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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 objectives of the present study was to assess 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, DPPH, 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 components were geranyl acetone (36.82%) and 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 against all of the test organisms, with maximum zones of inhibition of 32-33 mm against *C. 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 anion and metal-chelation.

Keywords:

Cassia singueana

Fabaceae

Essential oils

Antimicrobial

Antioxidant

Brine shrimp lethality assay

Agar diffusion method

DPPH

Introduction

Cassia singueana (Delile) Lock (family: Fabaceae; synonym: *Senna singueana*), commonly known as 'scrambled egg', 'sticky pod', 'winter cassia' or 'winter-flowering cassia', is a woody annual herb or under shrub between 1.2 and 1.5 m in height with 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 the 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 our knowledge, there is 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 our ongoing phytochemical and bioactivity studies on African medicinal plants (12-18), we now report, for the first time, the results of the GC-MS and GC-FID analyses of the essential oil of the flowers of *C. singueana*, and its antioxidant, antimicrobial properties and brine shrimp lethality/toxicity.

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 h 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 (GC-MS) 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/min. The GC oven was initially programmed at 50^{o}C (held for 1 min) and ramped at 80^{o}C/min finally to 300^{o}C (held for 5 min). Electron impact ionization (EI) was achieved with ionization energy of 7ev. The essential oil was diluted with *n*-hexane and 2µL 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/Kovats index and using analytical condition similar to that of GC-FID in reference with those of the chemical compounds gathered from the Adams' 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 (GC-FID)

The GC-FID analysis was performed on an Agilent 7890 series filter with a flame ionisation detector (FID) using the operating conditions as stated above. The FID was maintained at 260^{o}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 for light and proper ventilation at room temperature. After 48 h the brine shrimp larvae were collected by dropping pipette. About 1 mL portion of the essential oil was dissolved in 2 mL of *n*-hexane. 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 h 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% DMSO, which was used as a 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 (35g) was dissolved in 1 L of distilled water and autoclaved at 121°C for 15 min, 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 (1.5×10^8 CFU/mL) of each selected microbe (in triplicates) and spread with sterile cotton swab. The essential oil solutions (10 µ/mL) and the control were introduced into the holes containing the bacterial/ fungi inoculum and the plates were incubated micro aerobically at 37°C for 24 h. The diameters of zones of inhibition were recorded after 24 h 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 medium and incubated at 37°C for 24 h. A 2-fold serial dilutions of the essential oil were prepared in sterile distilled water to achieve a decreasing concentration ranging from 160 to 1.25 mg/mL in 9 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 h.. 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 h, the plates were examined for the growth of a bacterium 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* was determined by three different assays as outlined below.

DPPH free-radical scavenging activity: The 2,2-diphenyl-l-picryl hydrazyl radical (DPPH) free-radical scavenging activity of the essential oil was assessed using the method as 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 min and the absorbance at 517 nm was measured. About 2 mL of the solution of (0.25, 0.50, 1.00 and 1.50 mg/mL) of the oil extract in the *n*-hexane was prepared, 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 min and the absorbance at 517 nm of the solutions was measured against that of the control and the percentage inhibition was calculated using the following 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). A volume of 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) in n-hexane were mixed. 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 5 min at room temperature, the absorbance at 560 nm was measured. BHA, α-tocopherol and ascorbic acid were used as positive controls. The percentage inhibition of scavenging effect of anion superoxide anion was calculated the equation below.

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

Where, A_{blank} is the absorbance of the blank in 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 of $FeCl_2$ solution. After 30 min, 1.0 mL of 5 mM ferrozine solution was added. After 10 min 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 from the following equation.

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

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 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 components (in decreasing order) were geranyl acetone (36.82%), 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%).

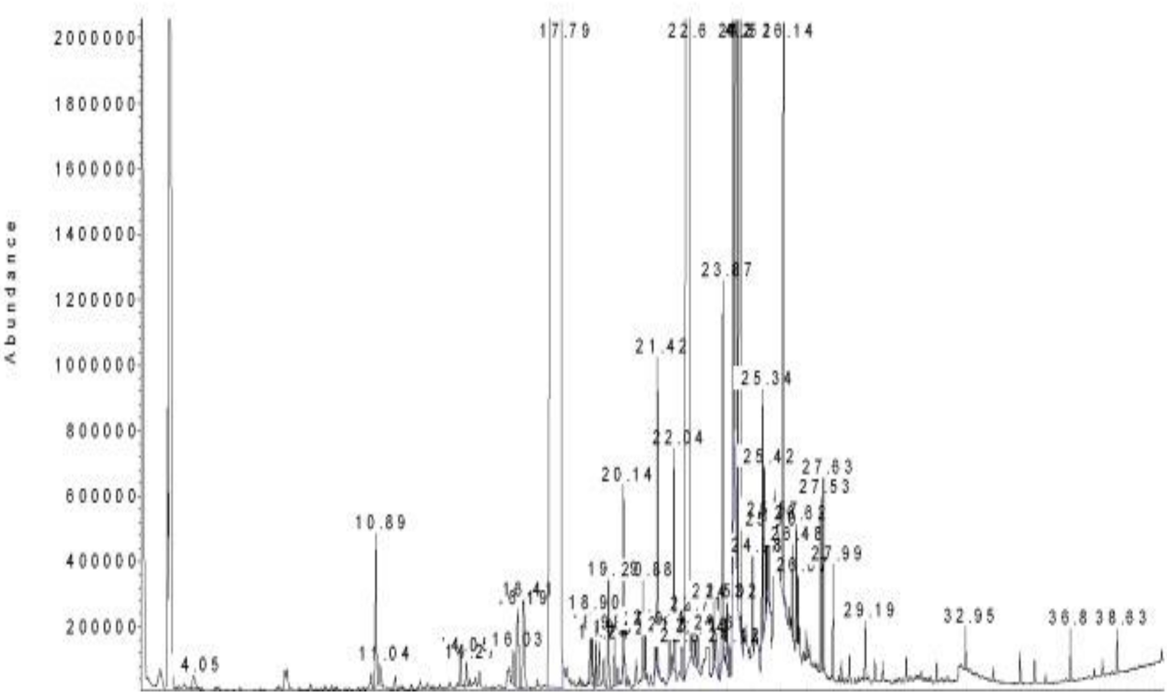

Figure 1 GC retention profile of the essential oil of *C. singueana*

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 as 18.7 μg/mL (after 24 h). The essential oils displayed concentration-dependent antimicrobial activity against all of the test organisms (Table 2), with maximum zones of inhibition of 32-33 mm against *C. albicans*, *Streptococcus pneumoniae* and *Staphylococcus aureus*. The broadest activity of the essential oil against most Gram-negative organisms was at 1.563 μg/mL as an MIC, while the MBC of 6.250 μg/mL was noted.

The essential oil showed considerable antioxidant activity as determined by all three assays. 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.

Table 1 Chemical composition of the essential oil of the flowers of *Cassia singueana*

	Compounds	**Retention time (min)**	**Kovats 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**

Table 2. Antimicrobial activity of the essential oil of the flowers of *C. singueana*

Samples	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*

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 our 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, onw 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 30mm 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 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-negative strains, while Esmaeili *et al*. (36) observed that the essential oil of *T. bioveana* was more active against Garm-positive bacteria than Gram-positive species.

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.

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

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 have established its potential as an antioxidant and antimicrobial agent. However, considerable level of toxicity towards brine shrimps indicated that caution must be taken before using this oil for any medicinal purposes.

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Conflict of interest statement

We declare that we have no conflict of interest.

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: 65-69.
2. Adzu B, Abbah J, Vongtau H, Gamaniel K. Studies on the use of *Cassia singueana* in malaria ethnopharmacy. J Ethnopharmacol 2003; 88: 261-267.
3. Ode OJ, Onakpa MM, Asuzu OV. Evaluation of *Cassia Singueana* extract on stomach HCl production and gastric emptying in rats. Int J Appl Biol Pharm Technol 2010; 1: 1352-1357.
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; 2:1443-1448.
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, and Wink M. *Senna singueana*: antioxidant, hepatoprotective, antiapoptotic properties and phytochemical profiling of a methanol bark extract. Molecules 2017; 22: article number 1502. DOI: 10.3390/molecules22091502
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: 392-399.
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: 609-615.

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: 709-719.
11. Mutasa SL, Khan MR, Jewers K. 7-Methylphyscion and cassiamin A from the root bark of *Cassia singueana*. Planta Medica 1990; 56: 244-245.
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: Article no. 410 (9 pages) doi:10.3390/molecules23020410
13. Guetchueng ST, Nahar L, Ritchie KJ, Ismail FMD, Wansi JD, Evans A, Sarker SD. (2017) Kaurane diterpenes from the fruits of *Zanthoxylum leprieurii* (Rutaceae), Records of Nat Prod 2017; 11: 304-309.
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: 293-298.
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 Scs 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*. Phytotherapy Res 2011; 25: 774-777.
17. Geroushi A, Auzi AA, Elhwuegi AS, Elzawam F, El Sherif E, Nahar L, Sarker SD. Antinociceptive and anti-inflammatory activity of *Ferula hermonis* root oil in experimental animals. Latin Am J Pharm 2010; 29: 1436-1439.
18. Tahsin T, Wansi JD, Al-Groshi A, Evans A, Nahar L, Martin C, Sarker SD. Cytotoxic properties of the stem bark of *Citrus reticulata* Blanco (Rutaceae). Phytotherapy Res 2017; 31: 1215-1219.
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**: 144-148.
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. Scholars Res Library, Anal Biol Res 2010; 1: 114-120.
23. Garba S, Salihu L, Bello MB. Aphyosemion Gardneri Test (AGT) for cytotoxicity. Nig J Sci Res 2009; 2: 56-57.
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: 384-387.
26. Lugasi A, Honvahrich P, Dworshark A. Addition information to the *in vitro* antioxidant activity of *Ginkgo biloba* L. Phototherapy Res 1999; 13:160-162.
27. Marijana ZK, Monika BI, Perkovićand BZ. Antiradical, chelating and antioxidant activities of hydroxamic acids and hydroxyureas. Molecules 2011; 16: 6232-6242.
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: 849–853.
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: 161–169.
30. Mizanur MR. Taslima S.M, 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-S2137.
31. Omotoyinbo O, Victor-Sanni V, David M. GC-MS analysis of phytocomponents from the leaves of *Senna alata* L. J Plant Scs 2015; 3:133-136.
32. Ode OJ, and Onakpa MM. Evaluation of *Cassia Singueana* extract on stomach HCl production and gastric emptying in rats. Int Journal of Applied Biol Pharm Technol 2013; 1; 1352-1357.
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: 84-89.
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: 742-750.

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: 2139-2150.
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: 1171-1182.
38. Finefrock AE, Bush AI, Doraiswamy PM. Current status of metals as therapeutic targets in Alzheimer's disease. J Am Geriatr Soc 2003; 8: 51–58.
39. Hillmann J. Reformulation key for consumer appeal into the next decade. J Food Res 2010; **37**:14–19.