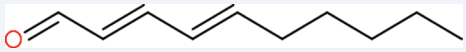
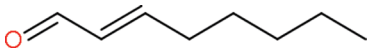
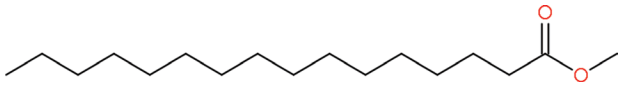
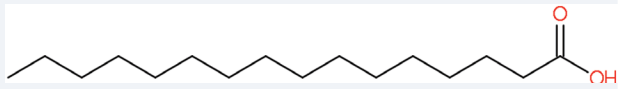
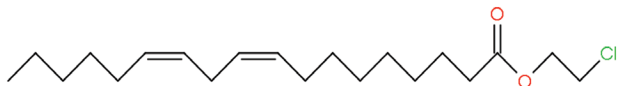
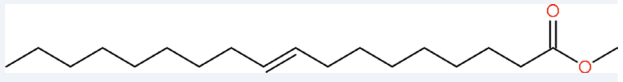
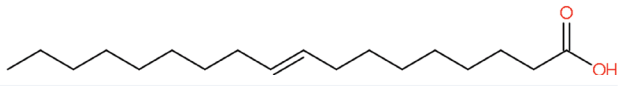
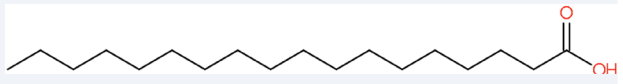
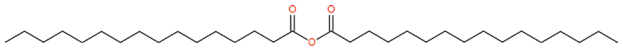


Fig. 3. GC-MS chromatogram of n-hexane extract of *A. squamosa* seed.

Table 3. Chemical constituents of n-hexane extract of *A. squamosa* seed

S/N	Compound	RT in m	PA, %	MF	MM	Structural formula
1	o-xylene	7.276	0.97	C_9H_{10}	106.16	
2	(E)-hept-2-enal	11.1	3.77	$C_7H_{12}O$	112.17	
3	Nonanal	17.1	1.37	$C_9H_{18}O$	142.24	
4	9-methyl-undec-1-ene	22.4	1.09	$C_{12}H_{24}$	168.32	
5	1-dodecanol	22.9	10.28	$C_{12}H_{25}O$	183.33	
6	(2E,4E)-deca-2,4-dienal	24.8	17.77	$C_{10}H_{16}O$	152.23	
7	(E)-oct-2-enal	26.3	3.15	$C_8H_{14}O$	126.20	
8	8-heptadecene	35.8	1.13	$C_{17}H_{34}$	238.45	
9	Methyl palmitate	38.5	2.06	$C_{17}H_{34}O_2$	270.45	
10	N-hexadecanoic acid	38.7	6.31	$C_{16}H_{32}O_2$	256.42	
11	2-chloroethyl linoleate	39.5	1.34	$C_{20}H_{35}ClO_2$	342.94	
12	(E)-methyloctadec-9-enoate	39.6	3.70	$C_{19}H_{36}O_2$	296.49	
13	(E)-octadec-9-enoic acid	39.8	26.37	$C_{18}H_{34}O_2$	282.46	
14	Octadecanoic acid	39.9	6.19	$C_{18}H_{36}O_2$	248.48	
15	Palmitic anhydride	40.8	1.21	$C_{32}H_{62}O_3$	494.47	

MF, molecular formula; MM, molecular mass; PA, peak area; RT, retention time.

tracts from *A. squamosa* against susceptible bacterial strains used for this study showed high antibacterial potency (Table 5). The lowest MIC obtained for the crude methanol extract of the fruit pod (S1) was 0.39 mg/mL, while the MBC was 1.56 mg/mL. The lowest MIC observed for the n-hexane fraction of the fruit pod was 1.56 mg/mL and the MBC was 3.13 mg/mL, while S3 and S4 showed low MIC and MBC values of 0.78 and 1.56 mg/mL, respectively. According to Achinto *et al.*,[31] any plant extracts exhibiting low MIC and MBC against susceptible pathogens possess high antimicrobial potency. This observation in *A. squamosa* extracts showed this extract to exhibit high antimicrobial potency. Such a plant can be used to produce potent antimicrobial compounds to combat the antimicrobial resistance experienced in many of these pathogenic infections.

Antioxidant activity

The TP recorded for the oil was 36.2 mg/kg (Table 6), and this value compared favorably with the 30.3 mg/kg recorded for groundnut oil[32] but was higher than the 14.4 mg/kg recorded for *Hibiscus rosa sinensis*.[33] Phenolic compounds have been associated with antioxi-

Table 4. Sensitivity patterns of zones of inhibition exhibited by the extracts against bacterial strains

Bacterial strains	Zones of inhibition in mm*				
	S1 (25 mg/mL)	S2 (25 mg/mL)	S3 (25 mg/mL)	S4 (25 mg/mL)	Strep (1 mg/mL)
Gram-positive					
Bacillus anthracis (LIO)	15	10	12	16	20
B. cereus (NCIB 6349)	12	10	0	08	21
B. polymyxa (LIO)	10	0	08	14	18
B. stearotherphilus (NCIB 8222)	13	09	08	12	19
B. subtilis (NCIB 3610)	16	12	10	17	20
Clostridium sporogenes (NCIB 532)	14	10	09	12	15
Corynebacterium pyogenes (LIO)	12	08	10	15	18
Staphylococcus aureus (NCIB 8588)	15	12	11	10	19
Enterococcus faecalis (LIO)	10	0	08	14	16
Gram-negative					
Escherichia coli (NCIB 86)	19	11	13	14	22
Klebsiella pneumoniae (NCIB 418)	13	10	07	18	16
Pseudomonas fluorescence (NCIB 3756)	16	10	12	11	17
Proteus vulgaris (NCIB 67)	20	13	10	21	22

S1, crude methanolic extract of the fruit pod; S2, n-hexane fraction of the fruit pod; S3, DCM fraction of the fruit pod; S4, n-hexane extract of the seed; 0, resistant; Strep, streptomycin; *, mean of three replicates.

dant activity; this implies that the oil could be a good source of antioxidants, which could prevent the oil from oxidative degeneration.

The antioxidant capacity of the oil determined from DPPH radical scavenging activity expressed as IC_{50} was 1.33; this value is higher than the 0.027 reported for rice bran[34] but lower than the 5.03 recorded for *Abrus precatorious* seed oil.[35] The IC_{50} value is inversely proportional to the antioxidant activity; the lower the value the better the radical scavenging ability. The low DPPH (IC_{50}) value positively correlated with high value of TP of the oil. Also, the ferric reducing power recorded for the oil was 34.8 mg AAE/g

Table 5. MIC and MBC exhibited by the extracts against susceptible bacterial strains

Bacterial strain	Extracts							
	S1		S2		S3		S4	
	MIC, mg/mL	MBC, mg/mL	MIC, mg/mL	MBC, mg/mL	MIC, mg/mL	MBC, mg/mL	MIC, mg/mL	MBC, mg/mL
Bacillus anthracis (LIO)	1.56	6.25	6.25	12.50	1.56	3.13	0.78	1.56
B. cereus (NCIB 6349)	3.13	6.25	3.13	6.25	ND	ND	1.56	6.25
B. polymyxa (LIO)	3.13	6.25	ND	ND	6.25	12.50	1.56	3.13
B. stearotherphilus (NCIB 8222)	3.13	6.25	6.25	12.50	6.25	12.50	3.13	6.25
B. subtilis (NCIB 3610)	1.56	3.13	3.13	6.25	3.13	6.25	1.56	3.13
Clostridium sporogenes (NCIB 532)	1.56	3.13	1.56	3.13	3.13	6.25	3.13	6.25
Corynebacterium pyogenes (LIO)	6.25	12.50	6.25	12.50	1.56	3.13	1.56	3.13
Escherichia coli (NCIB 86)	0.39	1.56	1.56	3.13	0.78	1.56	0.78	3.13
Klebsiella pneumoniae (NCIB 418)	3.13	6.25	3.13	6.25	6.25	12.50	0.78	1.56
Pseudomonas fluorescence (NCIB 3756)	0.78	1.56	3.13	6.25	1.56	3.13	3.13	6.25
Proteus vulgaris (NCIB 67)	0.78	1.56	1.56	3.13	3.13	6.25	0.78	1.56
Staphylococcus aureus (NCIB 8588)	1.56	3.13	3.13	6.25	3.13	6.25	3.13	6.25
Enterococcus faecalis (LIO)	3.13	6.25	ND	ND	6.25	12.50	1.56	3.13

S1, crude methanolic extract of the fruit pod; S2, n-hexane fraction of the fruit pod; S3, DCM fraction of the fruit pod; S4, n-hexane extract of the seed; ND, not done.

Table 6. Antioxidant activity of *A. squamosa* seed oil

Parameter	Value*
TP, mg GAE/kg	36.2 ± 0.3
FRP assay, mg AAE/g	34.8 ± 0.01
DPPH, IC_{50}	1.33 ± 0.001

*mean and standard deviation of triplicate analysis.

(Table 6); this value falls within the range of 7.79 to 56.4 reported for fruit juices.[36] The Fe(III) reduction can be used as an indicator of electron donating activity of primary antioxidants whose function is to prevent oxidative damage.[37] The higher the FRP value, the better the electron donating ability; therefore, from the values obtained, the oil could be said to have high antioxidant activity.

Chemical characteristics of seed oil

The results of the chemical characteristics of *A. squamosa* seed oil is presented in Table 7. Oil content of the seed was 19.65%; this value is lower than the 47% reported for groundnut[32] but the value recorded still categorized the seed as oil seed. The iodine value (IV), which is a measure of the degree of unsaturation of vegetable oil, was observed to be 109.8 g I_2/kg. This value was higher than the 91.90 g I_2/kg reported for groundnut oil.[32] The result shows that *A. squamosa* oil could be easily oxidized and may likely dry up when stored. Oil with high IV is preferred nutritionally, due to the presence of unsaturated fatty acids, but is prone to oxidative rancidity if not stored properly. Hence, the seed oil must be refined and protected with an antioxidant to increase storage time (shelf-life).

Saponification value (SV) provides information on the suitability or otherwise of vegetable oil for the production of soap. SV observed for this seed oil was 204.8 mg KOH/g, which is higher than that reported for groundnut oil (193.20 mg KOH/g).[32] The high SV indicated high content of triacylglycerols, which is consistent with a high ester value (>99%); this implies that the oil could complement or even substitute some conventional oils in soap making.

The acid value (AV) obtained for *A. squamosa* seed oil was 1.91 (as % oleic acid), which is lower than the 2.89 reported for groundnut[30] but comparable to the 1.49 reported for sunflower oil.[38] The low acid value indicates that triacylglycerol had not been appreciably hydrolyzed, which could indicate a good stability of the oil. The percentage free fatty acid (FFA) was 3.81; this value was significantly higher than the 2.82% recorded for acacia seed oil.[14] The high FFA value obtained in this study could be adduced to the activity of lipolytic enzymes during the preparation of the seed for oil extraction. The AV and FFA values provide information on the storage quality of vegetable oil. For example, FFA is more susceptible to oxidation compared to intact fatty acids. The result thus indicated that *A. squamosa* oil would have a longer shelf-life than some conventional oils, due to its high IV. However, the appropriate condition for storage should be observed. The seed oil could therefore be adjudged suitable as food for human consumption, medicinal as well as for industrial purposes in view of its biological and chemical characteristics.

Conclusion

The GC-MS analysis of the extracts showed that the plant contains some bioactive compounds which can contribute towards the biological activities of the plant. The extracts obtained from *A. squamosa* exhibited appreciable antibacterial potency against the panel of bacterial strains used for this study. The extracts exhibited broad spectrum activities and thus showed a significant therapeutic action for the treatment of infections caused by pathogens. This observation supported the usefulness of this plant in folklore remedies for the management of infections caused by microorganisms. The oil content of the seed (18.75%) is high enough for it to be considered as oil seed. Results from the chemical characteristics of the seed oil showed that the oil can be used both as edible and industrial oil. The seed oil also demonstrated a good antioxidant property.

Table 7. Chemical characteristics of *A. squamosa* oil

Parameters	Value*
Moisture content of seed	44.3 ± 2.0
Oil content	19.6 ± 0.9
AV as % oleic acid	1.91 ± 0.02
FFA (%)	3.81 ± 0.001
IV as g I_2/kg	109.8 ± 4.2
SV as mg KOH/g	204.8 ± 2.8
Ester value	203.3 ± 4.2

*mean and standard deviation of triplicate analysis.

Future directions

This current research is focused primarily on qualitative determinations on the fruit pod and seed oil of *A. squamosa*. Future research should focus on isolation of specific compounds and structure elucidation. Also, other parts of the plant (leaf, stem and root back and wood) should be further examined.

Acknowledgments

None.

Data sharing statement

No additional data are available.

Funding

None.

Conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

Study design and supervisor (JKA), performance of experiments, analysis and interpretation of data (AAA, IOO, DAA), manuscript writing (AAA), critical revision (JKA).

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