Journal of Agricultural, Food Science & Biotechnology

JAFSB, 1(3), 203-210, 2023
https://doi.org/10.58985/jafsb.2023.v01i03.24
ISSN (Print): 2959-3417

Research Article

Volatile oils of *Spondias mombin* L. leaves as potent antioxidant and antibacterial agent

Isaac Saint Njoku [1,2], **Olayinka Taiwo Asekun** [2] and **Oluwole Babjide Familoni** [2]

1. Department of Chemistry, Faculty of Basic Medical and Applied Sciences, Trinity University Yaba, Lagos, Nigeria
2. Department of Chemistry, Faculty of Science, University of Lagos, Akoka-Yaba, Lagos, Nigeria

Article Information
Received: 21 March 2022
Revised: 05 July 2023
Accepted: 21 July 2023

Academic Editor
Prof. Dr. Antonello Santini

Corresponding Author
Dr. Isaac Saint Njoku

E-mail:
saintnjokuphd@gmail.com

Keywords
Volatile oil, *Spondias mombin*, GC-MS, antibacterial, humulene, antioxidants

Abstract

Essential oils contain volatile compounds which have been used as potent antioxidant and antimicrobial agents for decades. This is because of the presence of many active components in the oils. In this research, the essential oil of air-dried leaves of *Spondias mombin* obtained through hydrodistillation was characterized by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry analyses (GC-MS). The antimicrobial assay was carried out using the agar diffusion method while the antioxidant assay was carried out using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant assay, ferric reducing antioxidant power, nitric oxide radical scavenging, total antioxidant capacity and lipid peroxidation assays on the oils. The oil yielded 1.20 % per dry weight basis of the sample. The oil was composed majorly of mono and sesquiterpenoids. The major components of the volatile oil of *S. mombin* were caryophyllene (35.78 %), δ-cadinene (21.56 %), humulene (11.33 %), γ-muurolene (5.86 %), (-)-isogermacrene D (4.47 %) and nerolidol (4.46 %).The highest sensitivity of the oil was on *K. pneumoniae* with minimum inhibitory concentration(MIC) and minimal bactericidal concentration (MBC) values of 62.40 and 122.80 µg/mL, followed *P. aeruginosa* (MIC and MBC values of 70.20 and 132.10 µg/mL) and *E. coli* (MIC and MBC values of 86.60 and 178.50 µg/mL). The volatile oil of *S. mombin* showed good antioxidant activity with 41.22 µg/mL, 5.55 µg/g and 83.24 % for DPPH, FRAP and β-carotene bleaching assays. The air-dried leaf oil showed strong activity against *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae,* as well as a promising antioxidant potency.

1. Introduction

The reduction of molecular oxygen in the cells produces superoxide, which is the precursor of most other reactive oxygen species [1]. Molecular oxygen (O_2) is the premier biological electron acceptor that serves vital roles in fundamental cellular functions [2]. These reduced species have damaging effects. These include apostasies, cardiovascular diseases, oxidative damage, ageing and cancer [3-7]. The use of synthetic antioxidants may produce many side effects. Natural antioxidants are the secondary metabolites of phyto-chemicals, and are preferred over synthetic anti-oxidants [8]. *Spondias mombin* (*S. mombin*) also known as yellow mombin or hog plum is a species of tree and flowering plant in the Anacardiaceae family. It is native to the tropical Americas, including the West Indies. It has been naturalized in parts of Africa, India, Nepal, Bangladesh and Sri Lanka. The mature fruit has a leathery skin and a thin layer of pulp. The seed has an oil content of 31.5% [9]. It is one of the medicinal herbs in Nigeria. In Nigeria, it is known by various names: *Ogheeghe* (Edo), *Iyawe* and *Akikaetikan* (Yoruba), *Akika* and *Ichikara* (Ibo), *Tsardarmasar*

(Hausa) Yoruba, *chabbuh* (Fulani) and *nsukakara* (Efik) [10-11]. Traditionally the fruit has been used as a diuretic and febrifuge. The bark is used as an emetic and for diarrhea, dysentery, hemorrhoids, gonorrhoea, and leucorrhea. The flowers and leaves are used to make tea for stomach ache, biliousness, urethritis, cystitis, and inflammation [12]. The leaves of *S. mombin* have been used locally in Nigeria by traditional medical practitioners in the treatment of stomach pain, cough, cuts, dizziness, eye ailments, thrush, yaw and as an expectorant. Scientific investigations have shown that it has anthelmintic, antioxidant, antimicrobial and anti-inflammatory actions [13-15]. Although some earlier studies revealed the medicinal attributes of *S. mombin* however, very little work has been done on the antioxidant and antimicrobial activities of the essential oil of this plant. It is on this backdrop that this research was undertaken.

2. Materials and methods

2.1 Plant material and essential oil extraction technique.

The healthy leaves of *Spondias mobin* were collected from the botanical garden of the University of Lagos, Akoka, Yaba Area of Lagos State, Nigeria in September, 2022. The botanical identification and authentication were done in the Herbarium of the Department of Botany, University of Lagos, Nigeria (herbarium number LUH 6567). The fresh leaves of *Spondias mombin* were air-dried for one week and pulverized using mechanical grinder prior to extraction. The essential oils from the air-dried leaves were obtained by hydro-distillation of 300g of each of the plant materials using the modified Clevenger-type apparatus [16]. The oil was dried over anhydrous sodium sulphate and stored in a refrigerator prior to analysis.

2.2 GC–FID and GC-MS analyses of volatile oils

The volatile oil samples were analyzed using a Varian CP-3800 gas chromatograph fitted with a flame ionization detector (FID) and dimethylpolysiloxane (100%) column (CP Sil-5 CB: 50 m length × 0.25 mm i.d. × 0.4 μm film thickness) (Varian, Netherlands). Nitrogen was the carrier gas with a 16-psi inlet pressure. Samples (0.2 μL) were injected in split mode with a ratio of 1:100. The column was initially held at 60°C for 5 minutes then heated to 220°C at a 5°C/minute ramp rate and was held for 3 minutes at that temperature. The temperature was further raised to 250°C at a 5°C/minute ramp rate and was held at this temperature for 4 minutes. The injector and detector temperatures were maintained at 250° and 300°C, respectively. The gas chromatography/mass spectrometry (GC-MS) analyses performed on a Perkin Elmer Turbo mass Clarus 600 Instrument at 70 eV ionization energy with a mass range of 40–500 amu, employing an Elite-5 column (5 % phenyl and 95 % dimethylpolysiloxane) of 30 m length, 0.25 mm internal diameter and 0.25 μm film thickness (PerkinElmer, USA). Helium (1 mL/min) was used as a carrier gas. The initial temperature was 60 °C (1 min), this was increased to 240 °C at a rate of 6 °C/min, remained at 240 °C for 6 min, and then continued to increase to 250 °C at a rate of 10 °C/min, with a final stage of 10 min at 250 °C. The oven temperature was programmed from 50 °C to 250 °C at a 5 °C/min dynamic rate, and remained for 15 min at 250 °C. Samples (0.1 μL) were injected with a split less mode.

2.3 Identification of volatile oil constituents

Component identification was accomplished by comparison of the retention indices (RI) of the GC peaks with those obtained using saturated n-alkanes (C8–C30) (Aldrich, St. Louis, MO, United States), those reported in the literature [17-20] and by comparison of the mass spectra of the peaks with those reported in the literature [21-22] and the NIST library. Peak area percentages were calculated from GC–FID response without employing correction factors. RI values were calculated for all components using a homologous series of *n*-alkane. Mixtures (C7-C30) were injected under conditions similar to those of the samples and computer matched with the NIST libraries.

2.4 Antioxidant assay

2.4.1 DPPH radical scavenging assay

The free radical scavenging capacity of the compounds was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [23, 24] with modifications. The volatile oil was allowed to react with stable free radical, DPPH for half an hour at 37 ^{o}C. The concentration of DPPH was 1 mM. The oils (10, 20, 30, 40 and 50 μL) were mixed with DPPH prepared in methanol. Ascorbic acid (4 mg/mL in methanol) was used as positive control. DPPH solutions at the same concentration without the tested oil was used as a negative control. Each sample, as

well as each control was analyzed in triplicate. The end volume for each sample was 100 µL in each well of the 96 well plate. After incubation, decrease in absorbance was measured at 517 nm using microplate reader (BMG Labtech Fluostar Omega UV-VIS microplate reader Instrument, Inc., Cary, NC, United States). Percentage radical scavenging activity was calculated using the formula:

$$Inhibition\ \% = \frac{AC - AS}{AC} \times 100$$

AC = Absorbance of control.
AS = Absorbance of Sample.

In order to calculate the IC_{50}, the essential oil was prepared in a series of concentrations of 1, 10, 20, 40, 60, 80, 200, 400, 800, and 2000 µg/mL. The test was repeated as described above for all concentrations of each oil in triplicates. Inhibition % was plotted against concentration and the IC_{50} was calculated graphically.

2.4.2 FRAP-ferric reducing antioxidant power assay

Ferric ion reducing capacity of the essential oil of *S. mombin* was conducted using the method described by Goodarzi et al. [25]. The ability of the volatile oil to reduce ferric tripyridyltriazine (Fe(III)-TPTZ) complex to its ferrous colored form (Fe(II)-TPTZ) at low pH was determined using a spectrophotometer. 1.5 mL of FRAP reagent (2.5 mL of 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3 M acetate buffer, pH 3.6) was added to 50 µL of each sample (100 µg/mL). After incubation at 37 °C for 10 min, the absorbance was measured at 593 nm. FRAP reagent without the sample was as blank and the experiment was performed in triplicate. Different concentration of aqueous solution of $FeSO_4{\cdot}7H_2O$ (in a range of 125-1000 µmol/L) was used for the calibration curve. The relative antioxidant activities of samples were reported as mmole Fe^{2+}/100 g of fractions.

2.4.3 β-Carotene bleaching test.

The β-carotene bleaching capacity of the volatile oil of *S. mombin* was conducted using the method of Kelvin *et al* [26] with slight modification; 10 mg of β-carotene was dissolved in 10 mL of chloroform. The carotene-chloroform solution, 0.2 mL, was pipetted into a boiling flask containing 20 mg linoleic acid and 200 mg Tween 40. Chloroform was removed using a rotary evaporator and 50 mL of distilled water was added slowly with vigorous agitation to the residue, to form an emulsion. Exactly 5 mL of the emulsion were added to a tube containing 2 mg of essential oils and the absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min period. Control samples contained 10 µL of water instead of volatile oils. Butylated hydroxy anisole (BHA) was used as a reference. The antioxidant activity was expressed as inhibition percentage with reference to the control after a 60 min incubation using the following equation:

AA = 100(DRC − DRS)/DRC

where AA is the antioxidant activity, DRC is the degradation rate of the control = [log (a/b)/60], DRS is the degradation rate in presence of the sample = [log (a/b)/60]; a is the absorbance at time 0; b is the absorbance at 60 min.

2.5 Antibacterial assay

The volatile oil of *S. mombin* was tested on three different bacterial strains. The strains were maintained at 4 °C and they are *P. aeruginosa* ATCC 21234, *K. pneumoniae* ATCC 15522 and *E. coli* ATCC 25922. The bacterial strains were cultured in a Thermo Scientific (Waltham, MA, United States) Oxoid Nutrient agar (NA) at 37 °C for 24 hours. The disc diffusion method [27] was used to determine the antimicrobial activities of the essential oils. Petri plates were prepared by pouring 20 mL Thermo Scientific Oxoid Nutrient agar (NA) and the solution was allowed to solidify. The plates were then dried, and 0.1 mL of the standardized inoculum containing 106-107 colony-forming units/mL of the bacterial suspension was poured, uniformly spread, and allowed to dry for 5 minutes. The volatile oil was prepared in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL. 100 µL was taken from this stock solution and was added to respective wells. The control well received only 100 µL DMSO. Gentamycin (positive control) was used as the reference antibiotic. The plates were left at room temperature to allow diffusion and then incubated at 37 °C for 24 hours for bacterial growth. The antimicrobial activity was evaluated by measuring the diameter of the zones of inhibition against the test organisms. The experiments were repeated in triplicate and the results are expressed as average values. The MIC was determined using the broth microdilution method

using 96-well microplates. The inoculum of the microbial strains was prepared from 24 to 48 broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Serial concentrations (500, 250, 125, 62.5, 31.3, 15.6, 7.81, 3.9, 1.95, 0.98, and 0.49 µg/mL) of essential oil were prepared. 100 µL from culture broth was mixed with 100 µL of different concentrations of the essential oils of *S. mombin* in the corresponding well and plates were incubated either at 37 °C for 24 hours for antibacterial activity. The lowest concentration of the tested oil showing no microbial growth was defined as the minimum inhibitory concentration (MIC). Minimum bactericidal concentration (MBC) values were determined by taking a part of the liquid from each well that showed no growth and incubating on agar plates at 37 °C for another 24 hours. The lowest concentration that disclosed no visible growth of bacteria or fungi was confirmed as MBC.

3. Results and discussion

3.1 Chemical composition

The yield of leaf the volatile oil obtained by hydrodistillation was 1.20 % (w/w relative to dry material weight basis). The analysis by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) identified 19 volatile compounds (Table 1 and Fig. 1), accounting for 99.00 % of the total extracted oil, which were identified by matching retention times of available authentic standards, retention indices (RIs), and mass spectra in the NIST 17 database (Table 1). The essential oil was mainly composed of monoterpenoids (21.05 %) and sesquiterpenoids (78.95 %). As shown in Table 1, the oil was composed majorly caryophyllene (35.78 %), δ-cadinene (21.56 %), humulene (11.33 %), γ-muurolene (5.86 %), (-)-Isogermacrene D (4.47 %) and nerolidol (4.46 %). In an earlier report, the fruit essential oil of *S. mombin* contained 25 compounds among which were (*E*)-ethyl cinnamate (14.06 %) and benzyl benzoate (12.27 %). Methyl salicylate (13.05 %) and heptacosane (12.69 %) [28]. In another report, the essential oil from the leaves of *S. mombin* contained β-C aryophyllene (19.99%), δ-cadinene (9.07%) α-humulene (6.67%); α-Muurolene (5.45%); α-gurjunene (4.27%); α-muurolene (3.38%) and 5-isocedranol (3.03%) [29]. There was an increase in the oxygenated monoterpenoid contents and a concomitant decrease in the amounts of sesquiterpenoid hydrocarbons observed on drying the leaves in the oil of the same *S. mombin* from Nigeria [30]. Other researchers have reported that the essential oil of *S. mombin* could contain a myriad of components occurring in relatively different compositions in the various chemotypes of this plant [31-33].

Table 1. Chemical composition of the volatile oil from *Spondias mombin*

Name	[a]RI	[b]RI	Composition (%)
Bicyclo[2.2.1]hept-2-ene, 2,7,7-trimethyl-	905	903	0.08
2-Bornene	907	907	0.04
o-Cymene	1021	1025	1.86
Benzeneethanol, α,β-dimethyl-	1194	1190	2.16
Copaene	1374	1376	0.75
(Z)-Caryophyllene	1406	1409	0.24
Caryophllene	1420	1419	35.78
(-)-Isogermacrene D	1435	1437	4.47
α-Muurolene	1442	1440	0.99
Humulene	1453	1451	11.33
γ-Gurjunene	1474	1470	0.23
Gamma-muurolene	1470	1472	5.86
Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	1469	1473	1.28
β-Eudesmene	1480	1482	2.19
(Z,E)-α-Farnesene	1486	1483	0.68
δ-Cadinene	1514	1516	21.56
Nerolidol	1548	1551	4.46
α-Cadinol	1642	1642	0.50
Caryophyllenyl alcohol	1677	1677	1.15
		Total	95.11

[a]RI: Retention index determined relative to *n*-alkanes (C7-C30) on the HP-5ms column. [b]RI: literature retention indices [17-21].

The volatile oil from this research had some components common to those earlier reported. These components include: caryophyllene, cadinene, humulene and muurolene. However, this research reports the presence of some major components not found in or present in the very low composition in the oils of *S. mombin* earlier reported. These variations and similarities observed in the chemical composition of the essential oil of this plant's essential oil may be due

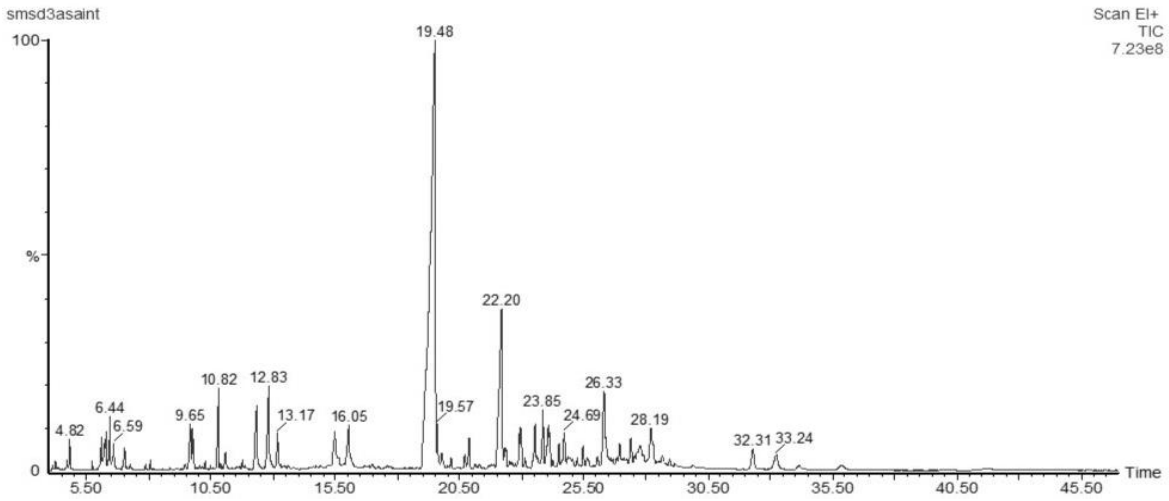

Figure 1. Total ion chromatogram of hydrodistilled oil of *Spondias mombin*.

Table 2. Zones of growth inhibition (mm), MICs, and MBCs of volatile oil from *Spondias mombin* against the growth of microorganisms[a]

Microorganisms	**Diameters of zones of inhibition** (mm),		**MICs**	**MCBs**
	Volatile oil	**Antibiotic**	**(µg/mL)**	**(µg/mL)**
Klebsiella pneumonia	22.50±0.2	23.00±0.2	62.40	122.80
Escherichia coli	22.90±0.1	23.00±0.3	86.60	178.50
Pseudomonas aeruginosa	20.20±0.1	21.00±0.2	70.20	132.10

Minimal Bactericidal Concentration (MBC); Minimum Inhibitory Concentration (MIC). Results were mean ± SD of triplicate values. Antibiotics used was gentamicin.

to geographical location or the possibility of a different chemotype.

3.2 Antimicrobial activity

The *in vitro* antimicrobial potency of the volatile oil of *S. mombin* against 3 pathogenic micro-organisms (*Klebsialla pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*) was evaluated using the disc diffusion method. The disc diameters of the zone of inhibition and the minimum inhibitory concentration (MIC) of the volatile oil for the tested microorganisms are shown in Table 2. The volatile oil was effective against *K. pneumoniae*, *E. coli* and *P. aeruginosa* with inhibition zones of 22.50, 22.90, and 20.20 mm respectively. The highest sensitivity of the oil was on *K. pneumoniae* with MIC and minimal bactericidal concentration (MBC) values of 62.40 and 122.80 µg/mL, followed by *P. aeruginosa* (MIC and MBC values of 70.20 and 132.10 µg/mL) and *E. coli* (MIC and MBC values of 86.60 and 178.50 µg/mL). Asante Ampadu et al., 2022 reported that the zones of inhibition of the oils from *S. mombin* ranged from 12 mm to 25 mm. They also stated that the biofilm inhibitory activities of the oils were dose-dependent [28]. The essential oil of *S. mombin* fresh and dried leaves were assayed for their potency against brine shrimps. The fresh leaf volatile oil was more active than that obtained from dried leaves, with LC_{50} values of 0.01 and 4.78 µg/mL, respectively [30] According to Plabon *et al*., 2021, the volatile oil of the peel oil showed the highest zone of inhibition against the *Aspergillus niger* (11.63 ± 0.0003 mm) and *Penicillium oxalicum* (13.67 ± 1.97 mm [34]. It is suggested that the potency shown by the essential oil of *S. mombin* could be due to the presence of major chemical components in the oil. The antimicrobial activity of humulene and caryophyllene has been reported [35-37]. The synergy between the various components of the oil could also be responsible for this potent action against these bacterial organisms.

3.3 Antioxidant activity

The antioxidant activity of the volatile oil of *S. mombin* was evaluated using three different methods. These are, the FRAP, DPPH and β-carotene bleaching assays respectively (Table 3). The volatile oil of *S. mombin* showed good antioxidant activity with 41.22 µg/mL, 5.55 µg/g and 83.24 % for DPPH, FRAP and β-carotene bleaching assays. Asante et al., 2022 evaluated the antioxidant potency of the essential oils of *S. mombin* using phosphomolybdenum, hydrogen peroxide

scavenging, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, and thiobarbituric acid reactive substances (TBARS) assays. The total antioxidant capacity of fruit and leaf volatile oils was 48.5 ± 0.7 μg/gAAE and 48.5 ± 0.7 μg/g AAE, respectively. The half maximal scavenging concentrations of the volatile oils in the hydrogen peroxide; DPPH and TBARS assays ranged from 252.2 μg/mL to 2288 μg/mL [28]. In another research, the oils from the fresh and dried leaves (1.0 mg/mL) scavenged hydroxyl radicals by 83% and 99.8%, respectively. The oils also reduced ferric ions significantly and compared favourably with vitamin C [30].

The antioxidant activity of the volatile oil of this plant could be attributed to the presence of some major components of its oil. The antioxidant property of caryophyllene has been earlier reported [38]. In the 3 Assays carried out, the essential oil of *S. mombin* showed antioxidant potency that was almost similar to that of the standard drug (Ascorbic Acid) used (Table 3).

Table 3. DPPH, FRAP and β-carotene bleaching antioxidant assay of the volatile oil from *Spondias mombin*.

Antioxidant Assay	*Spondias mombin* (Volatile oil)	ascorbic acid (Positive control)
DPPH Assay ((µg/mL)	41.22 ± 2.32	40.24 ±. 3.22
FRAP Assay (µg/g)	5.10 ± 0.02	5.55 ± 0.04
β-Carotene bleaching assay (%)	83.24 ± 2.24	84.82 ± 4.36

Results of antioxidant capability were reported in mean ± SD of triplicate values.

4. Conclusions

The volatile oil of *S. mombin* had mainly mono and sesquiterpenoids as its major component. The essential oil showed promising antibacterial and antioxidant capacity, which is suggested to be due to the presence of the major and minor components in the oil and their synergistic effect. The results indicate that the essential oil of *S. mombin* might be suitable for use as a natural antibacterial and antioxidant agent.

Authors' contributions

Conceptualization, O.T.A & I.S.N.; Methodology, O.T.A. and I.S.N.; Analysis; O.O.F, I.S.N and O.T.A; Resources, I.S.N.; Data curation, I.S.N; Writing-original draft and presentation, O.T.A; Writing-review and editing, O.T.A. and I.S.N.; Project administration, O.T.A.

Acknowledgements

Profound appreciation to the World Academy of Sciences (TWAS) for the award of the Scholarship that enabled this research to be conducted and to Comsats Institute of Information Technology, Abbottabad, Pakistan for been a worthy host.

Funding

This research was self-sponsored, with support from TWAS and Comsats Institute of Information Technology, Abbottabad, Pakistan.

Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors have declared that no competing interests exist.

References

1. Turrens, J.F. Mitochondrial formation of reactive oxygen species. J. Physiol. 2003, 552, 335-344.
2. Hayyan, M.; Hashim, M.A.; AlNashef, I.M. Superoxide ion: generation and chemical implications. Chem. Rev. 2016, 116(5), 3029-3085. doi:10.1021/acs.chemrev.5b00407.
3. Rada, B.; Leto, T.L. Oxidative innate immune defenses by Nox/Duox family NADPH oxidases. Trend. Inn. Imm. 2008, 15, 164-187.
4. Brooker, R. Genetics: Analysis and Principles (4th Edn.), New York: McGraw-Hill Science/ Engineering/Math 2011.
5. Patel, R.P.; Cornwell, T.; Darley-Usmar, V.M. The biochemistry of nitric oxide and peroxynitrite: implications for mitochondrial function. Oxidat. Str. Dis. 1999, 2, 39-56.
6. Van Raamsdonk, J.M.; Hekimi, S. Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. PLoS genetics 2009, 5, e1000361. doi:10.1371/journal. pgen.1000361. PMC 262 8729. PMID 19197346.
7. Dickinson, B.C.; Chang, C.J. Chemistry and biology of reactive oxygen species in signaling or stress responses. Nat. Chem. Biol. 2011, 7, 504-511.
8. Krishnaiah, D.; Sarbatly, R.; Bono, A. Phytochemical antioxidants for health and medicine a move towards nature. Biotechnol. Mol. Biol. Rev. 2007, 2, 97-104.
9. Eromosele, C.O.; Paschal, N.H. "Characterization and viscosity parameters of seed oils from wild

plants". Biores. Technol. 2003, 86, 203–205.
10. Uchendu, C.N.; Isek, T. Antifertility activity of aqueous ethanol leaf extract of *Spondias mombin* (Anacardiaceae) in rats. Afr. Health Sci. 2008, 8,163-167.
11. Kramer, A.; Mosquera, E.; Ruiz J.; Rodriguez E.; Ethnobotany and biological activity of plants utilized during pregnancy and child birth in the Peruvian amazon. Emanations from the rainforest of Carribean, Cornell University, 2002, 4. 24.
12. Ayoka, A.O.; Akomolafe, R.O.; Akinsomisoye O.S.; Ukponmwan O.E. "Medicinal and economic value of spondias mombin". Afr. J. Biom. Res. 2008, 11, 129–136.
13. Ademola, I.O.; Fagbemi, B.O.; Idowu, S.O. Antihelmintic activity of extract of *Spondias mombin* against gastrointestinal nematodes of sheep: Studies *in vitro* and *in vivo*. Trop. Anim. Health Prod. 2005, 37, 223-235. Doi: 10-1023/ B:Trop. 0000049296.47350.80.
14. Abad M.J.; Mermejo, P.; Carretero, E.; Martinez-Acitores, C. Anti-inflammatory activity of some medicinal plant extracts from Venezuela. J. Ethnopharmacol. 1996, 55, 63-68. Doi: 10.1016/s0378-8741(96)01478-X.
15. Abo, K.A., Ogunleye, V.O., Asindi, J.S. Antimicro-bialpotential of *Spondias mombin*, *Croton zambesicus* and *Zygotritonia crocea*. Phytother Res. 1999, 13, 494-497. PMID: 10479760.
16. Njoku I.S.; Ichide M.U.; Rahman N.; Khan A.M.; Otunomo I.; Asekun, O.T.; Familoni O.B. Variation in the sesquiternoid composition of the volatile oils of *Annona muricata* Linn. from South-Western Nigeria, caused by different drying methods. J. Chem. Soc. Nigeria. 2021, 46.
17. Njoku, I.S.; Ichide, M.U.; Rahman, N.; Khan, A.M.; Chibuko, N.A.; Asekun, O.T.; Familoni, O.B. Extraction, characterization and larvicidal activity of essential oil and hydrosol from *Sida acuta* Burm. f. leaves grown in Nigeria. Trop. J. Nat. Prod. Res. 2021, 5, 211-216.
18. Koenig, W.A.; Hochmuth D.H.; Joulain, D. Mass Finder 2.1 (Including the library) of terpenoids and related constituents of essential oils 1998, E-B Verlag: Hamburg.
19. El-Sayed, A.M. The pherobase: database of insect pheromones and semiochemicals 2011, See http://www.pherobase.com.
20. NIST LIBRARY. http://webbook.nist.gov/chem istry/(accessed 22 January 2020).
21. Jennings, W.; Shibamoto, T. Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary. Gas Chromatography.1989, Academic Press, New York.
22. Adams, R.P. Identification of essential oil components by gas chromatography/mass spectrometry 2007, 456, Carol Stream, IL: Allured Publishing Corporation.
23. Choudhary, M.I.; Begum, A.; Abbaskhan, A.; Musharraf, S.G.; Ejaz, A. Two new antioxidant phenylpropanoids from *Lindelofia stylosa*. Chem. Biodivers. 2008, 5, 2676-2683.
24. Clarke, G.; Ting, K.N.; Wiart, C.; Fry, J. High correlation of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. Antioxidants 2013, 2, 1-10.
25. Goodarzi, S.; Hadjiakhoondi, A.; Yassa, N.; Khanavi, M.; Tofighi. Essential oils chemical composition, antioxidant activities and total phenols of *Astrodaucus persicus*. Iran. J. Bas. Med. Sci. 2016, 19, 159.
26. Wang, W.; Wu, N.; Zu, Y.G.; Fu, Y.J. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chem. 2008, 108, 1019-1022.
27. Ha, C.T.; Thai, T.H.; Hien, N.T.; Anh, H.T.; Diep, L.N.; Thuy, D.T.; Setzer, W.N. Chemical composition and antimicrobial activity of the leaf and twig essential oils of *Magnolia hypolampra* growing in Na Hang Nature Reserve, Tuyen Quang Province of Vietnam. Nat. Prod. Comm. 2019, 14, 1934578X19860370.
28. Asante Ampadu, G.A.; Mensah, J.O.; Darko, G.; Borquaye, L.S. Essential oils from the fruits and leaves of *Spondias mombin* Linn.: Chemical composition, biological activity, and molecular docking study. Evid. Based Complement. Altr. Med. 2022.
29. Olufunke, M.D.; Kasali, A.A.; Olusegun, E. Constituents of the *Spondias mombin* Linn and the comparison between its fruit and leaf essential oils. J. Essent. Oil Bear. Plant. 2003, 6, 148-152.
30. Oladimeji, A.O.; Aliyu, M.B.; Ogundajo, A.L.; Babatunde, O.; Adeniran, O.I.; Balogun, O.S. Identification and comparison of the volatile constituents of fresh and dried leaves of Spondias mombin found in North-central Nigeria: in vitro evaluation of their cytotoxic and antioxidant activities. Pharm. Biol. 2016, 54, 2674-2678.
31. de Lima, E.Q.; de Oliveira, E.; de Brito, H.R. Extraction and characterization of the essential oils from *Spondias mombin* L.(Caj), *Spondias purpurea* L.(Ciriguela) and *Spondia ssp* (Cajarana do serto). Afr. J. Agric. Res. 2016, 11, 105-116.
32. Teai, T.; Claude-Lafontaine, A.; Schippa, C.; Cozzolino, F. Volatile compounds in fresh yellow mombin fruits (*Spondias mombin* L.) from French Polynesia. J. Essent. Oil Res. 2005, 17, 368-72.
33. Sagrero-Nieves, L.; De Pooter, H.L. Volatiles of the Jobo fruit (*Spondias mombin* L.). J. Essen. Oil Res. 1992, 4, 535-7.
34. Plabon, M.E.A.; Mondal, S.C.; Or Rashid, M.M.; Chowdhury, M.K.; Saeid, A.; Althobaiti, F.; Islam, M.S. Chemical composition and anti-microbial activity of

hog plum (*Spondias mombin* L.) peel oil extracted from different regions of tropical climates. Horticulturae. 2021, 7, 428.

35. Passos, G.F.; Fernandes, E.S.; da Cunha, F.M.; Ferreira, J.; Pianowski, L.F.; Campos, M.M.; Calixto, J.B. Anti-inflammatory and anti-allergic properties of the essential oil and active compounds from *Cordia verbenacea*. J. Ethnopharm. 2007, 110, 323-333.
36. Fernandes, E.S.; Passos, G.F.; Medeiros, R.; da Cunha, F.M.; Ferreira, J.; Campos, M. M.; Calixto, J.B. Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. Eur. J. Pharm. 2007, 569, 228-236.
37. Iacobellis, N.S.; Lo Cantore, P.; Capasso, F.; Senatore, F. 2005. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. *J. Agric. Food Chem.* 2005, 53, 57-61.
38. Dahham, S.S.; Tabana, Y.M.; Iqbal, M.A.; Ahamed, M.B.; Ezzat, M.O.; Majid, A.S.; Majid, A.M. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*. Molecules. 2015, 20, 11808-11829.