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Chemical Composition and Bioactivity of the Essential Oil of *Cassia singueana* Flowers Growing in Nigeria

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Abstract

Cassia singueana (Delile) Lock from the family Fabaceae is a well-known medicinal plant that grows abundantly in Nigeria and other African countries, and has long been used in the treatment of various ailments including malaria and other infectious diseases. The present study aimed at assessing the composition, and bioactivity of the essential oil of the flowers of *C. singueana* collected from Nigeria. The essential oil was extracted by hydrodistillation and the chemical composition was analyzed by gas chromatography (GC) coupled with a flame ionization detector (GC-FID) and GC coupled to mass spectrometry (GC–MS). The bioactivity of the oil was determined using the brine shrimp lethality assay, agar diffusion antimicrobial test, the 2, 2-diphenylpicrylhydrazyl, metal chelation, and superoxide anion antioxidant assays. The essential oil yield and the percentage of identified compounds were 1.58% and 97.91%, respectively. More than 20 compounds were identified. The major component was geranyl acetone (36.82%) followed by phytol (18.12%). The essential oil showed lethality against the brine shrimp larvae with an LC_{50} value of 18.7 µg/ml, and antimicrobial activity with largest inhibition zones of 32-33 mm against *Candida albicans, Streptococcus pneumoniae,* and *Staphylococcus aureus*. The oil also exhibited considerable antioxidant activity as evident from its ability to scavenge free-radicals such as DPPH, superoxide anions, and metal-chelation.

Keywords: *Cassia singueana,* Fabaceae, Essential oils, Antimicrobial, Antioxidant, Brine shrimp lethality assay

Introduction

Cassia singueana (Delile) Lock from the family Fabaceae-also called *Senna singueana*, and commonly known as scrambled egg, sticky pod, winter cassia, or winter-flowering cassia- is a woody annual herb or under shrub with 1.2 to 1.5 m height and small yellow flowers; it is widely distributed in India and tropical Africa including Western and Northern Nigeria (1, 2). The flowers of *C. singueana* have long been used traditionally in the treatment of typhoid, malaria, respiratory tract infections and as an antiulcer, antispasmodic, and anti-inflammatory agent by Yoruba, Fulani, and Hausa herbal medicine practitioners in Nigeria. Previous bioactivity studies on *C. singueana* showed its antidiabetic, antipyretic, antinociceptive, antioxidant, anticancer, antiplasmodial/antimalarial, hypolipidemic, and hepatoprotective properties (2-10), whilst the phytochemical investigations revealed the presence of anthraquinones, proanthocyanidins, other phenolics, fatty acids, amino acids, and triterpenoids in various parts of this plant (6, 10, 11). However, to the best of authors' knowledge, there was no report on the chemical composition and bioactivity of the essential oil of the flowers of *C. singueana* growing in Nigeria. As a part of authors ongoing phytochemical and bioactivity studies on African medicinal plants (12-18), the results of the GC-MS and GC-FID analyses of the essential oil of the flowers of *C. singueana*, and its antioxidant and antimicrobial properties and brine shrimp

lethality/toxicity were reported for the first time in the present paper.

Materials and methods

Plant material: Fresh flowers of *C. singueana* (Delile) Lock were collected from the premises of Ahmadu Bello University Zaria, Nigeria. The taxonomic identification of the plant was carried out at the herbarium section of the Department of Biological Science Ahmadu Bello University Zaria, Nigeria, where a voucher specimen (No. 1242) of this collection has been retained.

***Extraction of essential oil*:** Fresh flowers of *C. singueana* (1 kg) were subjected to hydrodistillation for 3.5 hours in a 5000 mL round bottom flask using a modified Clevenger-type hydrodistillation apparatus as described by the British Pharmacopeia (19). The oil was condensed and collected in *n*-hexane and rinsed with *n*-hexane to dissolve any oil particles adhering to the glass and kept in a freezer to allow separation of water from the essential oil. The concentrated oil was collected using a syringe, and then dried over anhydrous sodium sulfate. The pure oil kept at 4 °C in the dark for further analysis (20).

Gas chromatography-mass spectrometry analysis: The GC-MS analysis of the essential oil was performed on an Agilent 7890A GC coupled to a 5973 MSD mass spectrometer using an HP-5MS capillary column (30 m x 320 µm x 0.25 µm) and helium as a carrier gas at a flow rate of 3.3245 ml/minute. The GC oven was initially programmed at 50 ˚C for one minute and ramped at 80 ˚C/minute finally to 300 ˚C for five minutes. Electron impact ionization (EI) was achieved with ionization energy of 7ev. The essential oil was diluted with *n*-hexane and 2 µL of the diluted sample was injected. The data was acquired using GC-MS Analytical Chem-station software NIST-MS Library. Individual constituents were identified by comparing their retention data spectra with those stored in NIST0.8/Database/Kovat index and using analytical condition similar to that of GC-FID in reference with those of the chemical compounds gathered from the Adam table (21). The fragmentation patterns were also analyzed and compared with those stored in NIST-MS 0.8/Database (20).

Gas chromatography coupled to flame ionization detector: The GC-FID analysis was performed on an Agilent 7890 series filter with a flame ionization detector (FID) using the operating conditions as stated above. The FID was maintained at 260 ˚C. Split injection ratio was 1:20 (20).

Brine shrimp lethality assay: The brine shrimp lethality of the essential oil was determined using brine shrimp larvae (*Artemia salina)* in accordance with the method described by Oloyede *et al*. (22). In this assay, a drop of dimethyl sulfoxide (DMSO) was added to both test and control vials to enhance the solubility of the essential oil. Brine shrimp eggs (70 g) were hatched in a beaker containing 250 mL of seawater. The beaker was placed beside a window under sunlight for proper ventilation at room temperature. After 48 hours the brine shrimp larvae were collected by dropping pipette. About 1 mL of the essential oil was dissolved in 2 mL of *n*-hexane. Then, 50, 5, and 1 µg/L of the solution were drawn into vials; two drops of DMSO were added, and made up to 2 mL with distilled water corresponding to concentrations of 1000, 100, and 10 µg/ml, respectively. Each dosage was prepared in triplicates including the control. Ten shrimp larvae were added to each vial. The number of the surviving shrimp at each dosage and the control was recorded after 24 hours and the LC_{50} was computed using Finney Probit Analysis computer program (23).

Antimicrobial activity: Four bacterial strains, *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC10662), *Staphylococcus aureus* (NCTC6571), *Streptococcus pneumoniae* (NCTC7465) and one fungal strain, *Candida albicans* (NCIMB 3179), were used in the *in vitro* antimicrobial assay. DMSO was used to dissolve essential oil/drug, and the solution was diluted with sterile water to achieve the final concentration of 10%, which was used as the negative control. Levoxin, ofloxacin, and peflotab were used as the positive controls. The agar diffusion method using Muller-Hilton agar (MHA) (24) was used to determine the antimicrobial activity of the essential oil at different concentrations (1.0, 0.5, and 0.25 µg/ml). Agar (35 g) was dissolved in 1 L of distilled water and autoclaved at 121 °C for 15 minutes, cooled and poured into sterile Petri-dishes to solidify. A sterile cork-borer was used to make holes on each seeded agar plate for each concentration of the essential oil and the control. Plate count agar (PCA) plates were inoculated with 100 µL of standardized inoculum (1.5 x 10^8 CFU/ml) of each selected microbe (in triplicates) and spread with sterile cotton swab. The essential oil solutions (10 µg/ml) and the control were introduced into the holes containing the bacterial/ fungi inoculum and the plates were incubated microaerobically at 37 °C for 24 hours. The diameters of inhibition zones were recorded after 24 hours using a transparent ruler. The minimum inhibitory concentration (MIC) and MBC (minimum bactericidal concentration) of the essential oil were determined by microdilution agar method as described in the literature (24, 25). Active cultures for MIC determination were prepared by transferring a loopful of cells from the stock cultures to flasks, inoculated in MHA, and incubated at 37 °C for 24 hours. A 2-fold serial dilution of the essential oil was prepared in sterile distilled water to achieve a decreasing concentration

ranging from 160 to 1.25 mg/ml in nine sterile tubes labelled 1 to 9.

Sterile cork-borer of 8 mm diameter was used to bore well in the presolidified MHA plates and 100 µL of each dilution was added aseptically into the wells in that had microbe isolate seeded with the standardized inoculum (1.5 x 10^8 CFU/ml) and incubated at 37 °C for 24 hours. The lowest concentration of an extract showing a clear zone of inhibition after the macroscopic evaluation was considered as the MIC. In the determination of MBC, a 100 µL aliquot from the tube showing MIC was placed on MHA plate and spread over the plate and also incubated. After incubation at 37 °C for 24 hours, the plates were examined for bacterial growth to determine the concentration of the extract at which 99.9% killing of bacterial isolates was achieved.

Antioxidant activity: The antioxidant property of the essential oils of *C. singueana* flowers was determined by three different assays as outlined below:

DPPH free-radical scavenging activity: DPPH (the 2,2-diphenyl-l-picryl hydrazyl radical) free-radical scavenging activity of the essential oil was assessed using the method described by Lugasi *et al.* (26) and Marijana *et al*. (27). DPPH (39.4 mg) was dissolved in 100 mL of methanol to give a 1M solution. The solution was allowed to stand for 10 minutes and the absorbance was measured at 517 nm. About 2 mL of the oil extract solution (0.25, 0.50, 1.00, and 1.50 mg/ml) was prepared in the *n*-hexane, and 2 mL of 1M DDPH was added to 0.5 mL of each of the test solution. The mixture was shaken and left to stand for 10 minutes and the absorbance at 517 nm of the solutions was measured against that of the control and the percentage inhibition was calculated using the Equation 1. The same procedure was carried out using butyrate hydroxyl anisole (BHA), ascorbic acid, and α-tocopherol, which were used as positive controls.

$$Inhibition = \frac{(A_{DPPH} - A_S)}{A_{DPPH}} \times 100\%$$

A_{dpph} and A_S are the respective absorbance of the neat DPPH and test solutions, respectively.

Superoxide scavenging activity: The scavenging effect of the essential oil towards superoxide anion radicals were measured using the method published by Nishimiki *et al.* (28). However, 1 mL of nitroblue tetrazolium solution (156 µM in 100 mM phosphate buffer, pH 7.4), 1 mL of nicotine amide dinucleotide solution (468 µM in 100 mM phosphate buffer, pH 7.4), and 1 mL of sample solution (0.25, 0.5, and 1.0 mg/mL) were mixed in n-hexane. The reaction started with the addition of 100 µL of phenazine methosulfate solution (60 µM in 100 mM phosphate buffer, pH 7.4) to the mixture and after five minutes at room temperature, the absorbance was measured at 560 nm. BHA, α-tocopherol, and ascorbic acid were used as positive controls. The percentage inhibition of scavenging effect of superoxide anions was calculated using the Equation 2.

$$Inhibition = \frac{(A_{blank} - A_{Sample})}{A_{blank}} \times 100\%$$

Where A_{blank} is the absorbance of the blank in the absence of sample and A_{sample} is the absorbance in the presence of the sample.

Metal chelating activity: The chelation of ferrous ions by essential oil was estimated by the method described by Dinis *et al.* (29). The reaction mixture contained 0.5 mL of the oil in *n*-hexane (0.25, 0.5, 1.0, and 1.5 mg/ml), 1.5 mL of deionized water, and 0.5 mL of 2 mM $FeCl_2$ solution. After 30 minutes, 1.0 mL of 5 mM ferrozine solution was added. After 10 minutes of incubation at room temperature, the absorbance at 562 nm was measured. Ascorbic acid, BHA, and α-tocopherol were used as positive controls. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated using the Equation 3.

$$\%\ activity = \frac{(A_{control} - A_{Sample})}{A_{control}} \times 100\%$$

A_{dpph} and A_S are the respective absorbance of the DPPH and test solutions.

Where, $A_{control}$ is the absorbance of the blank in the absence of the sample and A_{sample} is the absorbance in the presence of the sample.

Results

***Extraction of essential oil and its composition*:** The average yield of the essential oil form the fresh flowers of *C. singueana* was 1.58%. The composition of the essential oil of this plant is presented in Table 1. The retention profile is presented in Figure 1. A total of 25 compounds making up 97.91% of the total essential oil, were identified. The major component was geranyl acetone (36.82%), followed by phytol (18.12%), squalene (10.84%), calarene (6.23%) α-terpineol (4.25%), menthol (3.51%), α-copaene (3.31%), α-bergermoten (2.19%), neomenthol (2.71%), geranyl acetate (1.67%), isomenthone (1.52%), *p*-mentha-1,5-dien-8-ol (1.47), limonene (1.34 %), and menthofuran (1.10%).

***Bioactivities*:** Brine shrimp toxicity of the essential oil of the flowers of *C. singueana* was determined by the brine shrimp lethality assay and the LD_{50} value was calculated 18.7 µg/mL after 24 hours. The essential oils displayed concentration-dependent antimicrobial activity against all of the test organisms (Table 2), with largest inhibition zones of 32-33 mm against *C. albicans, S. pneumoniae,* and *S. aureus*. The broadest activity of the essential oil against most Gram-negative bacteria was 1.563 µg/mL that was considered as MIC, while the MBC was 6.250 µg/mL. The essential oil showed considerable antioxidant activity as determined by all three assays.

Table 1 Chemical Composition of the Essential Oil of the Flowers of *Cassia singueana*

	Compound	Retention Time (min)	Kovat Index	Area (%)
1	**Limonene**	9.76	1033	1.34
2	**Phytol**	12.01	784	18.12
3	**α-Copaene**	13.61	1022	3.31
4	**α-Bergamoten**	13.90	983	2.19
5	**Caryophyllene oxide**	21.22	987	0.03
6	**Squalene**	24.41	1054	10.84
7	**α-Terpineol**	23.55	1175	4.25
8	**Calarene**	25.65	1420	6.23
9	**Cadinene**	26.34	1529	0.51
10	**Neophytadiene**	28.57	1223	1.04
11	**Neomenthol**	29.50	1159	2.71
12	**Geranyl acetone**	30.43	1237	36.82
13	**β-Caryophyllene**	31.01	1415	0.45
14	**Isomenthone**	32.02	1192	1.52
15	**Menthofuran**	32.50	1155	1.10
16	**Menthol**	33.82	1171	3.51
17	**1-Octen-3-ol**	34.50	969	0.06
18	**Germacrene D**	35.90	1474	1.05
19	**Geranyl acetate**	36.50	1352	1.67
20	**Terpinolene**	37.20	1080	0.53
21	**α-Caryophyllene**	39.03	1230	1.02
22	**α-Terpinene**	40.01	1057	0.08
23	**Menthone**	42.76	1142	1.03
24	**Terpinen-4-ol**	43.14	1159	0.03
25	***p*-Mentha-1,5-dien-8-ol**	45.00	1182	1.47
Total Constituents Identified				**97.91**
Yield (%)				**1.58**

The scavenging of the free-radicals by the essential oil increased with the increase in the concentration. In the DPPH assay, at a concentration of 1.5 µg/mL, the scavenging capacity of the essential oil was 93.3%, which was comparable to that of the positive control BHA (96.7%) and higher than those of ascorbic acid and α-tocopherol (75.8% and 17.3%, respectively). In the superoxide anion assay, at a concentration of 1.5 µg/mL, the percent scavenging by the essential oil was the same as that of BHA (82.6%) and higher than those of ascorbic acid and α-tocopherol (68.8% and 59.3%, respectively). The effectiveness of the antioxidants as superoxide anion scavenger ranged in the following descending order: BHA ≥ essential oil > ascorbic acid > α-tocopherol. In the metal chelation assay, the highest scavenging activity of the essential was 82.5%, which was higher than that of BHA, ascorbic acid, and α-tocopherol (41.0%, 47.1%, and 18.1%, respectively) at the concentration of 1.5 µg/mL. The effectiveness of the antioxidants as metal chelating agent was in the following descending order: essential oil > ascorbic acid > BHA > α-tocopherol.

Discussion

The yield (1.58%) of essential oils from the hydrodistillation of fresh flowers of C. singueana was higher than the yields reported for other plants industrially exploited as sources of essential oils, e g, lavender (0.8%-1.8%), mint (0.5%-1%), neroli (0.5%-1%), laurel (0.10.35%), and Lippia rotundifolia (0.01%) (20). However, the yield was similar to the values reported for other Cassia species, e g, C. senna-alata (1.43%), C. sophera (1.07%) and C. arrerehdel (1.47%) (30, 31).

This is the first report on the GC-MS and GC-FID analyses leading to the identification and quantification of the constituents of the essential oil of the flowers of C. singueana collected from Nigeria. However, reports on composition of the essential oils from other Cassia species- e g, C. senna-alata, C. sophera, and C. arrerehdel are available to date (30, 31). The essential oil composition of C. singueana as determined in the current study showed that geranyl acetone (36.82%), phytol (18.12%), squalene (10.84%), calarene (6.23%), and menthol (3.51%) were the major components (Table 1). These major components were also found in the essential oils of other Cassia species. Phytol was reported as one of the major compounds in the essential oil in another Cassia species (30).

Brine shrimp lethality of the essential oils of C. singueana flowers (LD_{50} = 18.7 µg/ml) indicated that the oil was more toxic than the extracts obtained from the leaves, stems, and roots (LD_{50}= 36.67, 39.78, and 20.9 µg/ml, respectively) (32). The higher cytotoxicity of the essential oil of the flowers might be partly attributed to various volatile components (20, 33). For example, phytol, one of the major compounds in the essential oil of the flowers of C. singueana is an antitumor and anti-inflammatory agent (34).

The essential oil was active against all tested microbial strains (Table 2), and the activity increased with the increase in concentrations. At a concentration of 1.0 µg/ml, the maximum zone of inhibition of 32 and 33 mm were recorded against C. albicans and S. aureus and 30 mm against S. pneumonia, which were comparable to those observed with the reference standards (positive controls) at the same concentration for these microbes. These results were in agreement with the observations made by Usman et al. (35) and Esmaeili et al. (36) for the essential oil of other plant species, e g, Euphorbia species and Tamarix boveana. However, Usman et al. (35) reported that the essential oil of Euphorbia species was more active against Gram-negative bacteria than Gram-positive ones, while Esmaeili et al., (36) observed that the essential oil of T. bioveana was more active against Gram-positive bacteria than Gram-negative ones.

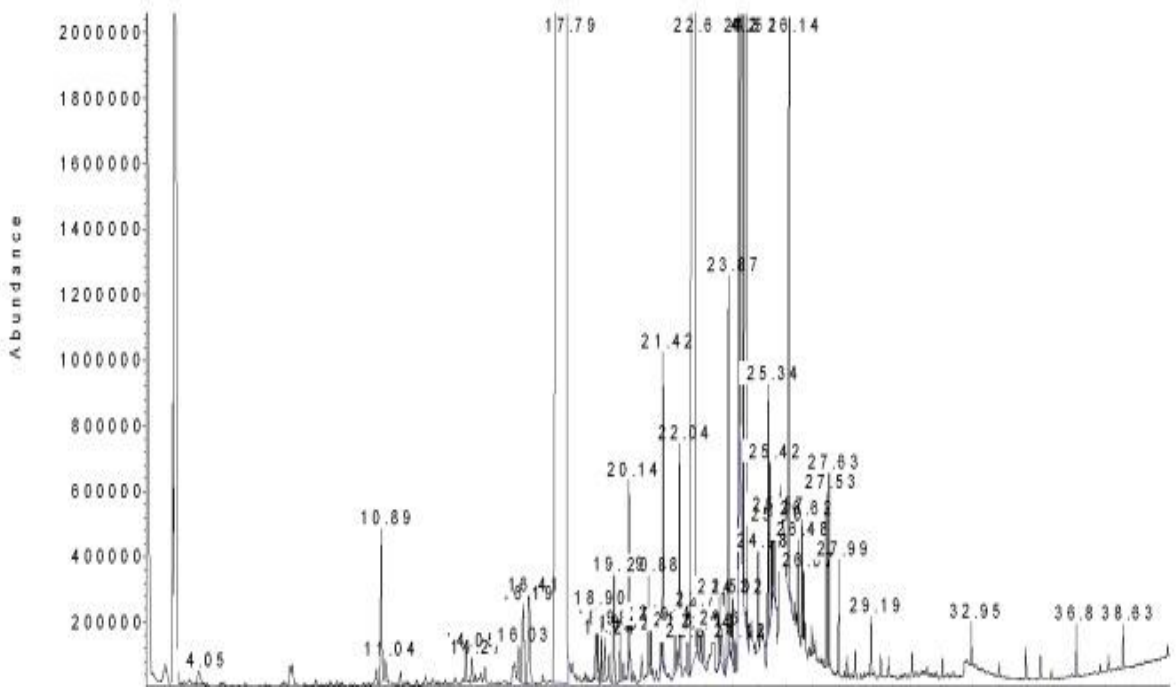

Figure 1 GC Retention Profile of the Essential Oil of *C. singueana*

Table 2 Antimicrobial Activity of the Essential Oil of the Flowers of *C. singueana*

Sample	Concentration (µg/ml)	Zones of Inhibition (mm)				
		SA	EC	PA	SP	CA
Essential oil	1.0	33 ± 0.03	27 ± 0.02	28 ± 0.21	30 ± 0.30	32 ± 0.22
	0.5	17 ± 0.50	13 ± 0.11	16 ± 0.03	14 ± 0.07	12 ± 0.23
	0.25	10 ± 0.01	10 ± 0.03	12 ± 0.04	10 ± 0.15	10 ± 0.24
Levoxin®	1.0	31 ± 0.02	27 ± 0.04	28 ± 0.23	30 ± 0.03	30 ± 0.04
	0.5	17 ± 0.03	13 ± 0.31	16 ± 0.41	14 ± 0.13	12 ± 0.05
	0.25	10 ± 0.30	10 ± 0.04	12 ± 0.32	10 ± 0.20	10 ± 0.24
Ofloxaxin®	1.0	31 ± 0.23	27 ± 0.12	28 ± 0.31	32 ± 0.04	30 ± 0.11
	0.5	17 ± 0.12	13 ± 0.03	16 ± 0.51	14 ± 0.33	12 ± 0.31
	0.25	10 ± 0.21	10 ± 0.31	12 ± 0.22	10 ± 0.41	10 ± 0.42
Peflotab ®	1.0	32 ± 0.04	27 ± 0.22	28 ± 0.08	30 ± 0.06	31 ± 0.03
	0.5	17 ± 0.03	13 ± 0.04	16 ± 0.05	14 ± 0.51	12 ± 0.22
	0.25	10 ± 0.06	10 ± 0.03	12 ± 0.51	10 ± 0.41	10 ± 0.06

SA = *Staphylococcus aureus;* EC = *Escherichia coli;* PA = *Pseudomonas aeruginosa;* SP = *Streptococcus pneumonia;* CA = *Candida albicans*

The essential oil of C. singueana was found to be a better metal chelating agent than radical scavenger (DPPH and superoxide anion). Overall, the essential oil displayed significant antioxidant property comparable or better than the positive controls used in this work. Iron is the most important lipid oxidation pro-oxidant among the transition metals (37). Lipid oxidation is accelerated by breaking down of hydrogen peroxide and lipid peroxides by the ferrous state of iron to reactive free radicals through the reaction shown below:

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + .OH + -OH +$ (Fenton reaction)

Radicals from peroxides are also produced by Fe^{3+} ion. These can lead to lipid oxidation, modification of protein and damage to DNA. Metal ions could be inactivated by chelating agents and the metal-dependent processes could potentially be inhibited (38). In the metal chelating assay, ferrozine can quantitatively form complexes with Fe^{2+}. Terpenoids, which are major constituents of this essential oil generally act as antioxidants by trapping free radicals, scavenge free radicals, and chelate metals as well (39). Three main ways of antioxidant actions of terpenoids are quenching of singlet oxygen, hydrogen transfer, or electron transfer. The present study established that the essential oil had proton donating ability and could serve as free–radical inhibitor.

The antimicrobial and antioxidant properties, and brine shrimp toxicity of the essential oil of the flowers of C. singueana as observed in the present study might provide some scientific rationale behind some of the traditional medicinal uses of this plant; particularly, the high level of antioxidant property of the essential oil could contribute to its traditional uses as an anti-inflammatory agent.

Conclusion

This is the first report on the analysis of the essential oil of the flowers of C. singueana growing in Nigeria, and geranyl acetone (36.82%) was identified as the major compound of this oil. Bioactivity studies on this oil established its potential as an antioxidant and antimicrobial agent. However, considerable level of toxicity towards brine shrimps indicated that caution should be taken before using this oil for any medicinal purposes.

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Authors' Contributions

Bilkisu Adedoyin carried out the lab work, all other authors contributed equally in coming up with the concept, project planning and editing the first draft submitted by Bilkisu Adedoyin.

Conflict of interest

Authors declared no conflict of interest.

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References

1. Olajide OO, Olusola A, Afolayan M, Khan IZ. Preliminary phytochemical and antimicrobial screening of the leaf extract of *Cassia singueana* Del. African J Pure Appl Chem 2011; 5:65-9.
2. Adzu B, Abbah J, Vongtau H, Gamaniel K. Studies on the use of *Cassia singueana* in malaria ethnopharmacy. J Ethnopharmacol 2003; 88:261-7.
3. Ottu OJ, Atawodi SE, Onyike E. Antioxidant, hepatoprotective and hypolipidemic effects of methanolic root extract of Cassia singueana in rats following acute and chronic carbon tetrachloride intoxication. Asian Pac J Trop Med 2013; 6:609-15.
4. Atawodi SE, Adekunle OO, Bala I. Antioxidant, organ protective and ameliorative properties of methanol extract of *Anogeissus leiocarpus* stem bark against carbon tetrachloride-induced liver injury. Int J Pharm Sci Res 2011;2:1443-8.
5. Hishe HZ, Ambech TA, Hiben MG, Fanta BS. Anti-nociceptive effect of methanol extract of leaves of *Senna singueana* in mice. J Ethnopharmacol 2018; 217:49-53.
6. Sobeh M, Mahmoud ME, Hasan RA, Cheng H, El-Shazly AM, Wink M. *Senna singueana*: antioxidant, hepatoprotective, antiapoptotic properties and phytochemical profiling of a methanol bark extract. Molecules 2017; 22: pii: E1502.
7. Ior L. *In vivo* assessment of the antimalarial activity of *Cassia singueana* and *Cymbopogon citrus*. Basic Clin Pharmacol Toxicol 2014; 115:126.
8. Ibrahim MA, Islam MS. Antidiabetic effects of the acetone fraction of Senna singueana stem bark in a type 2 diabetes rat model. J Ethnopharmacol 2014; 153:392-9.
9. Ottu OJ, Atawodi SE, Onyike E. Antioxidant, hepatoprotective and hypolipidemic effects of methanolic root extract of *Cassia singueana* in rats following acute and chronic carbon tetrachloride intoxication. Asian Pacific J Trop Med 2013; 6:609-15.
10. Ibrahim MA, Koorbanally NA, Islam MS. *In vitro* antioxidative activities and GC-MS analysis of various solvent extracts of *Cassia singueana* parts. Acta Poloniae Pharmaceutica 2013; 70:709-19.
11. Mutasa SL, Khan MR, Jewers K. 7-Methylphyscion and cassiamin A from the root bark of *Cassia singueana*. Planta Med 1990; 56:244-5.
12. Guetchueng ST, Nahar L, Ritchie KJ, Ismail FMD, Evans AR, Sarker SD. *Ent*-Clerodane diterpenes from the bark of *Croton oligandrum* Pierre *ex* Hutch. and assessment of their cytotoxicity against human cancer cell lines. Molecules 2018; 23:pii: E410
13. Guetchueng ST, Nahar L, Ritchie KJ, Ismail FMD, Wansi JD, Evans A, et al. Kaurane diterpenes from the fruits of *Zanthoxylum leprieurii* (Rutaceae). Records Nat Prod 2017; 11:304-9.
14. Wansi JD, Alain TT, Toze FAA, Nahar L, Martin C, Sarker SD. Cytotoxic acridone and indoloquinazoline alkaloids from *Zanthoxylum poggei*. Phytochem Letts 2016; 17:293-8.
15. Al-Groshi A, Evans AR, Ismail FMD, Nahar L, Sarker SD. Cytotoxicity of Libyan *Juniperus phoenicea* against human cancer cell lines A549, EJ138, HepG2 and MCF7. Pharm Sci 2018; 24:3-7.
16. Geroushi A, Auzi AA, Elhwegi AS, Elzawam F, El-Sherif A, Nahar L, Sarker SD. Anti-inflammatory sesquiterpenes from the root oil of *Ferula hermonis*. Phytother Res 2011; 25:774-7.
17. Geroushi A, Auzi AA, Elhwuegi AS, Elzawam F, El Sherif E, Nahar L, et al. Antinociceptive and anti-inflammatory activity of *Ferula hermonis* root oil in experimental animals. Latin Am J Pharm 2010; 29:1436-9.
18. Tahsin T, Wansi JD, Al-Groshi A, Evans A, Nahar L, Martin C, et al. Cytotoxic properties of the stem bark of *Citrus reticulata* Blanco (Rutaceae). Phytotherapy Res 2017; 31:1215-9.
19. British Pharmacopoeia. Hydrodistillation Clevenger apparatus method. Her Majesty's Stationary Office, London, 2007.

20. Onocha PA, Oloyede GK, Afolabi QO. Chemical composition, cytotoxicity and antioxidant activity of essential oils of *Acalypha hispida* flowers. Int J Pharmacol 2011; **7**:144-8.
21. Adams RP. Identification of essential oil components by gas chromatography/mass spectroscopy. Illinois: Allured Publishing Corporation 1995;1-69.
22. Oloyede GK, Akpomedeye PO, Soyinka J, Oguntokun O, Emmanuel T. Phytochemical screening, antimicrobial and antioxidant activities of four Nigerian medicinal plants. Anal Biol Res 2010; 1:114-20.
23. Garba S, Salihu L, Bello MB. Aphyosemion Gardneri Test (AGT) for cytotoxicity. Nig J Sci Res 2009; 2:56-7.
24. Andrews JM. Determination of minimum inhibitory concentration. J Antimicrob Chemother 2001; 48:5-16.
25. Irkin R, Korukluoglu M. Control of *Aspergillus niger* with garlic, onion and leek extracts. Afr J Biotechnol 2007; 6:384-7.
26. Lugasi A, Honvahrich P, Dworshark A. Addition information to the *in vitro* antioxidant activity of *Ginkgo biloba* L. Phototherapy Res 1999; 13:160-2.
27. Marijana ZK, Monika BI, Perkovićand BZ. Antiradical, chelating and antioxidant activities of hydroxamic acids and hydroxyureas. Molecules 2011; 16:6232-42.
28. Nishimiki M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Comm 1972; 46:849–53.
29. Dinis TCP, Madeira VMC, Almeida MLM. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994; 3:161–9.
30. Mizanur MR, Taslima SM, Yousuf AM, Masidur RS, Al-Reza M, Atiqur R. Chemical composition and antibacterial activity of the essential oil and various extracts from *Cassia sophera* L. against *Bacillus* sp. from soil. Arab J Chem 2017; 10:S2132-S7.
31. Omotoyinbo O, Victor-Sanni V, David M. GC-MS analysis of phytocomponents from the leaves of *Senna alata* L. J Plant Scs 2015 ;3:133-6.
32. Ode OJ, and Onakpa MM. Evaluation of *Cassia Singueana* extract on stomach HCl production and gastric emptying in rats. Int J Appl Biol Pharm Technol 2013; 1;1352-7.
33. Adedoyin BJ, Okeniyi SO, Garba S, Salihu L. Cytotoxicity, antioxidant and antimicrobial activities of essential oil extracted from *Euphorbia heterophylla* plant. Topclass J Herbal Med 2013; 2:84-9.
34. Ogunlesi M, Wesley O, Edith O, Osibote EA. Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn (Euphorbiaceae): A potential medication for asthma. Afr J Biotechnol 2009; 24:742-50.
35. Usman H, Abdulrahman FI, Ahmed IA, Kaita A, Khan IZ. Antibacterial effects of cyanogenic glucoside isolated from the stem bark of *Bauhinia rufescens* Lam. Int J Biol Chem Sci 2013; 7:2139-50.
36. Esmaeili M, Kanani M, Sonboli A. *Salvia reuterana* extract prevents formation of advanced glycation end products: An *in vitro* study. Iranian J Pharm Sci 2010; 6:33–50.
37. Roya K, Mostafa A, Ramtin P. Essential oil compositions and *in vitro* antioxidant and antibacterial activities of the methanol extracts of two *salvia* species (Lamiaceae) from Iran. Int J Agric Crop Science 2013; 5:1171-82.
38. Finefrock AE, Bush AI, Doraiswamy PM. Current status of metals as therapeutic targets in Alzheimer's disease. J Am Geriatr Soc 2003; 8:51–8.
39. Hillmann J. Reformulation key for consumer appeal into the next decade. J Food Res 2010; 37:14–9.