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Analysis of bioactive compounds from *Raphia taedigera* using gas chromatography–mass spectrometry

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Raphia taedigera seed is an underutilized seed with several bioactive compounds that have the tendency to regulate metabolic processes to promote the healthy living. In this research study, sixteen bioactive compounds were identified in the *Raphia taedigera* seed oil using Gas chromatography-mass spectrometry (GC-MS). GC-MS revealed the presence of Hexadecanoic acid, methyl ester (0.31%), n-Hexadecanoic acid (7.15%), trans-13-Octadecenoic acid methyl esters (0.91%), Oleic acid (10.83), Octadecanoic acid (10.23%), cis-13-Octadecenoic acid (2.75%), 6-Octadecenoic (3.26%), Cis-vaccenic acid (1.94%), Palmitoyl chloride (2.82%), Trans-13-Octadecenoic acid (1.78), 9, 12-Octadecadienoic acid (1.54%), 4, 4, 6a, 6b, 8a, 11, 11, 14b-Octamethyl-1, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a, 14b-octadecahydro-2H-picen-3-one (7.29%), 3-Methoxymethoxy-2, 3-dimethylundec-1-ene (8.88%), Cyclohexanecarboxylic acid (2.00%), undecylester, Beta.-Amyrin (8.31%) and Lup-20(29)-en-3-one (27.28%). The results show revealed the potential of the seed oil as an anti-inflammatory, anti-leishmanial, anti-cancer, antioxidant, antifungal and hypocholesterolemic.

KEYWORDS

Raphia taedigera; anti-inflammatory; antioxidant; anti-leukemia; anticancer; hypocholesterolemia.

Introduction

Nature has been the major source of medicinal plants for years and many researchers have reported the isolation and the deployment of numerous bioactive materials in traditional medicine [1]. Plants can store various liquids and fluid secondary metabolites including those that have been associated with therapeutic activities since prehistoric times [2]. On the other hand, *Raphia taedigera* is an evergreen and underutilized tree found in the tropical and sub-tropical ecosystems [3]. It belongs to the family of Aracaceae and identified as one of the oldest single cotyledon flowering plants [4]. The tree, stems erect often cespitose, single and clustering (more often), 3-12 m high and 25-60 cm in diameter, unarmed. *R. taedigera* reproduces by seed vegetative method and takes about a year before germination at swamp forest habitat. The mature tree produces fruits which is about 5-7 cm long and of 3-4 cm in diameter, covered by imbricate glossy reddish-brown scales containing one seed only. A well-dried seed is hard with brown outer part and white shinny inner and with an egg-size [5,6]. The plant is found in majorly in Southern part of Nigeria. The plant produces juice that is adjudged to be of good taste by the populace who drinks it for relaxation and also as dowry for traditional weddings and as medicine for treatment of measles by the rural populace [7].

Oluwaniyi, *et al*., 2014 [8] reported that *Raphia hookeri* and *Raphia farinifera* which are family of Aracaceae are rich in phytochemicals and other secondary plant metabolites which could function as antimicrobial effect, antioxidants, and anticancer [8]. These phytochemicals have the ability to fight or prevent infections and diseases [9, 10]. However, no detailed research has been conducted to identify the bioactive constituents in the plant seed oil of *R.taedigera* or its oil characterized for potency and quality. This arouses our interest in identifying the chemical constituents of the plant using the gas chromatography-mass spectrometer. Seeds of *R. taedigera* are reported to contain high percentage of carbohydrates (74.12%), which could make it to be a good alternate source of energy in animals, 4.56 % protein and rich in phytochemicals such as alkaloids, flavonoids and Tannin [11].

Materials and methods

Sample collection and preparation

The seeds (Figure 1b) were harvested from the Federal University of Technology, Akure-Nigeria teaching and research farm located along Akure–Ilesa expressway, Southwest, Nigeria. Harvested seeds were identified in the Department of Crop, Soil and Pests Department of the Federal University of Technology, Akure, Nigeria and thereafter washed under running water and air-dried. Air-dried sample was crushed and pulverized into powder which was later stored in an airtight plastic container.

FIGURE 1a Image of *Raphia taedigera* tree

FIGURE 1B The *Raphia taedigera* seed

Extraction

Oil from the seeds was extracted using soxhlet extraction techniques [12]. 5 g of the pulverized powder sample weighed using the Metler Toledo (model ME54T/00) weighing balance was extracted with 100 mL of petroleum ether. Anhydrous sodium sulphate was added to remove moisture from the oil and filter through 0.22 µm membrane filter and ready for GC-MS analysis. All the chemicals and solvents were analytical and chromatography grade respectively. Anhydrous sodium sulphate, petroleum ether,

n-Hexane were purchased from Sigma Aldrich.

Gas chromatography-mass spectrometry (GC-MS) analysis

Identification of the bioactive compounds presents in the seed oil was carried out using the method reported by Oboh *et al*., 2018 [13] with modification using gas chromatography coupled with mass spectrometer detector (GC-MSD) at the scan mode of high mass of 550 to low mass of 50 with a threshold of 150. The analysis of extracted oil was performed using the 7890A gas chromatograph coupled to 5975C inert mass spectrometer with electron-impact source (Agilent Technologies). The packed column used for the separation of the compounds was HP-1MS capillary column coated with 5% phenyl methyl siloxane (30 m length×250 µm diameter×0.25 µm film thickness) (Agilent 19091S-933HP-1MS). The carrier gas was helium used at a constant flow of 0.8 mL/min at pressure of 5.7667 psi and average velocity of 32.685 cm/sec. 1 µL volume of samples was injected in split mode (10:1) at an injection temperature of 110 °C. Septum Purge flow was 3 mL/min with a total flow of 11.762 mL/min; gas saver mode was switched on. The Oven was initially programmed at 60 °C (2 min) then ramped at 30 °C/min to 150 °C (5 min) then 30 °C/min to 280 °C (8 min). The total run time was 21.333 min with a 3 min solvent delay and post-run at 280 °C for 3 mins. The mass spectrometer was operated in electron ionization mode with ionization energy of 70 eV with ion source temperature of 230 °C, a quadrupole temperature of 150 °C, and transfer line temperature of 280 °C. Before the analysis, the mass spectrometer was auto-tuned to perfluorotributylamine (PFTBA) and the abundance of m/z 69, 219, 502, and other instruments optimal and sensitivity conditions were aligned. Analytical validation was conducted by running replicate samples in order to see the consistency of the constituent compound name, respective retention time, and molecular weight. The obtained results were identified using NIST11 library installed with the GC-MS with a corresponding mass spectrum showing the fragmentation pattern [13].

Results and discussion

The bioactive compounds identified and their retention time, peak area, molecular weight, molecular formula, and their physiological importance are demonstrated in Table 1. The separated compounds were matched with the National Institutes of Standards Technology mass spectrum database using the retention time and molecular mass. The fragmentation pattern of the identified compounds and their structures are demonstrated in Figure 3. Structures of the compounds were identified using the mass spectrometer as seen from the relative abundance of mass fragments of molecules (m/e) of the molecular ion (M^+). Stable molecular fragment formed possesses a large relative abundance and exists at a longer span [14,15]. The GC-MS chromatograms of the identified peaks of detected compounds are shown in Figure 2. First separated peak was identified to be Hexadecanoic acid, methyl ester (0.31%) followed by n-Hexadecanoic acid (7.15%) and other peaks are trans-13- Octadecenoic acid, methyl ester (0.91%), Oleic Acid (10.83%), Octadecanoic acid (10.23%), cis-13-Octadecenoic acid (2.75%), Cis-vaccenic acid (1.94%), Palmitoyl chloride(2.82%), Trans-13-Octadecenoic acid (1.78%), 9, 12-Octadecadienoicacid (1.54%), 4, 4, 6a, 6b, 8a, 11, 11, 14b-Octamethyl 1, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a, 14b-octadecahydro-2H-picen-3-one (7.29%), 3-Methoxymethoxy-2, 3-dimethylundec-1-ene (8.88%), Cyclohexanecarboxylic acid, undecylester (2.00%), and Beta.-Amyrin (8.31%), Lup-20(29)-en-3-one (27.28%). Lup-20 (29)-en-3-one has the highest

percentage of all constituents and is one of the most available triterpene [16,17] reported that Lup-20(29)-en-3-one inhibited the cell growth of leukemia. It inhibits tumor growth, cell cycle progression and induces the apoptosis of tumor cells both in vitro and invo situations [18]. It is also known to demonstrate antibacterial and antifungal properties [19, 20]. Lup-20(29)-en-3-one serves as anti-tumor, anti-inflammatory and also as anti-epilepsy [21]. Oleic acid is the second prominent constituent in the seed oil with 10.23%. Its addition to the cell membrane lipids modifies the membrane structure and alters its biophysical properties [22]. It was found to be useful in pathogen control, treatment of prostrate and breast cancer [23]. Oleic acid, as an essential monounsaturated fatty acid, its importance in human nutrition cannot be over-emphasized as it can reduce the low-density cholesterol and glycemic index [24]. The biological benefits of other bioactive compounds found are presented in Table 1.

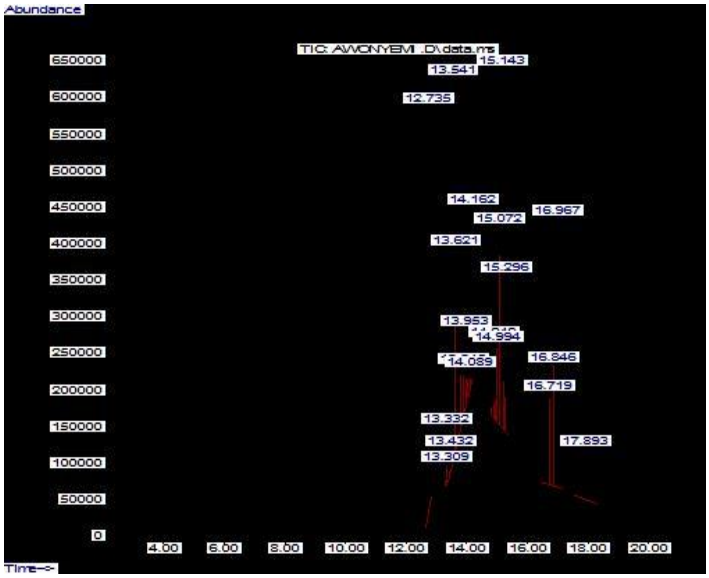

FIGURE 2 Total ion chromatogram for *Raphia taedigera* seed oil

TABLE 1 List of Bioactive compounds from *Raphia taedigera* seed oil and their biological activities

S/N	Compound Name	Retention time (min)	Peak Area (%)	Molecular Formula	Molecular Weight	Biological Activities
1	Hexadecanoic acid,methyl ester	12.518	0.31	$C_{17}H_{34}O_2$	270.256	Anti-inflammatory. hypocholesterolemic,cancer preventive,hepatoprotective, nematicide,insectifuge,antihistaminic, anticorona, antiarthritic [25].
2	n-Hexadecanoic acid	12.735	7.15	$C_{13}H_{32}O_2$	256.24	Antioxidant, Hypocholesterolemic, Nematicide, Anti-androgenic, pesticides, Hemolytic [26].
3	Trans-13-octadecenoic acid,methyl ester	13.330	0.91	$C_{19}H_{36}O_2$	296.272	Anti-inflammatory, anti-amdrogenic,dermatitigenic, anaemiagenic, insecticides,flavor [27].

4	Oleic Acid	13.542	10.83	$C_{18}H_{34}O_2$	282.256	Antifungal, anti-inflammatory, antioxidants, antibacterial [28]
5	Octadecanoic acid	13.617	10.23	$C_{18}H_{36}O_2$	284.272	Antifungal, antitumor and antibacterial [29]
6	Cis-13-Octadecenoic acid	13.845	2.75	$C_{18}H_{34}O_2$	282.256	Therapeutic uses in medicine, surgery [29]
7	6-Octadecenoic acid	13.954	3.26	$C_{18}H_{34}O_2$	282.256	Anti-cancer [30].
8	Cis-Vaccenic acid	14.086	1.94	$C_{18}H_{34}O_2$	282.256	Anti hypercholesterolemic, anti-inflammatory [31]
9	Palmitoyl chloride	14.160	2.82	$C_{16}H_{31}ClO$	274.206	Antibacterial [32]
10	Trans-13-Octadecenoic acid	14.229	1.78	$C_{18}H_{34}O_2$	282.256	Anti-inflammatory, anti-amdrogenic,dermatitigenic, anaemiagenic, insecticides,flavor [33].
11	9,12-Octadecadienoic acid(Z,Z)	14.847	1.54	$C_{18}H_{32}O_2$	280.240	Anti-inflammatory, hypocholesterolemic, cancer preventive, insectifuge, antiarthritic, hepatoprotective, antiandrogenic, nematicide, antihistaminic, antieczemic [34].
12	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	15.076	7.29	$C_{30}H_{48}O$	424.371	Anti-bacteria, antioxidant,antitumor,cancer preventives [35].
13	3-Methoxymethoxy-2,3-dimethylundec-1-ene	15.144	8.88	$C_{15}H_{30}O_2$	242.225	No Activity reported
14	Cyclohexanecarboxylic acid,undecyl ester	15.293	2.00	$C_{18}H_{34}O_2$	282.256	No Activity reported
15	.Beta-Amyrin	16.088	8.31	$C_{30}H_{50}O$	426.386	Antihyperglycemic, hypolipidemic anti-inflammatory, antinociceptive, antioxidant, antipruritic, gastroprotective and hepatoprotective effects [36, 37].
16	Lup-20(29)-en-3-one	16.964	27.28	$C_{30}H_{48}O$	424.371	Antileukemia, anti-inflammatory,anticarcinogenicity [38,39].

Conclusion

This study revealed that, all the compounds found in the seed oil of R. taedigera are of different biological importance which shows its potency as therapeutics and could also serve as functional foods following its initial recommendation that the whole seed can serve as good components of animal feed for energy supply. The study has also provided a baseline for the considering this plant as a prospective source of new drugs for treatment of cancer and antileukamia. Further studies are ongoing on the toxicological assays.

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