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Variations in Confinement of Bioactive Components in Different Sections of *Spondias mombin* Tree

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ABSTRACT: Good combination and significant concentration of *phytochemicals* and minerals are responsible for medicinal potentials of most plants. *Spondias mombin* is a medicinal plant, whose parts are relied upon for herbal remedies to numerous disease conditions. Result obtained from the GC-MS analysis of the the leaf extract shows n-Propyl 11-octadecenoate, Squalene and 2,2-Dimethyl-3-vingl-bicyclo (2,2.1) heptane as the most predominant amongst the seventeen bioactive components with percentage values of 19.675, 16.569 and 14.997. 9, 12-Octadecadienoic acid was the highest amongst the four bioactive components observed in the stem bark with a percentage value of 75.720 followed by n-Hexadecanoic acid with a total percentage concentration of 21.186. Amongst the eleven bioactive components in the root bark extract, 9, 12-Octadecadienic acid was also the most predominant with a total percentage value of 91.423, while n-Hexadecanoic acid, and Diethyl phthalate had percentage concentrations of 3.359 and 2.046 respectively. These bioactive components exhibited various pharmacological and biochemical properties which may be responsible for the use of this plant in traditional medicine.

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The application of herbs in the treatment and management of human and animal diseases dated to the era of prehistoric humans. Though so many plants were locally examined and crudely experimented upon, most of them showed high efficacy and yielded the required results. Amongst the exceptional plants, whose medicinal properties were highly utilized by the then ancient locals is *Spondias mombin*. The medicinal potentials of this plant is still been tapped by traditional medical practitioner especially those of the Eastern part Nigeria in the treatment and management of different disease conditions. Its efficacy as herbal remedy can be attributed to significant concentration and synergetic combination of different bioactive compounds possibly present in different sections of the plant. Bioactive confinement has been very important in the selection of plant sections for specific herbal treatments. It is responsible for the application of different parts of the same plant in the treatment of different diseases by traditional medical practitioners. For instance, different sections of *S. mombin* has been reported to be of medicinal relevance in both human and veterinary medicine. The leaves are used as beverage for stomach ache, biliousness, urethritis, cystitis, and anti-inflammation substance for humans (Ayoka *et al*., 2008), it is also used locally to aid delivery and post-natal placental discharge is ruminants. Scientific reports on the astringent nature of the stem bark and its use as emetic and for the treatment of diarrhea, dysentery, haemorrhoids,

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gonorrhoea, and leucorrhoea (Corthout *et al*., 1994). Its fruits has also been applied traditionally as diuretic and febrifuge in humans (Aiyeloja and Bello 2006). This present study will determine the possible confinement of bioactive components in different sections of the *S. mombin* tree, thereby revealing the individual medicinal potentials of the various sections.

MATERIALS AND METHODS

Collection, Preparation and Extraction of Samples: Leaves, stem and root bark of *S. mombin* were harvested from a healthy *S. mombin* tree in the botanical garden of the University of Port Harcourt, Rivers State, Nigeria. The plant parts were washed and allowed to dry at room temperature for five days in a clean closet. The dried pieces were pulverized separately and extracted by introducing 50 mL of 98% dichrolomethane (analytical standard) into different well stoppered bottle containing 10.0 g of a specific pulverized plant sample. The mixtures were vigorously agitated and allowed to stand for 72 hours. This procedure was repeated twice and the two aliquots of each sample were combined and concentrated in a steam bath to 5 mL. The concentrates were purified by introduction into a Pasteur pipette packed with silica gel and anhydrous Sodium sulphate. The pure extracts were air dried to 1 mL and used for the gas chromatographic analysis.

Gas chromatography and spectrometry analyses of Extracts: The extracts were analyzed using Agilent Technology HP 6890 model of Gas chromatography and spectrometer model 5973 fitted to a capillary column HP-5 MS (5% phenyimethylsiloxane) 30.0m x 250μm x 0.25μm, using Helium gas at initial column temperature 120 ºC as a carrier gas for 5 minutes. Subsequent increase in temperature was done at 5 ºC per minute to 320 ºC and the setup was held for 5 minutes at 320 ºC and ionization energy of 70eV was used for mass spectroscopy electron impact ionization. The pure extracts were diluted with 98% hexane and a volume of 2 μL was auto-injected into Agilent Technology mass spectrometer model 5973. A Chem-office software connected to Microsoft Library was employed to identify the constituent bioactive components in the extracts, while National Institute of Standards and Technology (NIST) database was used in the confirmation of the names and structures of the bioactive components.

RESULTS AND DISCUSSION

Highest peaks in the chromatogram of the bioactive components of leaf extract of *S. mombin* were observed at 19.378 mins, 29.603 mins and 19.442 mins, while lowest peaks were observed at 13.129 mins. 16.224 mins and 17.714 mins respectively (see fig.1a). Chromatogram peaks of the bioactive components of the stem bark extract were highest at 19.754 mins, 17.661 mins and 18.527 mins, while the lowest peaks were observed at 16.742 mins, and 18.073 mins (see fig. 1b). For root bark extracts, highest peaks were observed at 19.718 mins, 23.506 mins and 19.054 mins, while the lowest peaks were observed at 12.019 mins, 27.039 mins and 36.243 mins respectively (see fig. 1c).

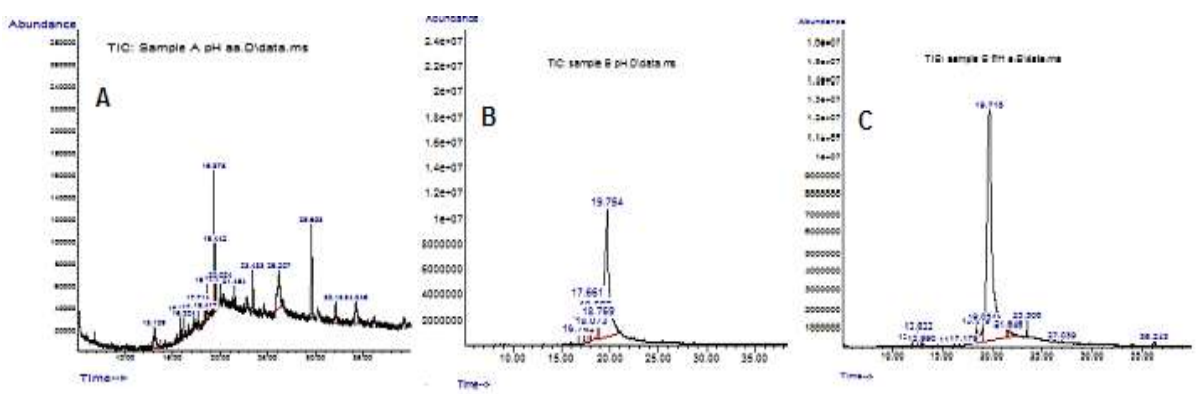
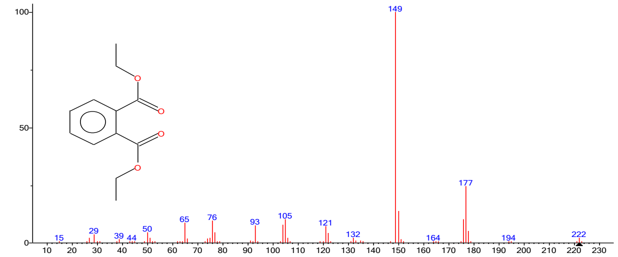
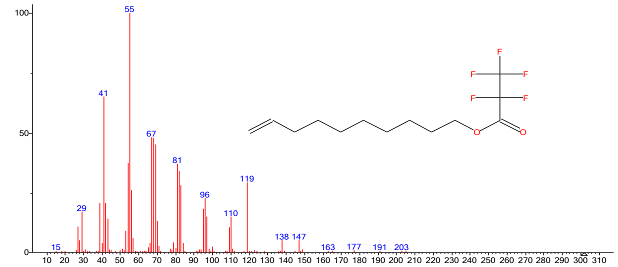
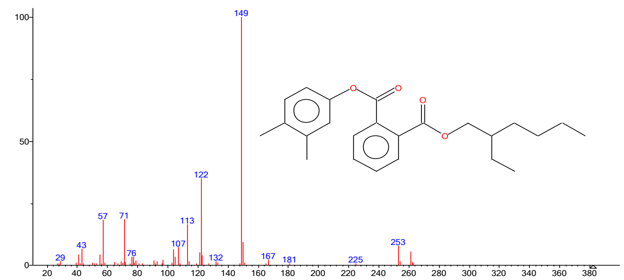
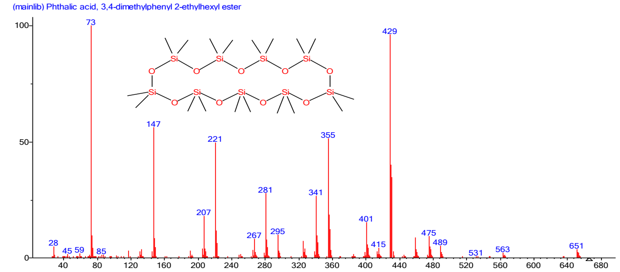
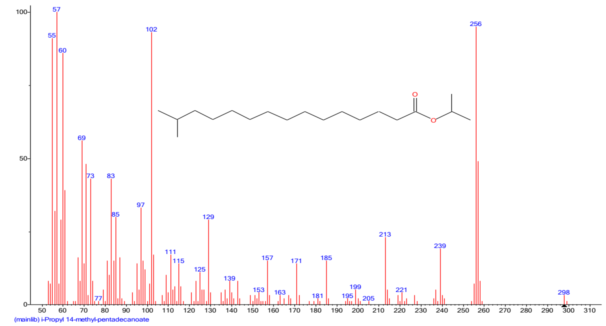
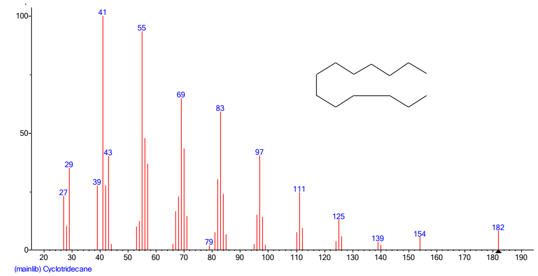
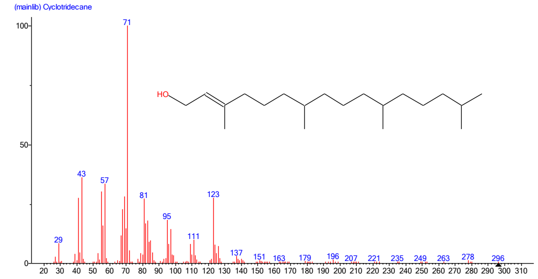
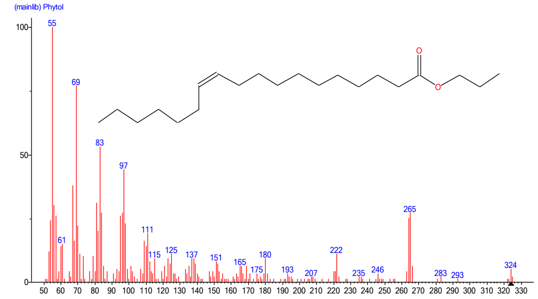


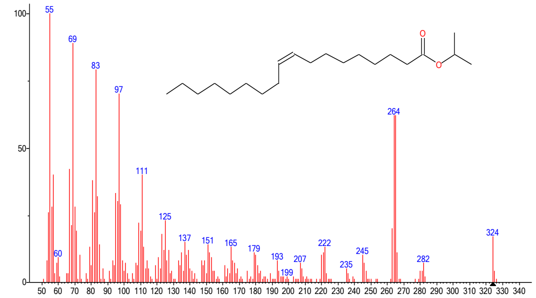
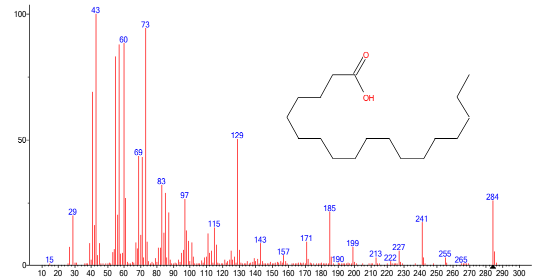
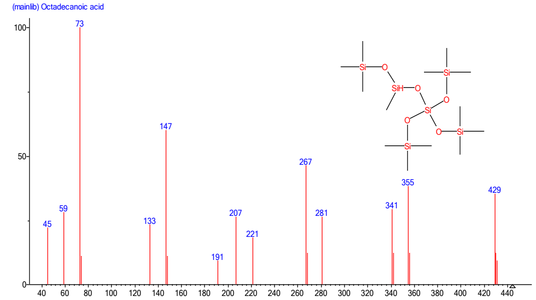
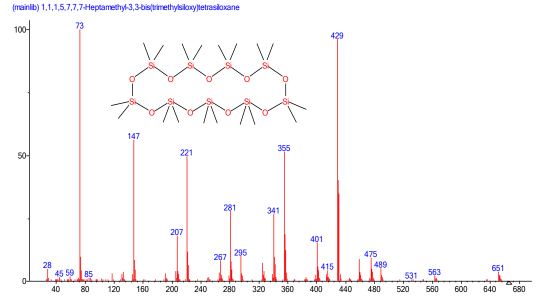
Fig.1. Chromatograms of bioactive components of: a. Leaf; b. Stem bark and c. Root bark extracts of *S. mombin*

Amongst the seventeen bioactive components observed in the leaf extract of *S. mombin*, n-Propyl 11-octadecenoate, Squalene and 2,2-Dimethyl-3-vingl-bicyclo (2,2.1) heptane had the highest concentration with percentage values of 19.675, 16.569 and 14.997, while Diethyl Phthalate, Cyclotridecane, and Cyclononasiloxane had the lowest concentration with percentage values of 0.675, 0.850 and 1.376 respectively (table 1). 9,12-Octadecadienoic acid had the highest percentage concentration amongst the bioactive components observed in the stem bark extract of *S. mombin* with a value of 75.720, while n-Hexadecanoic acid had a total percentage concentration of 21.186.

Table 1: Bioactive components of leaf extract of *Spondias mombin*

S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight (g/mol)	Structure
1	Diethyl Phthalate	13.129	0.675	$C_{12}H_{14}O_4$	222.2372	
2	9-Decen-1-ol, pentafluoropropionate	15.765	1.983	$C_{13}H_{19}F_5O_2$	302.2808	
3	Phthalic acid, 3,4-dimethylphenyl 2-ethylhexyl ester	16.224	1.904	$C_{18}H_{18}O_4$	298.3000	
4	Cyclononasiloxane,octa decamethyl-	17.201	1.376	$C_{18}H_{54}O_9Si_9$	667.3855	

5	i-Propyl 14-methyl-pentadecanoate	17.714	2.414	$C_{16}H_{32}O_2$	256.4200	
6	Cyclotridecane	18.417	0.850	$C_{14}H_{28}$	196.3721	
7	Phytol	18.628	5.988	$C_{20}H_{40}O$	296.5310	
8	n-Propyl 11-octadecenoate	19.378	19.675	$C_{21}H_{40}O_2$	324.5410	

9	i-Propyl 9-octadecenoate	19.442	9.893	$C_{21}H_{40}O_2$	324.5411	
10	Octadecanoic acid	19.615	2.622	$C_{18}H_{36}O_2$	284.4772	
11	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetra siloxane	20.024	2.476	$C_{21}H_{40}O_2$	324.5411	
12	Cyclononasiloxane,octa decamethyl-	21.484	1.951	$C_{18}H_{54}O_9Si_9$	667.3855	

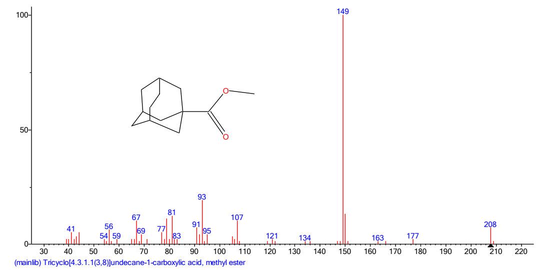
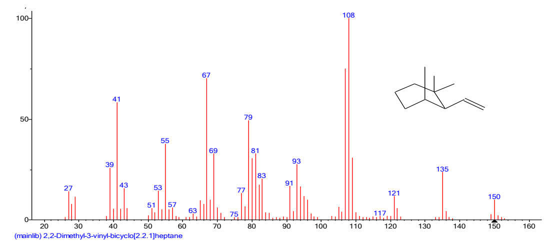
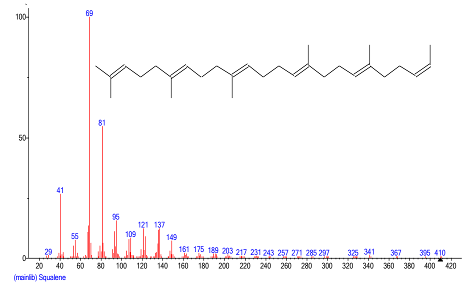
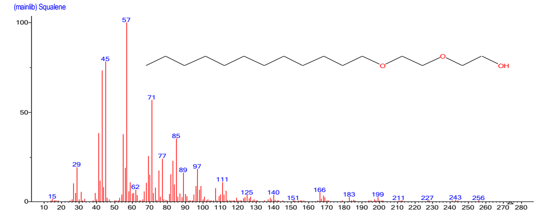
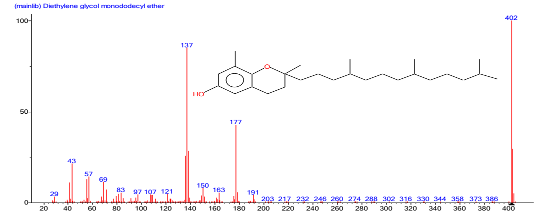
13	Tricyclo[4.3.1.1 (3,8)]undecane-1-carboxylic acid, methyl ester	23.433	5.539	$C_{13}H_{20}$	208.297	
14	2,2-Dimethyl-3-vinyl-bicyclo (2,2.1) heptane,	26.207	14.997	$C_{11}H_{18}$	150.26	
15	Squalene	29.603	16.569	$C_{30}H_{50}$	410.718	
16	Diethylene glycol monododecyl ether	32.130	5.560	$C_{16}H_{34}O_3$	274.4394	
17	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	34.249	5.526	$C_{27}H_{46}O_2$	402.6529	

Table 2: Bioactive components of stem bark extract of *Spondias mombin*

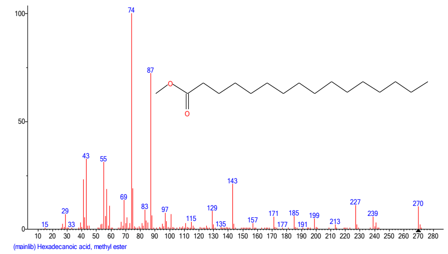
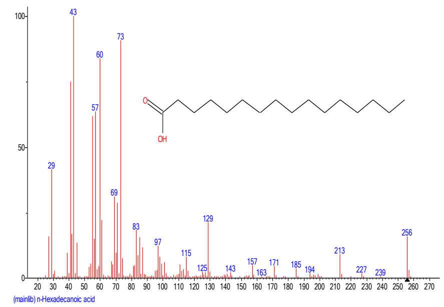
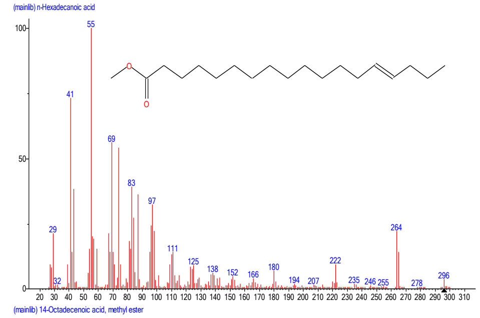
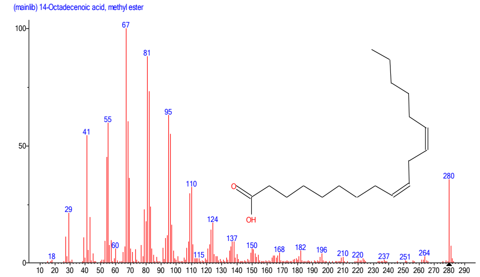
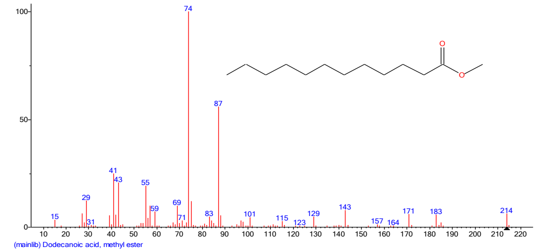
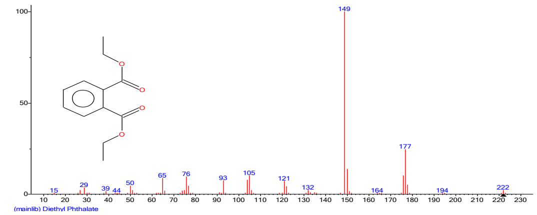
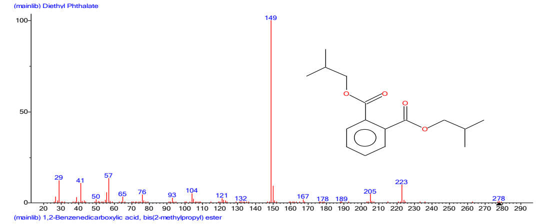
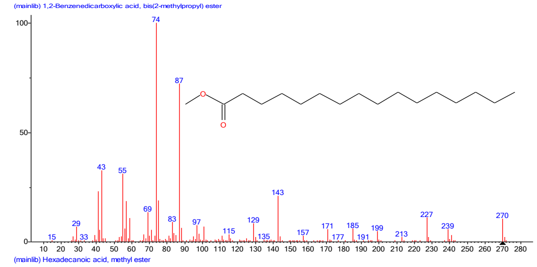
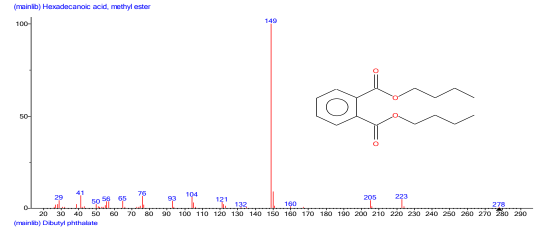
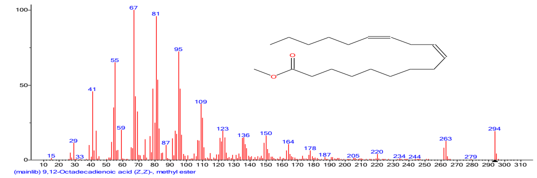
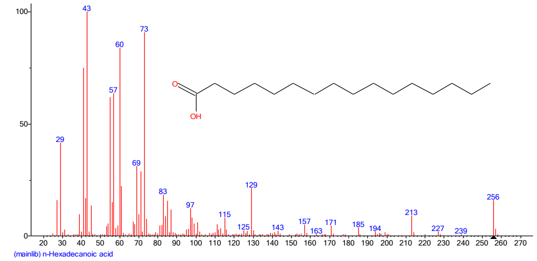
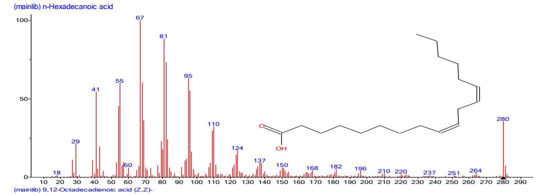
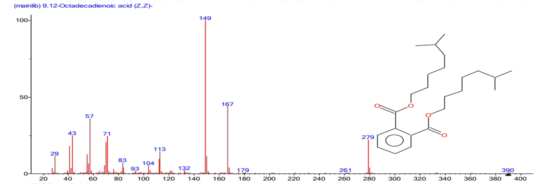
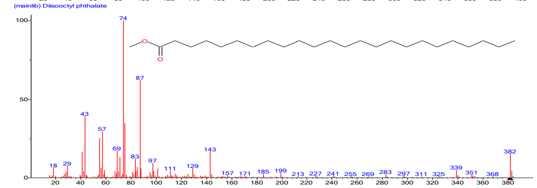
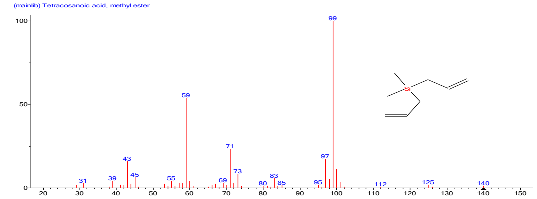
S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight (g/mol)	Structure
1	Hexadecanoic acid, methyl ester	16.742	0.306	$C_{17}H_{34}O_2$	270.4507	
2	n-Hexadecanoic acid	17.661 16.742 18.073 18.473 18.769	8.255 1.539 2.303 5.903 3.186	$C_{16}H_{32}O_2$	256.4241	
3	14-Octadecenoic acid, methyl ester	18.527	2.787	$C_{19}H_{36}O_2$	296.4879	
4	9,12-Octadecadienoic acid (Z,Z)-	19.754	75.720	$C_{18}H_{32}O_2$	280.4455	

Table 3: Bioactive components of root bark extracts of *Spondias mombin*

S/No	Compound	RT (min)	% Tot	MF	MW (g/mol)	Structure
1	Dodecanoic acid, methyl ester	12.015	0.148	$C_{13}H_{26}O_2$	214.3443	
2	Diethyl Phthalate	12.622 12.990	1.691 0.355	$C_{12}H_{14}O_4$	222.2372	
3	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	16.163	0.098	$C_{16}H_{22}O_4$	278.3435	
4	Hexadecanoic acid, methyl ester	16.698	0.068	$C_{17}H_{34}O_2$	270.4507	
5	Dibutyl phthalate	17.179	0.064	$C_{16}H_{22}O_4$	278.3435	

6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.419	0.996	$C_{19}H_{34}O_2$	294.4721	
7	n-Hexadecanoic acid	19.054	3.359	$C_{16}H_{32}O_2$	256.4241	
8	9,12-Octadecadienoic acid (Z,Z)-	19.718 21.509 21.646	89.732 0.584 1.407	$C_{18}H_{32}O_2$	280.4455	
9	Diisooctyl phthalate	23.506	0.842	$C_{24}H_{38}O_4$	390.5561	
10	Tetracosanoic acid, methyl ester	27.039	0.144	$C_{25}H_{50}O_2$	382.6633	
11	Silane,dimethyldi-2-propenyl-	36.243	0.512	$C_8H_{16}Si$	140.2981	

RT = retention time; % Tot = Percentage of the total; MF = molecular formula; MW = molecular weight

The methyl esters of Hexadecanoic acid and 14-octadecanoic acid were the least bioactive compounds observed in this extract with percentage concentration of 0.306 and 2.787 respectively (table 2). 9,12-Octadecadienic acid was also the most predominant amongst the eleven bioactive components in the root bark extract of the *S. mombin*, with a percentage value of 91.423. n-Hexadecanoic acid, and Diethyl phthalate also showed a slightly high concentrations with percentage values of 3.359 and 2.046 respectively. However, Dibuthyl phthalate, Hexadencanoic acid methyl ester, 12-Benzenedicarboxylic acid, bis(2-methyl propyl) ester had the lowest concentrations with percentage values of 0.064, 0.068 and 0.098 respectively (table 3).

This study showed n-propyl-11-Octadenoate as the predominant bioactive compound in the dichrolomethane extract of *S. mombin* leaf. As a waxy 18-carbon saturated fatty acid, n-Propyl-11-Octadenoate may be responsible for the waxy texture and shiny appearance of *S. mombin* leaf. This high concentration of n-propyl-11-Octadecanoate may also be responsible for the use of *S. mombin* leaf as an organic supplement in the preparation of biogenic soap. This collaborates the work of Ayoka *et al*., (2008), where the use of *S. mombin* leaf extract in the production of soaps, cosmetics and detergents was reported. Wright *et al*., (2002), also reported n-propyl-11-Octadecanoate as a surfactant and softening agent. His findings imply that the high concentration of n-propyl-11-Octadecanoate in this plant leaf may be responsible for the traditional application of *S. mombin* leaves as stool softener and purgative in ruminants. The high concentration of squalene observed in the leaf of this plant may also play a significant role in the use of *S. mombin* leaf as an organic supplement in the preparation of biogenic soap. Squalene is an isoprenoid compound structurally similar to beta-carotene and serves as an intermediate metabolite in the synthesis of cholesterol (Kelly, 1999). In humans, about 60% of dietary squalene is absorbed, transported in synergy with low density lipoproteins and distributed ubiquitously in human tissues, with its highest concentration observed in the skin (Lopez *et al*., 2014). Due to low susceptibility of squalene to peroxidation and as a major components of human skin surface lipids, it functions mainly as a suppressor of reactive oxygen species and other free radicals (Njoku *et al*., 2007), thereby eliciting a protective effect on human skin surface against lipid peroxidation due to exposure to UV and other sources of ionizing radiation. In humans, squalene might serve as a useful additive to potentiate the effects of some cholesterol-lowering drugs. However, squalene is primarily use therapeutically as an adjunctive therapy in most cancer treatment (Idowu *et al*., 2014). This also indicates that *S. mombin* leaf and its extract may be of therapeutic use in cancer treatment and management. As a bicyclic monoterpene, 2,2-Dimethyl-3-vinyl-bixyclo heptane is readily volatile and possible serves as flavouring agent to enhance palatability of this plant part. This corroborates the work of Kafuru *et al*., (1994), which indicates the use of 2,2-Dimethyl-3-vinyl-bxyclo heptane as a flavouring additive in foods. The high concentrations of both 9,12- Octadecadienoic acid and n-Hexadecanoic acid observed in both the root bark and stem bark indicates a partially restricted or unrestricted circulation of bioactive components within the stem bark and the root bark. It also shows a closer medicinal and therapeutic relationship between the stem bark and root bark. The high concentration of 9,12-Octadecadienoic acid (stearic acid) observed in these sections of the plant indicates that both the root bark and stem bark of *S. mombin* can be used therapeutically in the treatment of viral and inflammatory diseases and also as a thickener and hardener in the treatment of chemically induced burns and skin lesion (Abo *et al*., 1999). The slight increase in concentration n-Hexadecanoic acid and decrease in 9,12- Octadecadienoic acid observed in the stem bark extract of this plant may be attributed to the conversion 9,12-Octadecadienoic acid present in its root to n-Hexadecanoic acid and its enantiomers or isomers. This finding agrees with the work of (Griinari, and Bauman, 1999), which reported a possible isomerization of *cis,cis*-9,12- Octadecadienoic acid to either *cis,trans*-9,11-octadecadienoic acid (under normal condition) and (or) *trans,cis*-10,12-octadecadienoic acid (milk-fat-depressing conditions). The hydrogenation of these conjugate acids yields a *trans*-11-octadecaenoic acid and/or a *trans*-10-octadecaenoic acid, whose conversion yields n-Hexadecanoic acid as its final monoenoic acid (Bauman and Griinari, 2003). This may be responsible for the different retention times observed for n-Hexadecanoic acid in the stem bark (see table 2). This also corroborates the findings of (Kiralan *et al*., 2021), which reported that an alteration in the physicochemical properties of fatty acids when changing from *cis* to *trans* isomerization. The rate limiting step in the isomerization reaction (conversion of *cis,cis*-9,12-octadecadienoic acid to *cis,trans*-9,11-octadecadienoic acid and (or) *trans,cis*-10,12-octadecadienoic acid) is by catalyzed linoleate isomerase (Kiralan *et al*., 2021). The enzyme Linoleate isomerase is obtained from *Lactobacillus plantarum* which is widely found in soils mostly associated with rhizosphere (Landete *et al*., 2021). This indicates that the *L. plantarum* present in the soil may have attached themselves to the root barks and may be responsible for the first enzymatic step in the conversion 9,12-Octadecadienoic acid to n-Hexadecanoic acid. The first intermediary metabolites in the conversion process may be responsible for the different enantiomers, which contributed to the different retention times of 9,12-Octadecadienoic acid observed in the root bark (see table 3), while the subsequent intermediates may be transported (via the transpiration pull) to the stem bark, thereby leading to the slight increase in the concentration of n-Hexadecanoic acid and its enantiomers and/or isomers in the stem bark.

Conclusion: The confinement of bioactive components in different sections of this plant indicate that each section has been naturally endowed with therapeutic potentials against specific disease conditions. However, the similarity observed in the predominant compound in both the stem bark and the root bark shows a possible therapeutic relationship amongst the two sections. Isolation and purification of these novel compounds may be an important step in obtaining nature-based pharmaceutical compounds.

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