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Phytochemical screening, gc-ms analysis and antioxidant activity of methanol stem bark extract of *Cassia sieberiana* DC

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Abstract

The over production of free radicals in the human body is known to cause oxidative stress which plays an important role in the pathogenesis of many diseases. Therefore, the search for natural antioxidants would continue to be a major area of research for many decades. This study was to investigate the *in vitro* antioxidant activities and to determine the phytochemicals present in *Cassia sieberiana*. The methanol stem bark extract of *C. sieberiana* was subjected to phytochemical screening and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Its antioxidant activities were assayed *in vitro* using nitric oxide scavenging assay, ferric reducing antioxidant potential assay, lipid peroxidation inhibitory assay and 2-diphenyl-1-picrylhydrazyl scavenging assay. The phytochemical screening of the stem bark extract of *C. sieberiana* showed saponins, alkaloids, flavonoids, steroids, tannins, phlobatannins, terpernoids, cardiac glycosides, phenolics and reducing sugar. The GC-MS Table revealed that the methanol extract has 51 bioactive compounds with 3-0-Methyl-d-glucose having the highest percentage of 12.16 % while 5.alpha-Androstan-3-beta-ol,4,4-dimethyl- having the lowest percentage of 0.09 %. The antioxidant study showed concentration-dependent radical scavenging activity of the methanol stem bark extract of *C. sieberiana*. This concentration-dependent result was also observed when compared with ascorbic acid used as the standard drug. The presence of different phytochemicals such as alkaloids, saponins, tannins, phenols, flavonoids, among other things, might be responsible for *C. sieberiana* antioxidant activity. The study concluded that the methanol stem bark extract of *C. sieberiana* contains 51 bioactive compounds and showed good antioxidant activity which could be therapeutic in several human diseases.

Key words: *Cassia sieberiana*, Phytochemical, Antioxidant, Pharmaceuticals

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Introduction

Infections caused by pathogenic microbes occur when the microbes invade and multiply in a host by overcoming the host's immune system (Olajuyigbe et al., 2018). For instance, infectious diseases have been reported to cause 15 million deaths each year, accounting for approximately 27.12% of deaths worldwide (Dye, 2014). They are also responsible for 560,000 of the 2.7 million neonatal deaths registered each year (WHO, 2021). The use of plant-based medicine has become popular among individuals and communities in Nigeria and other developing countries; where the practice of medicinal plants derived remedies in traditional health care practices is common and widespread (Sugita et al., 2020). Currently, about 80% of the World's population still depends on the traditional medicine practices for the management of various human and other animal diseases (WHO, 2021, Wintola et al., 2021). Plants produce various bioactive compounds that contain components of therapeutic value making them a rich source of medicines (Sugita et al., 2020). These Plants possess powerful therapeutic potentials with great number of phytochemicals and antioxidants embodied in them which are known to prevent diseases and are also used to facilitate the treatment of several illnesses (Wintola et al., 2021).

One plant that is mostly and widely used by traditional health practitioners due to its therapeutic value is *C. sieberiana* (Mary-Ann et al., 2019). *C. sieberiana* commonly known as West African laburnum is a tropical deciduous small tree. It is characterized by bright yellow flowers that form into groups (Mary-Ann et al., 2019). It belongs to the kingdom Plantae; subkingdom Tracheobionta; phylum Angiospermophyta; superdivision Spermatophyta; division Magnoliophyta; class Magnoliopsida; sub-class Rosidae; order Fabales; family Fabaceae; genus *Cassia* and species *sieberiana* (von Maydell, 1986). In Nigeria, *C. sieberiana* is locally called Dankila (Hausa), Ugba-oyibo (Igbo) and Aridan-tòòrò (Yoruba). Its various parts have been used to treat fever, malaria, gastroenteritis, leprosy, jaundice, inflammation, gonorrhoea, HIV/AIDS, sleeping sickness, venereal disease, sterility disorder, ulcer and so on (Mary-Ann et al., 2019). Despite the fact that there are scientific evidences affirming the ethnomedicinal value of *C. sieberiana*, the bioactive constituents of its stem bark and its antioxidant activity remain vague. Therefore, the objectives of the present study were to identify the chemical compositions in the methanol stem-bark extract of *C. sieberiana* using phytochemical screenings; evaluate its bioactive compounds using gas chromatography-mass spectrometry (GC-MS) analysis; and to determine its antioxidant activity using standard procedures.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh stem bark of *C sieberiana* was purchased from traditional herbal vendor at Oke-Aje market, Ijebu-Ode, Ogun State, Nigeria in June, 2021. The plant was identified and authenticated by Forestry Research Institute of Nigeria (FRIN) Ibadan with a voucher specimen number FHI 113313. The specimen was subsequently deposited at the herbarium for future reference.

Extraction of Plant Material

The stem back of *C sieberiana* was washed with distilled water, sliced and air-dried at room temperature (25^{o}C) for 14 days. It was then ground into fine powder

using laboratory pestle and mortar. The pulverized stem back (20 g) was poured into a sterilized 500 mL conical flask and was macerated with 400 mL of methanol. The content was mixed thoroughly and incubated in an intelligent thermostatic shake cultivation cabinet (ZHP – 100 England) at reduced temperature for 72 h. The methanolic extract was filtered using Whatman filter paper No. 1. The filtrate was concentrated to dryness under reduced pressure using rotary evaporator (model, place of manufacture). The concentrated extract was stored at 4°C in air-tight screw-capped glass vials for the qualitative and quantitative phytochemical analysis.

Phytochemical screening of *C. sieberiana*

The phytochemical constituents of *C. sieberiana* stem bark was determined in the methanol extract using the standard methods as described by Friday et al., 2018; Gracelin et al., 2013; Ashafa, 2013 Soladoye and Chukwuma, 2012; Sofowora, 1993; Edeoga et al., 2005 and with some modifications.

Saponins (Frothing Test)
Two grams of pulverized *C. sieberiana* stem-bark was weighed and poured into a test tube. Twenty millilitres of methanol was dispensed into the test tube and placed in a water bath for 5 min and filtered. 10 ml of the filtrate was mixed with 5 ml of ethanol and shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously again and then observed for the formation of emulsion as indication of saponins.

Alkaloids
Half gram of pulverized *C. sieberiana* stem bark methanol extract was stirred in 5 mL of 1 % aqueous hydrochloric acid on a water bath at 60 C for 3 min and filtered using Whatman number 1 filter paper. I mL of the filtrate was treated with few drops of Mayer's reagent and a second portion was treated same way only with Drangendorff's reagent. Turbidity of precipitation with either of those reagents was taken as preliminary evidence for the presence of alkaloids in the extract.

Flavonoids
One gram of pulverized *C. sieberiana* stem bark methanolic extract was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered using Whatman number 1 filter paper and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. Development of yellow colouration was taken as indication of flavonoids.

Steroids
Two millilitres of acetic anhydride was added to 0.5 g of *C. sieberiana* stem-bark methanol extract with 2 mL of H_2SO_4. The colour changed from violet to blue or green as indication of steroids in the extract.

Tannins
Half gram of pulverized *C. sieberiana* stem bark was mixed in 20 mL of methanol in a test tube. It was boiled in a water bath at 60 ° C for 3 min and filtered using Whatman number 1 filter paper. Few drops of 0.1 % ferric chloride were added and observed for brownish green or a blue black colouration as indication of tannins in the extract.

Phlobatanins
Half gram of the pulverized *C. sieberiana* stem bark was extracted in 10 mL of methanol. 4 mL of the filtered methanol extract was boiled with 1 % aqueous hydrochloric acid in a water bath at 60 ° C for 3 min and observed for deposition of red precipitate as indication of phlobatannins in the extract.

Terpenoids (Salkowski test)

Five milliliter of the pulverized *C. sieberiana* stem bark methanol extract was mixed with 2 mL chloroform and 3 mL H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface was indication of terpenoids in the extract.

Cardiac glycosides (Keller-Killani test)
Five milliliter of pulverized *C. sieberiana* stem bark methanolic extract was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 mL of concentrated H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides in the extract.

Phenols [FeCl3 test]
To 2 mL of *C. sieberiana* stem bark methanolic extract were added a few drops of an aqueous solution of FeCl3 2% [w/v]. A dark blue and dark green colour indicated the presence of phenols in the extract.

Reducing sugar
Fehling's test: In a test tube containing 5 mL of *C. sieberiana* stem bark methanolic extract, were added Fehling's reagent [5 mL]. The formation of a brick red precipitate after 3 min heating in a water bath at 70 ° C indicated the presence of reducing sugars in the extract.

Tollens's test: In a test tube containing 5 mL of the same extract, Tollens's reagent [5 mL] was added, then ammonium hydroxide was dripped until the disappearance of the precipitate. The formation of a silver mirror indicated the presence of reducing sugars.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanol stem bark extract of *C. sieberiana* and Identification of bioactive compounds

The quantitative phyto-constituents of *Cassia sieberiana* methanol stem bark extract was determined using GC-MS technique after extraction as explained above. Before performing the GC-MS analysis, the extract was reconstituted and filtered with the use of sterilized syringe filter. It was then subjected to GC-MS analysis (GC–MSQP2010E SHIMADZU, JAPAN) Plus system equipped with RTX-5 m.s. capillary column (0.25 mm X 30 m X 0.25 mL). Helium gas (99.999 %) was used as carrier gas with a constant flow rate of 16.3ml/min. Column flow rate was maintained 1.2 mL/min. Column temperature was started at 50°C, held for 2 min, ramped to 250 °C for 6 min and finally ramped at 280 °C and held for 22 min. The sample was injected in a volume of 20 µL.

The Identification of the bioactive constituents of *Cassia sieberiana* methanolic extract was determined by comparing their retention times, percentage composition (area %) and retention indices with those of known standards and mass spectral fragmentation patterns. The identification was further confirmed by searching using the National Institute of standards and Technology (NIST) database (NIST/EPA/NIH mass spectral library (2014) with those of published data (Babushok et al., 2007; Stein, 2011). The name, molecular weight, and structure of the compounds of the test materials were ascertained. The average peak area of the total areas was calculated by comparing relative percentage amount of each compound.

Antioxidant Assay

The antioxidant activities of the stem bark methanol extract of *Cassia sieberiana* were determined by using nitric oxide (NO) scavenging, ferric reducing antioxidant potential (FRAP), lipid peroxidation (LP) inhibitory and 2,diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays.

Nitric Oxide Radical Scavenging Assay (Panda et al., 2009)

The sample was prepared from a 10 mg/mL methanol crude extract. These were then serially diluted with distilled water to make concentrations from 25−100 μg/mL and the standard ascorbic acid. They were stored at 4◦C for later use. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the methanol extracts (25−100μg/mL) and incubated at 25◦C for 180 mins. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The colour tubes contained methanol extracts at the same concentrations with no sodium nitroprusside. A volume of 150 μL of the reaction mixture was transferred to a 96-well plate. The absorbance was measured at 700 nm using a UV/VIS TG 50 plus UV-Vis microplate reader (Molecular Devices, GA, USA). Ascorbic acid was used as the positive control. The percentage inhibition of the extract and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the methanol extracts and Ascorbic acid were calculated using the following formula:

Nitric oxide scavenged (%) = **$\frac{(A\text{control} - A\text{test}) \times 100}{A\text{control}}$**

Where *A*control = absorbance of control sample and *A*test = absorbance in the presence of the samples of extracts or standards.

Ferric Reducing Power Assay (Pham-Hu et al., 2008)

1 mL of the plant sample (25-100 µg/mL), was mixed with 2.5 mL of 0.1 M Sodium phosphate buffer (pH 6.6) and 2.5 mL of 1%, w/v Potassium ferrocyanate [$K_3Fe(CN)_6$] in a 250 mL conical flask and then incubated at 50°C for 20 min. After which, addition of 2.5 mL trichloroacetic acid (10%, w/v), the mixture was centrifuged at 5000rpm for 10 min. The upper layer (5 mL) was mixed with 0.5 mL of fresh $FeCl_3$ (0.1%, w/v), and the absorbance at 700 nm was measured against a blank. Ascorbic acid was used as the standard.

FRAP Scavenging effect (%) = [(A_0-A_1)/A_0] x 100

Lipid Peroxidation (LP) (Cervantes et al., 1984)

10 µL of samples at different concentrations of 25, 50, 75 and 100 µg/mL of standard solution 1,1,3,3-tetramethoxypropane (TEP) and 40 µL of 20 mM phosphate buffer (pH 7.0) were added to an test tube on ice bath. In each tube, 50 µL of 3% sodium dodecyl sulfate (SDS), 200 µL of 0.1 N HCl, 30 µL of 10% phosphotungstic acid, and 100 µL of 0.7% of 2-thiobarbituric acid (TBA) were added. The tubes were firmly closed and boiled at 100°C for 30 min in water bath. The reaction mixture was mixed with 400 µL of n-butanol and then centrifuged at 3000 rpm for 10 min. Supernatants were collected and pass through a UV/VIS spectrophotometer at a wavelengths of 515 nm/555 nm.

Lipid Perioxidation (%) = **$\frac{(A\text{control} - A\text{test}) \times 100}{A\text{control}}$**

Where *A*control = absorbance of control sample and *A*test = absorbance in the presence of the samples of extracts or standards.

DPPH radical scavenging assay (Pham-Huy et al., 2008)

0.1 mM solution of DPPH in methanol was prepared; 1ml of the solution was added to 1 ml of extract in water at different concentrations (25-100 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a UV-Visible Spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using the following equation

DPPH Scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the standard (ascorbic acid). The IC_{50} value represented the concentration of the compounds that caused 50% inhibition of DPPH radical scavenging activity.

Statistical analysis

Data were expressed as means ± standard deviations (SD) of three replicate determinations and then analysed by using Statistical Package for Service and Solution (SPSS), version 23.0 (Corporation, Chicago, IL). Where applicable, One way analysis of variance (ANOVA) and the Duncan's New Multiple-range test (DNMRT) were used to determine the differences among the means; $P < 0.05$ were regarded to be significant.

RESULTS

The phytochemical screening of the methanol stem bark powdered sample of *Cassia sieberiana* showed the presence of saponins, alkaloids, flavonoids, steroids, tannins, phlobatannins, terpernoids, cardiac glycosides, phenols and carbohydrates as presented in Table 1. The result of GC-MS analysis showed that its methanol extract has 51 bioactive compounds with 3-0-Methyl-d-glucose having the highest Area percentage (12.16%) while 5. alpha-Adrostan-3-beta-ol,4,4-dimethyl- having the lowest Area percentage (0.09%) as indicated in Table 2. This study also showed an increasing trend in the antioxidant activity of the methanol stem bark extract of *C. sieberiana* with respect to concentration. The study reveals that as the concentration (25 - 100µg/mL) of the extract increases, the NO, FRAP, LP and DPPH radical scavenging activity of the plant extract increases significantly in comparison with that of Ascorbic acid ($\rho < 0.05$). Interestingly, Ascorbic acid used as the standard drug also showed increasing trend as the concentration increases (Figures 1-4). Data were presented as means ± standard deviation of three replicate with significant increases from both samples tested and standard drug used.

Table 1: Phytochemical screening of methanol stem bark powdered sample of *C. sieberiana*

phytochemical	**Present**
Saponins	+
Alkaloids	+
Flavonoids	+

Steroids	+
Tannins	++
Phlobatannins	+
Terternoids	+
Cardiac glycosides	+
Phenols	+
Reducing sugar	+

Key: + represent slightly present, ++ represent strongly present

Table 2: GC-MS Analysis of methanol stem bark extract of *C. sieberiana*

Peak	R. Time	Area	Area%	Height	Height%	Name of compound
1	4.673	4304868	0.49	1278422	0.76	Ethanol, 2-butoxy-
2	4.817	101688921	11.52	27862303	16.61	Ethanol,2-(2-butoxyethoxy)-
3	5.013	23018985	2.61	2320467	1.38	Ethanol, 2-butoxy-
4	8.145	1619540	0.18	828283	0.49	.alpha-Terpineol
5	8.550	1877340	0.21	484373	0.29	Catechol
6	8.607	7864546	0.89	1091365	0.65	Benzofuran, 2,3-dihydro-
7	9.428	67812991	7.68	3679556	2.19	Resorcinol
8	10.157	71486459	8.10	9410555	5.61	Phenol, 4-propyl-
9	10.454	21502789	2.44	2513263	1.50	Orcinol
10	10.675	4371350	0.50	734112	0.44	Tetradecane
11	10.771	1985693	0.23	508128	0.30	Naphthalene, 2,6-dimethyl-
12	11.298	981522	0.11	449049	0.27	Furanacetic acid, 4 hexyl-2,5-dihydr-2,5-di
13	11.683	19614316	2.22	1543909	0.92	D-Allose

14	11.913	2324370	0.26	498243	0.30	Indane, 2-methoxy-3-(2-methyl-1-propenyl-1)
15	12.112	2182822	0.25	460437	0.27	Naphthalene,1,4,6-trimethyl-
16	12.257	1746550	0.20	1227700	0.73	Dodecanoic acid
17	13.147	2267122	0.26	1370108	0.82	Ar-tumerone
18	14.126	2764701	0.31	1536682	0.92	Tetradecanoic acid
19	15.002	107294381	12.16	4388150	2.62	3-O-Methyl-d-glucose
20	15.278	848407	0.10	515770	0.31	3,7,11,15-Tetramethyl-2-bexadecen-l-ol
21	15.533	10989490	1.25	7578055	4.52	Hexadecanoic-acid, methyl ester
22	15.671	5073824	0.57	1131484	0.67	Hexadecanoic acid, Z-11-
23	15.939	98730754	11.19	20987762	12.51	n-Hexadecanoic acid
24	16.100	2123851	0.24	1309937	0.78	Hexadecanoic acid, ethyl ester
25	16.499	3167384	0.36	1050630	0.63	Cis-10-Heptadecenoic acid
26	16.689	2237497	0.25	783152	0.47	Heptadecanoic acid
27	16.925	3438418	0.39	2061102	1.23	Methyl 10-trans,12-cis-octadecadienoate
28	17.002	7744040	0.88	4674135	2.79	9-Octadecenoic acid, methyl ester (E)-
29	17.430	106057995	12.02	16322832	9.73	9,12-Octadecadienoic acid (Z,Z)-
30	17.497	55142699	6.25	15938771	9.50	9,-Octadecenoic acid (E)-
31	17.681	12193797	1.38	4466965	2.66	Octadecanoic acid
32	18.302	873873	0.10	410554	0.24	Hexylresorcinol

33	18.418	12196827	1.38	758244	0.45	.alpha-Tocophenyl acetate
34	19.086	13464630	1.53	3600665	2.15	1,8,9-Anthracenetriol, 3-methyl-
35	19.311	14184958	1.61	3818743	2.28	9,10-Anthracenedione, 1,8-dihydroxy-3-methy
36	19.450	949102	0.11	441556	0.26	Cis-Cyclohexane-1,3-methyl-
37	20.491	15815707	1.79	1808523	1.08	2-Propen-l-one, 1-(2-hydroxyphenyl)-3-(3-hydroxyl)
38	20.779	4745292	0.54	1497522	0.89	Hexanoic acid, heptadecyl ester
39	21.095	1247985	0.14	614232	0.37	Phthalic acid, di(2-propylpentyl) ester
40	21.318	5837083	0.66	1216064	0.72	Ergost-5-en-3-ol, (3.beta.)-
41	21.937	13034686	1.48	2128882	1.27	Stigmasterol
42	22.150	1801192	0.20	722467	0.43	1-Benzoimidazol-l-yl-3-(4-iodo-phenoxy)-pro
43	22.194	3366459	0.38	1105131	0.66	3-(2,6-Dimethylocta-2,7-dienyl)-lH-indole
44	22.242	798613	0.09	588857	0.35	5.alpha-Androstan-3 beta-ol, 4,4-dimethyl-
45	22.484	1407112	0.16	397324	0.24	Obtusifoliol
46	22.933	22891713	2.59	6328101	3.77	.gamma-Sitosterol
47	23.133	1537974	0.17	241436	0.14	Pregnance-3,11,20-trio, (3,alpha., 11.beta.,20
48	23.283	2929971	0.33	389144	0.23	.beta-Armyrin
49	23.402	986419	0.11	565102	0.34	1,5,9,-Decatriene, 2,3,5,8-tetramethyl-

50	23.931	6800768	0.77	1542847	0.92	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-din
51	24.256	3080278	0.35	569560	0.34	Stigma-3,5-dien-70-one

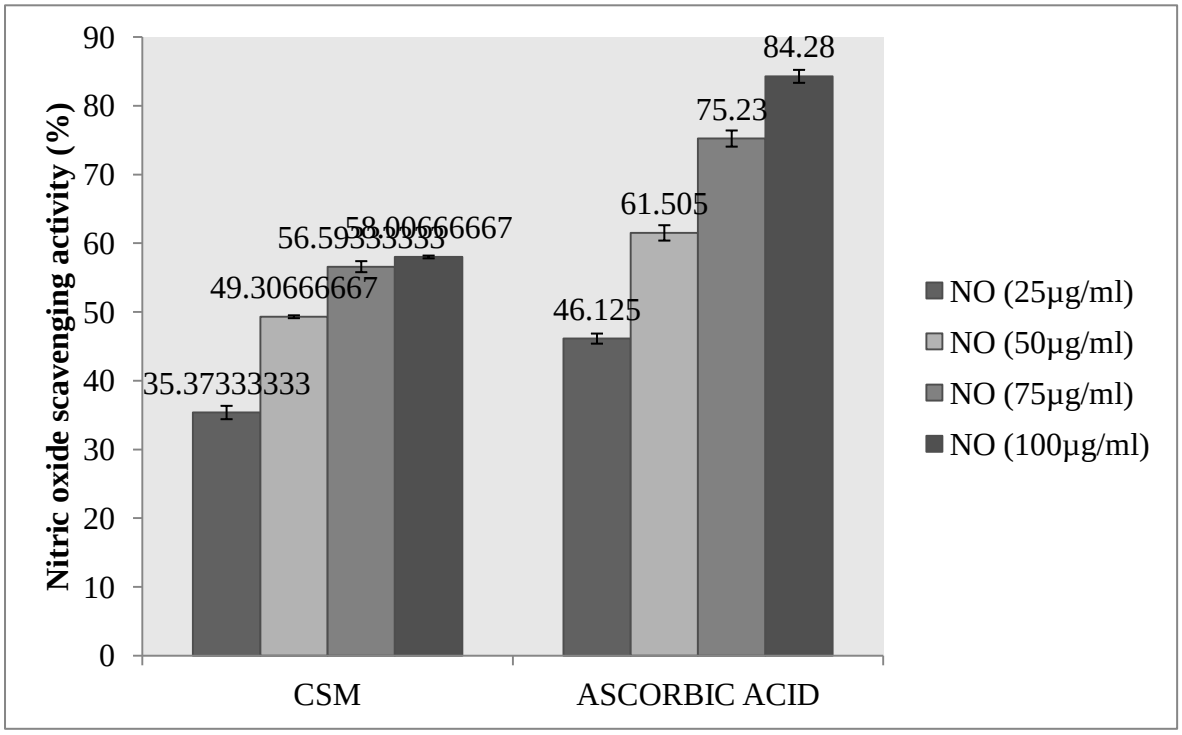

Figure 1: Nitric Oxide Antioxidant Assay

Note: Each result is an expression of mean ± Standard Deviation ($P < 0.05$)
Key: NO represent Nitric Oxide, CSM represent *Cassia sieberiana* Methanol

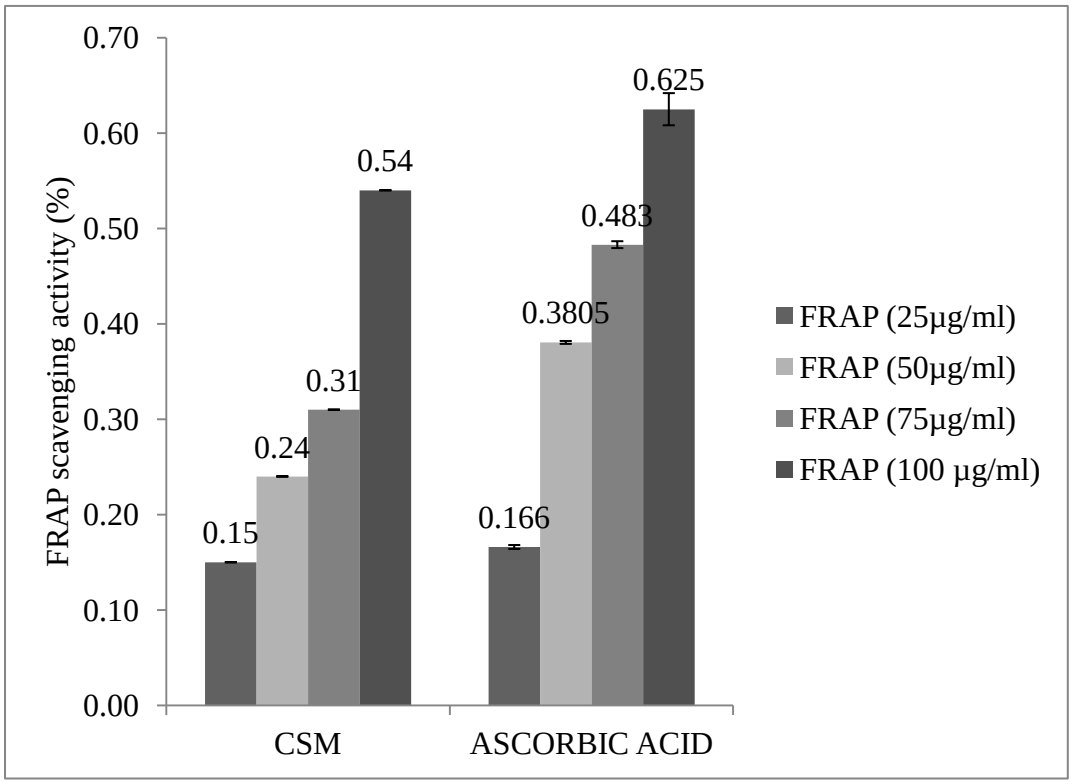

Figure 2: Ferric reducing antioxidant potential (FRAP) assay
Note: Each result is an expression of mean ± Standard Deviation ($P < 0.05$)
Key: FRAP represent ferric reducing antioxidant potentials; CSM represent *Cassia sieberiana* Methanol

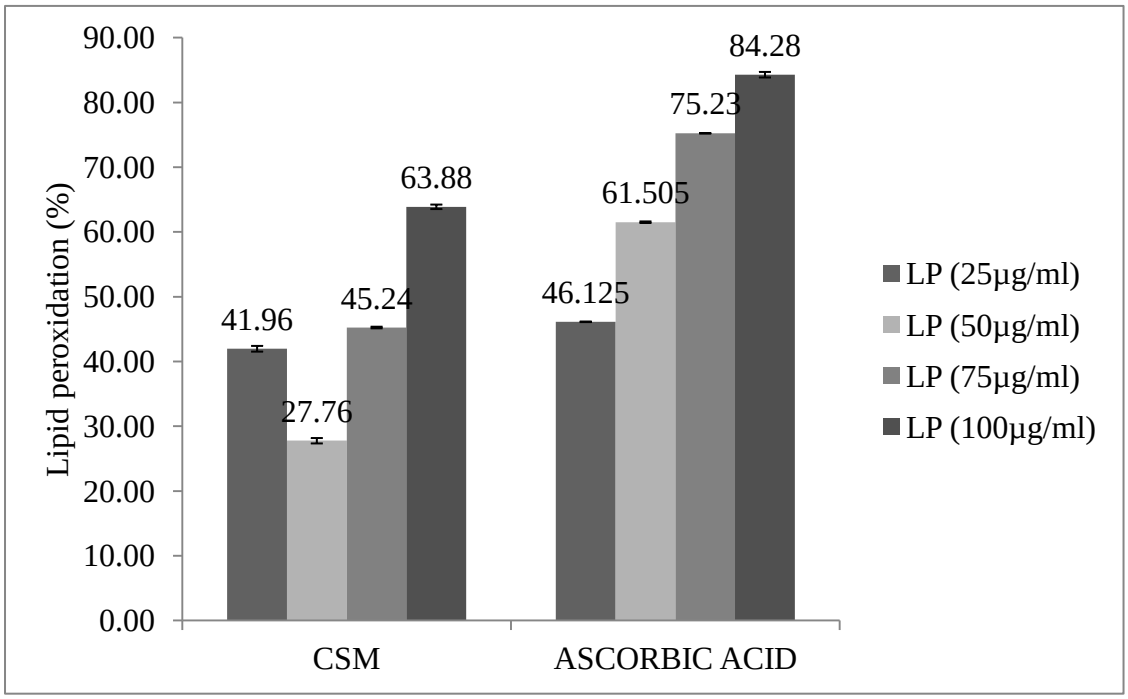

Figure 3: Lipid Peroxidation Antioxidant Assay

Key: LP represent Lipid Peroxidation; CSM represent *Cassia sieberiana* Methanol

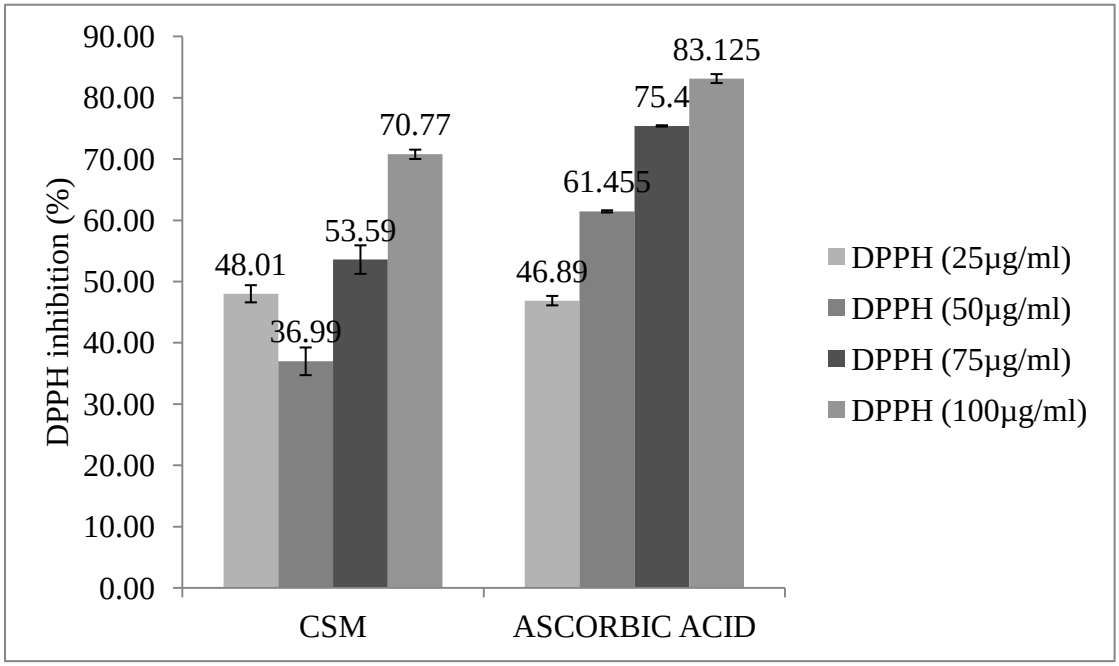

Figure 4: DPPH radical scavenging assay.

Note: Each result is an expression of mean ± Standard Deviation ($P < 0.05$)
Key: DPPH represent 2, diphenyl-1-picrylhydrazyl; CSM represent *Cassia sieberiana* Methanol

DISCUSSION

Plants have long been known to have antimicrobial and anticancer effects. Increased unfavourable negative impacts induced by several chemotherapeutic treatments may have been the primary motivating factor for using alternative therapies with the aim of finding a better and safer cure for infectious and non-infectious diseases (Moon and Shibamoto, 2009). The majority of people all over the world currently use herbal remedies as part of their medical system, indicating that plant-based traditional medicine will continue to play an important role in human healthcare in the coming years (Baliyan et al., 2022). Growing expenditures on prescribed drugs to preserve good health and well-being have revived interest in traditional medicines in healthcare delivery. Medicine discovered from plants might be less expensive, have little or no toxicity due to bioprospecting (Ndhlala et al., 2010).

Medicinal plants are considered as arsenal of bioactive compounds for the synthesis of novel drugs in the twenty-first century. Secondary metabolites such as phytochemicals are natural products that are found in plants. These products are biologically active chemical compounds found in nature (Hasibuan et al., 2021). They are commonly used in drug synthesis, development and discovery. Many known plant's secondary metabolites have been reported to contain medicinal properties (Hasibuan et al., 2020). They are compounds that have no nutritional value but they play a crucial role in an organism's survival in the environment (Venugopala et al., 2013).

The majority of secondary metabolites can be used as a pharmaceutical component (Batiha et al., 2020). Tannins, flavonoids, alkaloids, steroids, terpenoids, phenols, and other chemical substances found in plants can be used as therapeutic medicines (Biswas et al., 2020). The phytochemicals in the medicinal plants varies with species and according to the environmental conditions of the areas in which they are grown (Srivastava et al., 2021). Hitherto, the methanol stem bark extract of *C. sieberiana* has only been screened qualitatively; however, it has not been explored for its GC-MS analysis. Therefore, the present study was carried out to investigate the phytochemicals, bioactive compounds and antioxidant activity in this plant. The phytochemical screening of the medicinal plants was mainly performed to determine the secondary metabolites (Pandey and Grover, 2020). The phytochemical screening showed the presence of saponins, alkaloids, flavonoids, steroids, tannins, phlobatanins, terpenoids, phenols, cardiac glycosides and reducing sugars as shown in Table 1. Olajuyigbe and Afolayan, (2011) also presented similar report on alcoholic and aqueous extracts of *Acacia mearnsii* De Wild. Plant extract having flavonoid, terpenoids, tannins were considered significant sources of potential therapeutic compound for many human diseases (Srivastava et al., 2021). Phytochemicals are basically divided into two groups that is, primary and secondary metabolites based on the function in plant metabolism. Primary metabolites comprise the common reducing sugars/carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and so on (Prajapati *et al*., 2007). The distribution of these compounds differs from plant to plant and part to part. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries and the constituents play a significant role in the identification of novel drugs (Savithramma et al., 2011). In this study, the crude powdered form and extracts of the medicinal plant revealed the presence of important bioactive constituents which could form the basis for their medicinal use in the treatment of various ailments by the indigenous people of Africa. Tannins, phenols, saponins, terpenoids and alkaloids have been linked with antioxidant, hypoglycemic, antibacterial and anti-viral activity (Saio and Syiem, 2015). Steroids, phlobatannins and reducing sugars in modern clinical studies have supported their role as anti-inflammatory and analgesic agents (Singh, 2006) are also of importance and of interest in pharmacy due to their relationship with sex hormones (Okwu, 2001). Cardiac glycosides are diuretic, help to transfer fluids from tissues and circulatory system to the urinary tract, thereby lowering blood pressure (Prajapati et al., 2007). Flavonoids are phenolic substances which exhibit biological activities such as antioxidative, anti-allergenic, anti-viral, anti-inflammatory and vasodilating actions (Pietta, 2000). Having reported the total phenolic content of medicinal plants along with their *in-vitro* antioxidant activity (Syiem et al., 2009), this study also investigated the presence of flavonoids which are one of the main groups of phenolic compounds that are most importantly known for their antioxidant potential, due to their ability to reduce and scavenge free radicals (Pietta, 2000), prevent oxidative cell damage and have strong anti-cancer activity (Salah et al., 1995).

GC-MS analysis of methanol stem bark extract of *C. sieberiana* showed a total number of 51 bioactive compounds as shown in Table 2. The peak, retention time (RT), area and name of various bioactive compounds in the methanol extract were identified. The identified compounds comprise mainly hydrocarbons, fatty acids, alcohols, esters and phenols; 3-0-Methyl-d-glucose has the highest percentage of

12.16 % while 5.alpha-Androstan-3-beta-ol,4,4-dimethyl- has the lowest percentage of 0.09 %. Among the identified bioactive compounds, 3-O-Methyl-d-glucose (12.16%), n-Hexadecanoic acid (11.19%) and 9,12-Octadecanoic acid (Z,Z)- (12.02%) have been reported to exhibit antioxidant, anti-inflammatory and antibacterial activity (Gomathi et al., 2015; Pu et al., 2010).

The antioxidant activities of the plant extract using nitric oxide scavenging assay, ferric reducing antioxidant potential assay, lipid peroxidation inhibitory assay and DPPH radical scavenging assay were also determined. The methanol stem bark extract of *C. sieberiana* showed excellent antioxidant activities as shown in Figures 1-4. There were considered to constitute the potent anti-oxidative property of herbal extract (Wangchuk et al., 2013). Phenolic compounds were known to exhibit significant free radical scavenging activity because of the presence of hydrogen or electron donating agent and metal ion chelating property. This report agreed with other studies that showed a close relationship between total phenolic content and high antioxidant activity (Olajuyigbe and Afolayan, 2011b; Chimi et al., 1991). Similarly, flavonoids inhibit free radical mediated event by its chemical structure as these transfers electrons, chelate metal catalyst, activates antioxidant enzymes and inhibit oxidases (Chimi et al., 1991). Flavonoids are plant nutrients that when consumed in the form of fruits and vegetables are non-toxic as well as potentially beneficial to the human body (Ejikeme et al., 2014).

Antioxidants are required by the body because they limit the beginning and progression of substrate oxidation. Beta-hydroxy acid, tert-butyl hydroquinone, propyl gallate, and butylated hydroxytoluene are examples of synthetic antioxidants that have been widely employed across the world (Tonolo et al., 2020; Yan et al., 2020). However, these synthetic antioxidants have negative side effects on the heart and lungs, including carcinogenic and cytotoxic consequences (Baschieri et al., 2021). Since antioxidants are used for the prevention and treatment of free radical-related disorders (Middleton et al., 2000) as well as being essential in the prevention of diseases (Di Matteo and Esposito, 2003), the presence of phenolic and flavonoid contents of methanol stem bark extract of *C. sieberiana* may contribute to its potential antioxidant activity by neutralizing free radicals (Figures 1-4). The negative consequences of nitric oxide (NO) overexpression in the host organism have traditionally been linked to a variety of illnesses, including human vascular and cardiac ailments (Lubis et al., 2022). The reductive ability of the extracts reflected the reducing power of the *C. sieberiana* as a potential source of antioxidants (Williams et al., 2004). The best known antioxidants are phenolic compounds and flavonoids (Vessal et al., 2003; Molina et al., 2003; Paganga et al., 1999), exhibiting extensive free radical scavenging activities through their reactivity as hydrogen or electron-donating agents and metal ion chelating properties (Olajuyigbe and Afolayan, 2011b). Antioxidant activity of DPPH radical scavenging and lipid peroxidation inhibition were equally determined. The reaction of DPPH with numerous antioxidants has earlier been published and the stoichiometry characterized (Lubis et al., 2022). The DPPH antioxidant assay is based on the principle that 2,2-diphenyl-1-picryl-hydrazyl (DPPH) is able to decolourise in the presence of free radical scavengers commonly known as antioxidants. The study demonstrated strong free radical scavenging inhibitions with IC_{50} values; NO (0.58%), FRAP (0.54%), LP (0.63%) and DPPH (0.70%) as shown in Figures 1-4. This study, therefore, suggests that the recorded antioxidant capacity resulted from the contribution of different phytochemicals present in the plant and

the reducing capacity of the extract may serve as a significant indicator of the antioxidant potential of *C. sieberiana.*

CONCLUSION

The phytochemical screening of methanol stem bark powdered sample of *C. sieberiana* showed that the plant contained medicinal constituents such as saponins, alkaloids, flavonoids, steroids, tannins, phlobatannins, terpernoids, cardiac glycosides, phenolics and reducing sugar. The GC-MS analysis revealed that its methanol extract has 51 bioactive compounds with 3-0-Methyl-d-glucose having the highest percentage (12.16%) and 5.alpha-Androstan-3-beta-ol,4,4-dimethyl- having the lowest percentage (0.09%). From these results, it could be concluded that the methanol stem bark extract of *C. sieberiana* possess phytochemicals, bioactive compounds and good antioxidant activities which could be therapeutic in several human diseases. Therefore, further quantitative and molecular analysis in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds and their roles in anti-oxidative and antimicrobial activities is recommended.

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CONFLICT OF INTEREST

The author declared no conflicts of interest with respect to the research, authorship, and/or publication of this research article.

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