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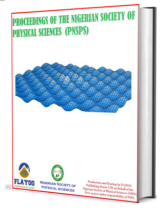

Phytochemical profiles and evaluation of free radical scavenging activities of *Cassia sieberiana* using 1,1-diphenyl-2-picryl hydrazyl and 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)

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ABSTRACT

Cassia sieberiana is used in traditional medicine in West Africa, including Nigeria. This study evaluated the phytochemical profile and free radical scavenging activities of *C. sieberiana* root bark extracts (weighing 100 g). The powdered root bark was extracted with petroleum ether, methyl acetate, acetone, chloroform, methanol (MeOH), and 70% MeOH using a Soxhlet extractor, and the resulting extracts were concentrated under reduced pressure. Methyl acetate, MeOH, and 70% MeOH extracts were analyzed qualitatively and quantitatively using standard phytochemical methods, and their antioxidant activities were assessed using 1,1-diphenyl-2-picryl hydrazyl (DPPH) and 2,2-azinobis-ethyl benzothiozoline-6-sulphonic acid (ABTS) assays. Qualitative analysis indicated the presence of saponins, quinones, phenols, steroids, tannins, flavonoids, terpenoids, anthraquinones, cardiac glycosides, alkaloids, carbohydrates, glycosides, and coumarins in the extracts. The highest contents of flavonoids, phenolics, tannins, saponins, steroids, and alkaloids were recorded in methyl acetate (38.74 μg QE/g), MeOH (259.78 μg GAE/g), methyl acetate (228.67 μg TAE/g), MeOH (961.88 μg DE/g), 70% MeOH (108.08 μg βSSE/g), and 70% MeOH (1.81 μg CE/g), respectively. The extracts showed substantial free radical scavenging activities, with IC_{50} values of 30.19 μg/mL for the methyl acetate extract in the DPPH assay and 15.20 μg/mL for the methyl acetate extract in the ABTS assay. These findings support the antioxidant potential of *C. sieberiana* root bark and its possible value as a source of natural antioxidants.

Keywords: *Cassia sieberiana*, Phytochemicals, DPPH, ABTS, Antioxidant.

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1. INTRODUCTION

Plants produce many secondary metabolites that are important for their defense systems and biological functions. These compounds are the basis of many pharmaceutical products because of their health benefits, and each secondary metabolite has a specific function [1]. Plant secondary metabolites are bioactive substances with various pharmacological effects that may be beneficial to human health [1, 2]. They contribute to plant appearance, taste, and aroma and protect plants from injury and disease [3]. Fruits, vegetables, herbs, and spices contain different dietary phytochemicals [4]. Phytochemicals exhibit biological

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properties, including antioxidant activity, antimicrobial effects, modulation of detoxification enzymes, and anticancer properties [5]. Their biological significance is due mainly to their capacity to neutralize reactive oxygen species (ROS) [6]. Oxidative stress occurs when pro-oxidants exceed antioxidant defenses and has been linked to long-term illnesses such as cardiovascular disease, cancer, and diabetes [7].

Antioxidants reduce these effects by stabilizing free radicals and preventing cellular damage. Many plants have been used because they possess antioxidant or antimicrobial properties derived from compounds produced through secondary metabolism [8]. Research indicates that the protective properties of some foods correlate with their antioxidant components, including vitamin C, vitamin E, carotenoids, and phenolic compounds, which are predominant antioxidants in many plants [9]. Concerns about the possible toxic and mutagenic effects of synthetic antioxidants have increased research interest in naturally occurring antioxidants [10, 11]. In addition, interest in plant-derived natural alternatives has made medicinal plants important sources of bioactive compounds with potential therapeutic applications. Natural antioxidants may therefore protect cellular organelles from damage caused by free radicals. *Cassia sieberiana*, a plant prevalent in tropical Africa, has been used in traditional medicine for the treatment of diverse ailments. Despite its wide use, limited detailed studies have examined its phytochemical content and antioxidant capacity. This study therefore examined the phytochemical profiles and antioxidant activities of *C. sieberiana* root bark extracts using standard analytical techniques.

2. MATERIALS AND METHODS

2.1. SAMPLE COLLECTION, IDENTIFICATION, AND PREPARATION

A fresh root sample of *Cassia sieberiana* was obtained from a wild source in southern Kaduna State, Nigeria. A botanist in the Department of Biological Sciences, Gombe State University, identified the medicinal plant, and a voucher specimen assigned the number FHJ-653 was deposited in the herbarium. The root samples were air-dried in the shade for two weeks on a clean surface at room temperature. The dried root sample was ground into powder using a pestle and mortar and stored in a brown paper envelope for later use.

2.2. REAGENTS AND CHEMICALS

High analytical-grade chemicals from Riedel-de Haen Chemical Ltd., Germany, were used. Low-grade solvents were purified, where necessary, before use unless otherwise stated.

2.3. EXTRACTION PROCEDURE

Extraction solvents, namely petroleum ether, methyl acetate, acetone, chloroform, methanol, and 70% methanol/water, were used in order of increasing polarity to extract *C. sieberiana* root samples. A rotary evaporator under reduced pressure was used to concentrate the solvent extracts and remove residual solvents. The extracts were dried, weighed, and kept in desiccators for subsequent analysis [12].

2.4. QUALITATIVE ESTIMATION OF PHYTOCHEMICALS

Qualitative phytochemical analysis of *C. sieberiana* roots was conducted to detect notable phytochemical classes, including alkaloids, carbohydrates, tannins, terpenoids, glycosides, steroids, saponins, phenols, cardiac glycosides, flavonoids, quinones, anthraquinones, and coumarins, using established methods [13, 14, 16], with slight modifications as specified by Ref. [15].

2.5. QUANTITATIVE ESTIMATION OF PHYTOCHEMICALS

Standard spectrophotometric methods were used to determine the phytochemical contents of the extracts.

2.5.1. Total flavonoid content

Total flavonoid content was determined using quercetin as the standard. Methanol (300 μL) was added to 100 μL of sample. Sodium potassium tartrate (100 μL) and aluminum chloride (100 μL) were added to the mixture. The solution was diluted by shaking with 300 μL of distilled water. After 30 min of incubation, absorbance was measured at 415 nm. A standard calibration plot was prepared at 415 nm using quercetin. Flavonoid content in the test sample was calculated from the calibration plot and expressed as μg quercetin equivalent per gram of sample.

2.5.2. Total phenolic content

Total phenolic content was determined using gallic acid as the standard. Distilled water (900 μL) was added to 100 μL of the sample, followed by 150 μL of Folin–Ciocalteu reagent. The mixture was incubated at room temperature for 5 min. Sodium carbonate (400 μL; 20%, w/w) was added, mixed, and kept at room temperature for 30 min. Absorbance was measured against a blank at 765 nm using a UV–Vis spectrophotometer. A standard curve was prepared from absorbance and gallic acid concentration. Phenolic content in the test sample was calculated from the calibration plot and expressed as μg gallic acid equivalent per gram of sample.

2.5.3. Total saponin content

Total saponin content was determined using diosgenin as the standard. The test extract was mixed with 80% methanol. Vanillin in ethanol (2 mL) was added and mixed thoroughly. Sulfuric acid solution (2 mL; 72%) was then added and mixed. The test solution was heated in a water bath at 60 °C for 10 min with occasional shaking. Absorbance was measured at 544 nm against the reagent blank. A standard calibration plot was prepared at 544 nm using diosgenin. Saponin content was calculated from the calibration plot and expressed as μg diosgenin equivalent per gram of sample.

2.5.4. Total steroid content

Total steroid content was determined using β-stigmasterol as the standard. The test sample (1 μg/mL) was dissolved in the respective solvent. Sulfuric acid (2 mL; 4 N) and iron(III) chloride (2 mL; 0.5%, w/v) were added, followed by potassium ferricyanide solution (0.5 mL; 0.5%, w/v). The test solution was heated in a water bath maintained at 70 ± 2 °C for 30 min with occasional shaking. Absorbance was measured at 780 nm against the reagent blank. A standard calibration plot was prepared at

780 nm using β-stigmasterol. Steroid content in the test sample was calculated from the calibration plot and expressed as μg β-stigmasterol equivalent per gram of sample.

2.5.5. Total tannin content

Total tannin content was determined using tannic acid as the standard. Water (7.5 mL) was added to 100 μL of the sample, followed by 500 μL of Folin–Denis reagent and 1000 μL of 35% sodium carbonate. The test solution was made up to 10 mL. After 30 min of incubation, absorbance was measured at 725 nm. A standard calibration plot was prepared at 725 nm using tannic acid. Tannin content in the test sample was calculated from the calibration plot and expressed as μg tannic acid equivalent per gram of sample.

2.5.6. Total alkaloid content

Total alkaloid content was determined using capsaicin as the standard. Phosphate buffer (5 mL; pH 4.7) was added to 1 mL of test extract, followed by 5 mL of bromocresol green (BCG) solution, and the mixture was shaken. Chloroform (4 mL) was added to the mixture. The extracts were collected in a 10 mL volumetric flask and diluted to volume with chloroform. Absorbance was measured at 470 nm against the reagent blank. A standard calibration plot was prepared at 470 nm using capsaicin.

2.6. ABTS RADICAL SCAVENGING ASSAY

ABTS radical scavenging activity was measured at 734 nm [16]. The ABTS radical cation was prepared and then mixed with extracts at different concentrations. Absorbance was measured at 734 nm, and percentage inhibition was calculated at each concentration. Log-probit analysis was used to calculate the IC_{50} values.

2.6.1. Calculation

$$\%\ \text{Inhibition} = \left(\frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}}\right) \times 100. \quad (1)$$

2.7. DPPH ANTIOXIDANT ASSAY

DPPH radical scavenging activity was assessed at 510 nm [9, 30]. The activity was evaluated by measuring the decrease in absorbance at 510 nm after the extracts were mixed with the test sample. A control reaction was prepared without the test sample. The IC_{50} values were compared to evaluate antioxidant strength.

2.7.1. Calculation

$$\%\ \text{Inhibition} = \left(\frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}}\right) \times 100. \quad (2)$$

3. RESULTS AND DISCUSSION

Standard methods are used to extract medicinally active constituents from plant tissues using selective solvents. Plant-derived products are relatively complex mixtures of metabolites in liquid or semisolid form or, after solvent removal, as dry powders. They may be intended for oral use or topical application [17]. Table 1 presents the percentage yields and physicochemical evaluation of *C. sieberiana* root bark extracts. Extraction yield varied with solvent polarity. The highest extract yields were obtained with 70% MeOH and methyl acetate (MA), which gave 30.684% dark brown solid and 24.625% dark brown solid extracts, respectively. This confirms that solvent choice affects phytochemical recovery [18]. The performance of the MA extract suggests that medium-polarity solvents may be effective for extracting bioactive antioxidant compounds, probably because they can solubilize both polar phenolics and moderately nonpolar flavonoid aglycones. This finding aligns with reports emphasizing the importance of solvent selection in phytochemical extraction efficiency.

Phytochemical screening established the presence of various bioactive compounds, indicating the plant's potential therapeutic significance. Saponins, quinones, phenols, steroids, tannins, flavonoids, terpenoids, anthraquinones, cardiac glycosides, alkaloids, carbohydrates, glycosides, and coumarins were detected in methyl acetate, MeOH, and 70% MeOH extracts (Table 2). Quantitative analysis further demonstrated substantial variation in phytochemical concentrations, indicating that solvent selection is important for extraction efficiency and reflects differences in phytochemical solubility across solvents, extraction methods, plant parts, and species [19–21]. This differential extraction indicates which solvent is most suitable for obtaining active constituents from medicinal plants [22, 23]. These variations were documented for flavonoids, phenolics, tannins, saponins, steroids, and alkaloids in methyl acetate, methanol, and 70% methanol extracts [24].

The alkaloid contents are presented in Table 3, while the flavonoid contents are shown in Table 4. The total flavonoid assay of the 70% MeOH, MA, and MeOH extracts revealed flavonoid contents of 27.34, 38.74, and 33.53 μg QE/g, respectively, corroborating previous findings [25, 26]. The MA extract contained the highest level of flavonoids (38.74 μg QE/g) compared with the other extracts (Table 4 and Figure 2). The total phenolic assay showed that the MeOH extract had the highest phenolic content, at 259.78 μg GAE/g (Table 5 and Figure 3). The highest amounts of alkaloids, saponins, steroids, and tannins were recorded in 70% MeOH (1.81 μg CE/g; Table 3 and Figure 1), MeOH (961.88 μg DE/g; Table 6 and Figure 4), 70% MeOH (108.08 μg βSSE/g; Table 7 and Figure 5), and MA (228.67 μg TAE/g; Table 8 and Figure 6), respectively. Thus, the 70% MeOH extract had the highest alkaloid and steroid contents, the MA extract had the highest flavonoid and tannin contents, and the MeOH extract had the highest phenolic and saponin contents. These results may support the potential use of these phytochemical-rich fractions in nutraceuticals, functional foods, pharmaceuticals, and natural food preservatives [27]. Quantitative analysis demonstrated variability in phenolic and flavonoid contents among extracts, with methanol exhibiting the highest phenolic concentration and MA exhibiting the highest flavonoid content.

The DPPH assay for standard quercetin showed an IC_{50} value of 3.644 μg/mL (Figure 8), while the MA, 70% MeOH, and MeOH extracts showed dose-dependent DPPH radical scavenging activity, with IC_{50} values of 30.19, 81.43, and 90.59 μg/mL, respectively (Table 10). These results show dose-dependent DPPH radical scavenging activity of 75.84% for the MA extract, with a lower IC_{50} value of 30.19 μg/mL, compared with the quercetin standard value of 81.43% activity and IC_{50} of 3.644 μg/mL (Figure 8). The 70% MeOH and MeOH extracts of *C.*

Table 1. Percentage yields and physicochemical evaluation of *Cassia sieberiana* root bark extracts.

S/N	Extraction solvent	Recovery (g)	Yield (%)	Colour	Texture
1	Petroleum ether	0.774	0.516	Brown	Oily
2	Methyl acetate	36.938	24.625	Dark brown	Solid
3	Acetone	1.021	0.681	Brown	Solid
4	Chloroform	0.331	0.221	Dark brown	Oily
5	Methanol	6.953	4.635	Dark brown	Solid
6	70% MeOH	30.684	30.684	Dark brown	Solid

Yield (%) = yield obtained/theoretical yield × 100.

Table 2. Qualitative phytochemical screening of *C. sieberiana* root bark.

S/N	Phytochemical	Type of test	Methyl acetate	MeOH	70% MeOH
1	Saponins	Frothing test; foam test	−/+	+/+	+/+
2	Quinones	Conc. HCl	+	+	-
3	Phenols	$FeCl_3$ test	+	+	+
4	Steroids	Salkowski's test; Liebermann–Burchard test	+/+	+/+	+
5	Tannins	Braymer's test	+	+	+
6	Flavonoids	Lead acetate; iron(III) chloride	+/+	+/+	+/+
7	Terpenoids	Salkowski's test	+	+	+
8	Anthraquinones	10% ammonia solution	+	+	+
9	Cardiac glycosides	Keller–Killiani test; Salkowski's test	+/+	+/+	+/+
10	Alkaloids	Dragendorff's test; Wagner's test	+/+	+/+	+/+
11	Carbohydrates	Molisch's test	+	+	+
12	Glycosides	Borntrager's test	+	+	+
13	Coumarins	NaOH	+	+	+

− = negative; + = positive.

Table 3. Alkaloid content of *Cassia sieberiana* in the extraction solvents.

Sample	Conc. (μg/mL)	Abs	Conc. alkaloid (μg CE/200 μg)	Conc. alkaloid (μg CE/g)
70% MeOH	200	0.3876	0.36	1.81
MA	200	0.3761	0.33	1.66
MeOH	200	0.3848	0.36	1.78

Table 4. Flavonoid content of *Cassia sieberiana* in the extraction solvents.

Sample	Conc. (μg/mL)	Abs	Conc. flavonoids (μg QE/1600 μg)	Conc. flavonoids (μg QE/g)
70% MeOH	1600	0.2133	43.74	27.34
MA	1600	0.2673	61.99	38.74
MeOH	1600	0.2426	53.64	33.53

Table 5. Phenolic content of *Cassia sieberiana* in the extraction solvents.

Sample	Conc. (μg/mL)	Abs	Conc. phenols (μg GAE/400 μg)	Conc. phenols (μg GAE/g)
70% MeOH	400	1.4060	594.05	237.62
MA	400	1.4435	611.09	244.44
MeOH	400	1.5279	649.45	259.78

sieberiana root bark had activities of 64.28% and 66.20%, with IC_{50} values of 81.43 and 90.59 μg/mL, respectively.

Table 9 presents the ABTS assay results for *C. sieberiana* root bark extracts. The MA extract showed dose-dependent ABTS radical scavenging activity of 87.45%, with a lower IC_{50} value of 15.20 μg/mL compared with the standard quercetin value of 97.96% activity with an IC_{50} of 1.711 μg/mL. The 70% MeOH and MeOH extracts showed 66.23% and 89.18% activity, with IC_{50} values of 32.48 and 22.67 μg/mL, respectively (Table 9 and Figure 7). These antioxidant assays demonstrated that all ex-

Table 6. Saponin content of *Cassia sieberiana* in the extraction solvents.

Sample	Conc. (μg/mL)	Abs	Conc. saponin (μg DE/400 μg)	Conc. saponin (μg DE/g)
70% MeOH	400	0.050	83.38	208.44
MA	400	0.111	236.69	591.72
MeOH	400	0.171	384.75	961.88

Table 7. Steroid content of *Cassia sieberiana* in the extraction solvents.

Sample	Conc. (μg/mL)	Abs	Conc. steroid (μg βSSE/200 μg)	Conc. steroid (μg βSSE/g)
70% MeOH	200	1.852	21.62	108.08
MA	200	1.481	16.15	80.77
MeOH	200	1.602	17.94	89.71

Table 8. Tannin content of *Cassia sieberiana* in the extraction solvents.

Sample	Conc. (μg/mL)	Abs	Conc. tannin (μg TAE/100 μg)	Conc. tannin (μg TAE/g)
70% MeOH	100	0.0941	11.37	113.67
MA	100	0.1286	22.87	228.67
MeOH	100	0.1095	16.50	165.00

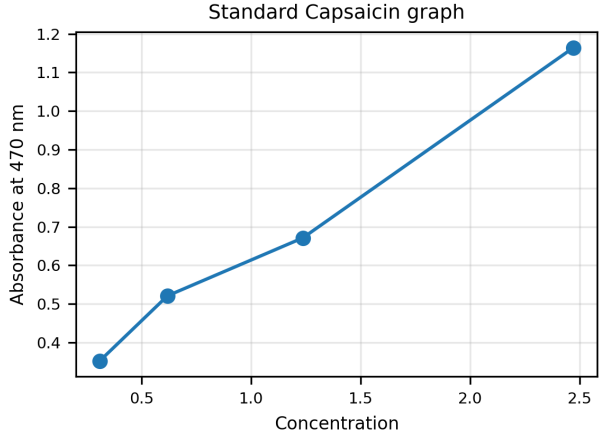


Figure 1. Standard capsaicin graph.

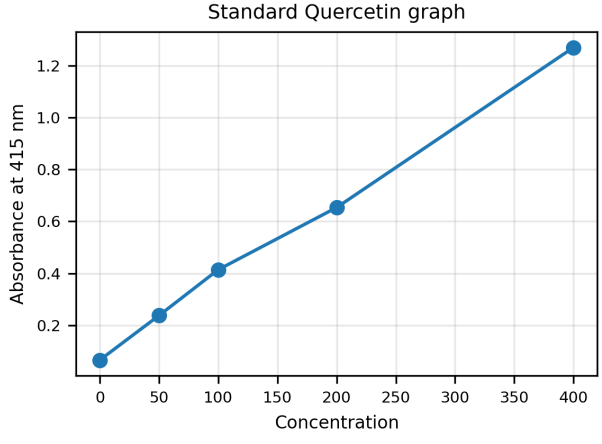


Figure 2. Standard quercetin graph.

tracts exhibited concentration-dependent radical scavenging activity. Among the tested samples, the methyl acetate extract showed superior performance in both DPPH and ABTS assays, indicating strong capacity to neutralize free radicals. The ob-

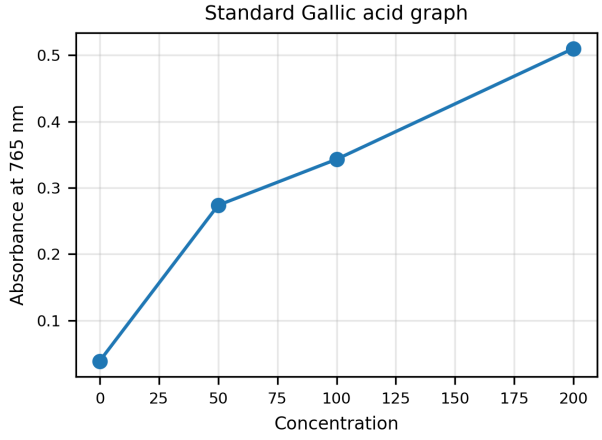


Figure 3. Standard gallic acid graph.

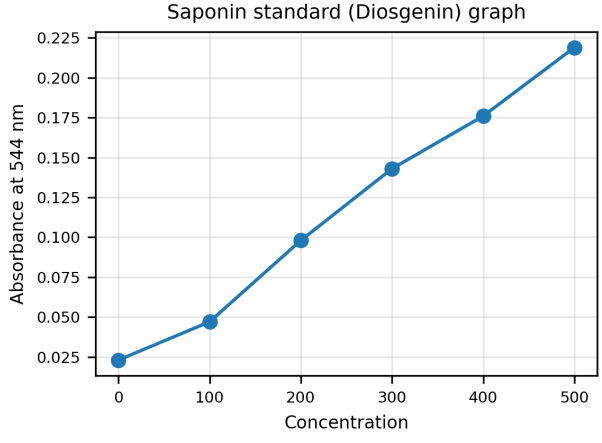


Figure 4. Saponin standard (diosgenin) graph.

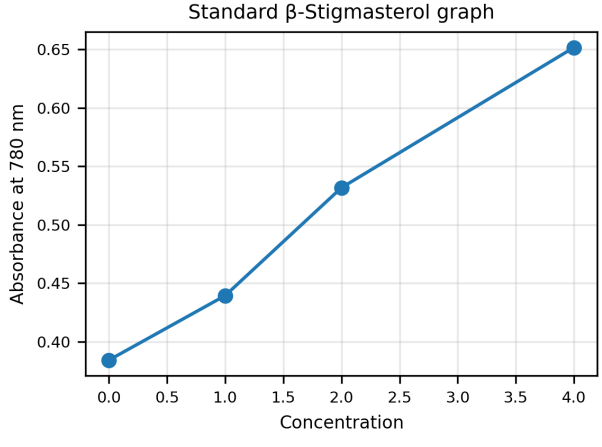


Figure 5. Standard β-stigmasterol graph.

served antioxidant activity may be attributed to phenolic compounds and flavonoids, which can donate electrons or hydrogen atoms to stabilize reactive species [28, 29]. The correlation between phytochemical content and antioxidant activity further supports this relationship, and the antioxidant capacity observed in this study aligns with previous reports on medicinal plants, reinforcing the importance of plant-derived compounds in combating oxidative stress and related diseases. These results suggest

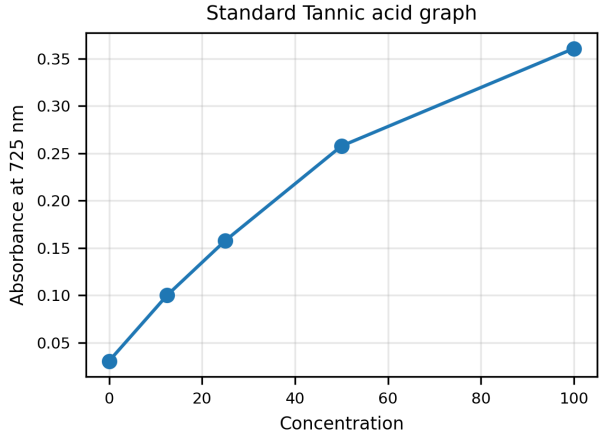


Figure 6. Standard tannic acid graph.

Table 9. ABTS radical scavenging activity of *Cassia sieberiana* root bark.

Sample	Conc. (μg/mL)	OD at 734 nm	Inhibition (%)	IC_{50} (μg/mL)
Solvents only	C	0.7495	0	
Quercetin	0.35	0.6402	14.59	1.711
	0.613	0.5425	27.62	
	1.25	0.4127	44.94	
	2.5	0.2486	66.83	
	5	0.1282	82.90	
	10	0.0153	97.96	
Methyl acetate	3.125	0.6569	12.36	15.20
	6.25	0.5562	25.79	
	12.5	0.4763	36.45	
	25	0.3479	53.58	
	50	0.2352	68.62	
	100	0.1690	87.45	
70% MeOH	3.125	0.6876	8.26	32.48
	6.25	0.6512	13.12	
	12.5	0.5636	24.81	
	25	0.4782	36.20	
	50	0.3109	58.52	
	100	0.2531	66.23	
MeOH	3.125	0.6585	12.14	22.67
	6.25	0.5691	24.07	
	12.5	0.4358	41.86	
	25	0.3485	53.50	
	50	0.1764	76.46	
	100	0.0811	89.18	

that *C. sieberiana* could serve as a valuable source of natural antioxidants for therapeutic applications.

In comparison, both assays showed concentration-dependent antioxidant activity. The ABTS assay showed slightly higher inhibition than DPPH, suggesting broad-spectrum radical scavenging ability of the extract, and the IC_{50} line indicates effective antioxidant performance at moderate concentrations (Figure 9). The antioxidant activity of *C. sieberiana* root bark extracts observed in this study demonstrates notable potency when compared with established antioxidant-rich plants such as *Camellia sinensis* and *Moringa oleifera* (Table 11). The methyl acetate extract of *C. sieberiana* exhibited strong radical scavenging activity, with DPPH and ABTS IC_{50} values of 30.19 and 15.20 μg/mL, respectively. These values fall within the range reported for *C. sinensis*, where DPPH IC_{50} values typically range from 23.26 to 69.51 μg/mL, depending on extraction method, plant origin, and processing conditions. This comparison suggests that *C. sieberiana* possesses antioxidant strength comparable to green tea, a

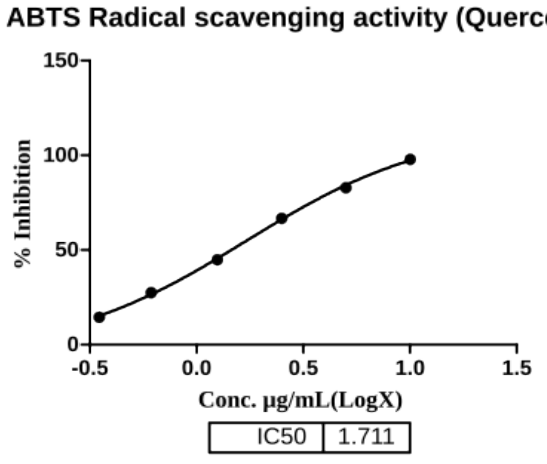


Figure 7. ABTS standard (quercetin) graph.

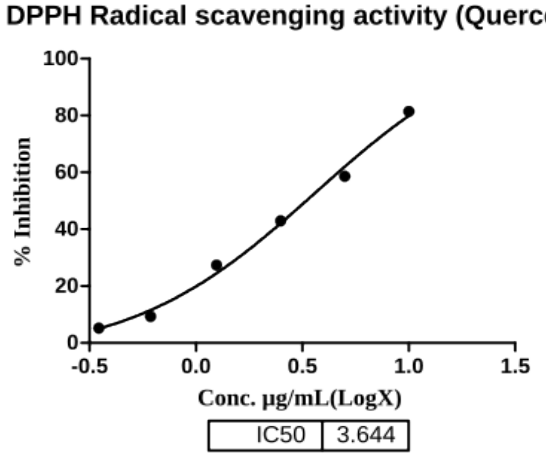
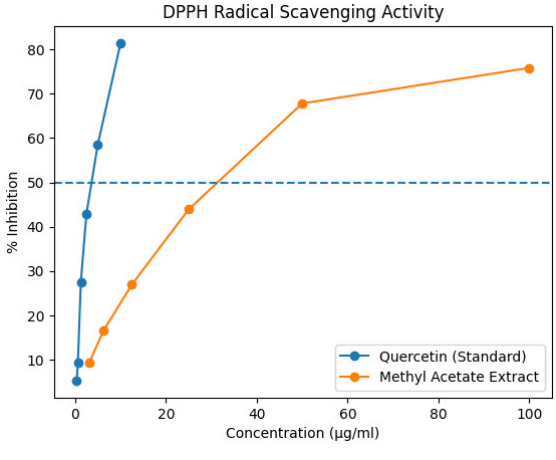
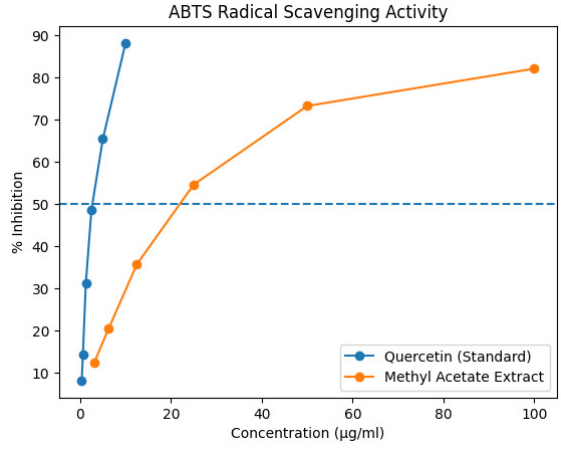


Figure 8. DPPH standard (quercetin) graph.



(a) DPPH radical scavenging activity.



(b) ABTS radical scavenging activity.

Figure 9. Comparative antioxidant activity of *Cassia sieberiana* extracts.

widely recognized natural antioxidant source [30].

According to commonly accepted classification systems, plant extracts with IC_{50} values below 50 μg/mL are categorized as strong antioxidants, while values between 50 and 100 μg/mL indicate moderate activity [31]. Based on this classification, the methyl acetate extract of *C. sieberiana* qualifies as a strong an-

Table 10. DPPH radical scavenging activity of *Cassia sieberiana* root bark.

Sample	Conc. (μg/mL)	OD at 510 nm	Inhibition (%)	IC_{50} (μg/mL)
Solvents only	C	0.3225	0	
Quercetin	0.35	0.3056	5.24	3.644
	0.613	0.2925	9.30	
	1.25	0.2341	27.41	
	2.5	0.1839	42.97	
	5	0.1335	58.60	
	10	0.0599	81.43	
Methyl acetate	3.125	0.2925	9.30	30.19
	6.25	0.2692	16.52	
	12.5	0.2356	26.94	
	25	0.1809	43.91	
	50	0.1038	67.81	
	100	0.0779	75.84	
70% MeOH	3.125	0.3031	6.01	81.43
	6.25	0.2898	10.14	
	12.5	0.2644	18.01	
	25	0.2204	31.66	
	50	0.1911	40.74	
	100	0.1152	64.28	
MeOH	3.125	0.3202	0.71	90.59
	6.25	0.3166	1.83	
	12.5	0.2886	10.51	
	25	0.2334	27.63	
	50	0.1927	40.25	
	100	0.1090	66.20	

Table 11. Comparative antioxidant activity (IC_{50}) of *Cassia sieberiana* and selected antioxidant-rich plants.

Plant	Extract	DPPH IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)	Reference
Cassia sieberiana	Methyl acetate	**30.19**	**15.20**	Present study
Cassia sieberiana	Methanol	90.59	22.67	Present study
Cassia sieberiana	70% MeOH	81.43	32.48	Present study
Camellia sinensis (green tea)	Aqueous/MeOH	23.26–69.51	~15–30	Multiple studies
Moringa oleifera	Methanol	49.30–57.12	~20–40	Reported studies
Moringa oleifera	Optimized extract	~17.50	~16.40	Advanced extraction

tioxidant, whereas the methanol and 70% methanol extracts exhibit moderate activity.

In comparison, *M. oleifera* leaf extracts have been widely reported to exhibit moderate to strong antioxidant activity, with DPPH IC_{50} values ranging from 49.30 to 57.12 μg/mL in conventional methanolic extracts. However, optimized extraction techniques have produced lower IC_{50} values, as low as 17.50 μg/mL for DPPH and 16.40 μg/mL for ABTS, indicating enhanced bioactive compound recovery. Relative to these findings, the methyl acetate extract of *C. sieberiana* demonstrates superior activity compared with standard *M. oleifera* extracts and comparable activity to optimized fractions [32–34]. The high antioxidant activity observed in *C. sieberiana* may be attributed to its rich phytochemical composition, particularly its elevated levels of phenolic compounds, flavonoids, and tannins. These compounds exert antioxidant effects through mechanisms such as electron donation, hydrogen atom transfer, and stabilization of ROS. The relationship observed between phytochemical content and antioxidant activity in this study further supports this mechanistic explanation. Although *C. sinensis* and *M. oleifera* have been extensively studied and commercialized worldwide, *C. sieberiana* remains relatively underutilized despite its abundance in tropical regions, particularly in West Africa. The comparable antioxidant performance demonstrated in this study highlights its potential as a cost-effective and locally accessible source of natural antioxidants.

4. CONCLUSION

This study demonstrates that *Cassia sieberiana* root bark contains a variety of phytochemicals with notable antioxidant properties. The findings show that different solvents affect both the composition and efficacy of the extracts. The differences in

phytochemical contents across solvents indicate the importance of extraction methods. The methyl acetate extract showed the strongest antioxidant activity, while the methanol extract had higher phenolic and saponin contents. These results provide scientific support for the traditional use of *C. sieberiana* and indicate its potential for the formulation of natural antioxidants, pharmaceuticals, and nutraceuticals. However, the bioactive phytocompound responsible for the observed antioxidant activity was not isolated, and no *in vivo* analysis was carried out in this work. Further investigations are suggested to isolate and characterize the bioactive compounds responsible for these biological activities.

DATA AVAILABILITY

The data will be available on request from the corresponding author.

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References

[1] T. S. Geetha & N. Geetha, ''Phytochemical screening, quantitative analysis of primary and secondary metabolites of *Cymbopogon citratus* leaves from Kodaikanal Hills, Tamil Nadu'', International Journal of PharmTech Research **6** (2014) 521. https://sphinxsai.com/2014/PTVOL6/PT=17(521-529)AJ14.pdf.

[2] S. Santhiravel, A. E.-D. A. Bekhit, E. Mendis, J. L. Jacobs, F. R. Dunshea, N. Rajapakse & E. N. Ponnampalam, ''The impact of plant phytochemicals on the gut microbiota of humans for a balanced life'', International Journal of Molecular Sciences **23** (2022) 8124. https://doi.org/10.3390/ijms23158124.

[3] F. Abbas, Y. Zhou, D. O'Neill Rothenberg, I. Alam, Y. Ke & H.-C. Wang, ''Aroma components in horticultural crops: chemical diversity and usage of metabolic engineering for industrial applications'', Plants **12** (2023) 1748. https://doi.org/10.3390/plants12091748.

[4] P. Halagali, A. Inamdar, J. Singh, A. Anand, P. Sadhu, R. Pathak, H. Sharma & D. Biswas, ''Phytochemicals, herbal extracts, and dietary supplements for metabolic disease management'', Endocrine, Metabolic & Immune Disorders–Drug Targets **25** (2025) 993. https://doi.org/10.2174/0118715303287911240409055710.

[5] A. A. A. Aljabali, M. A. Obeid, R. M. Bashatwah, E. Qnais, O. Gammoh, A. Alqudah, V. Mishra, et al., ''Phytochemicals in cancer therapy: a structured review of mechanisms, challenges, and progress in personalized treatment'', Chemistry & Biodiversity **22** (2025) e202402479. https://doi.org/10.1002/cbdv.202402479.

[6] B. Halliwell, ''Understanding mechanisms of antioxidant action in health and disease'', Nature Reviews Molecular Cell Biology **25** (2024) 13. https://doi.org/10.1038/s41580-023-00645-4.

[7] P. Chaudhary, P. Janmeda, A. O. Docea, B. Yeskaliyeva, A. F. A. Razis, B. Modu, D. Calina & J. Sharifi-Rad, ''Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases'', Frontiers in Chemistry **11** (2023) 1158198. https://doi.org/10.3389/fchem.2023.1158198.

[8] J. Tauchen, L. Huml, M. Jurasek, J. M. Regenstein & F. Ozogul, ''Synthetic and semi-synthetic antioxidants in medicine and food industry: a review'', Frontiers in Pharmacology **16** (2025) 1599816. https://doi.org/10.3389/fphar.2025.1599816.

[9] L. A. Magashi, H. M. Adamu, A. M. Kolo & U. F. Hassan, ''Extraction, phytochemicals screening and free radical scavenging activity of methyl acetate extracts from the root barks of *Cassia sieberiana*'', International Journal of Medical, Biological and Pharmaceutical Sciences **11** (2020) 92. https://www.researchgate.net/publication/348944995.

[10] B. Akbari, N. B. Yazdi, M. Bahmaie & F. M. Abhari, ''The role of plant-derived natural antioxidants in reduction of oxidative stress'', BioFactors **48** (2022) 611. https://doi.org/10.1002/biof.1831.

[11] H. S. Elshafie, I. Camele & A. A. Mohamed, ''A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based on plant origin'', International Journal of Molecular Sciences **24** (2023) 3266. https://doi.org/10.3390/ijms24043266.

[12] M. M. Rahaman, R. Hossain, J. Herrera-Bravo, M. T. Islam, O. Atolani, O. S. Adeyemi, O. A. Owolodun, et al., ''Natural antioxidants from some fruits, seeds, foods, natural products, and associated health benefits: an update'', Food Science & Nutrition **11** (2023) 1657. https://doi.org/10.1002/fsn3.3217.

[13] S. H. Hassanpour & A. Doroudi, ''Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants'', Avicenna Journal of Phytomedicine **13** (2023) 354. https://www.researchgate.net/publication/373650286_Review_of_the_antioxidant_potential_of_flavonoids_as_a_subgroup_of_polyphenols_and_partial_substitute_for_synthetic_antioxidants.

[14] AOAC, *Official Methods of Analysis*, 22nd ed., AOAC International (2023). https://www.aoac.org/official-methods-of-analysis/.

[15] J. B. Harborne, *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, Chapman and Hall Ltd., London (1973) 279. https://www.scirp.org/reference/referencespapers?referenceid=2852380.

[16] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang & C. Rice-Evans, ''Antioxidant activity applying an improved ABTS radical cation decolorization assay'', Free Radical Biology and Medicine **26** (1999) 1231. https://doi.org/10.1016/s0891-5849(98)00315-3.

[17] A. N. M. Alamgir, ''Herbal drugs: their collection, preservation, and preparation; evaluation, quality control, and standardization of herbal drugs'', in *Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1: Pharmacognosy*, Cham: Springer International Publishing, 2017, pp. 453-495. https://content.e-bookshelf.de/media/reading/L-10237160-be444024e3.pdf.

[18] P. Kapadia, A. S. Newell, J. Cunningham, M. R. Roberts & J. G. Hardy, ''Extraction of high-value chemicals from plants for technical and medical applications'', International Journal of Molecular Sciences **23** (2022) 10334. https://doi.org/10.3390/ijms231810334.

[19] A. Cetinkaya, S. Yayla, M. M. Hurkul & S. A. Ozkan, ''The sample preparation techniques and their application in the extraction of bioactive compounds from medicinal plants'', Critical Reviews in Analytical Chemistry (2025) 1. https://doi.org/10.1080/10408347.2025.2503437.

[20] R. Darwin, R. Valmon, S. Chithanna, S. H. Galla, S. H. Syed, A. A. M. Billah, K. T. K. Reddy & U. V. N. V. Arjun, ''Sustainable extraction and purification of phytochemicals: a review of green solvents and techniques'', Chemical Methodologies **9** (2025) 356. https://doi.org/10.48309/CHEMM.2025.504050.1892.

[21] M. S. Munuo, M. J. Martin, L. F. Shechambo & I. Gervas, ''Effect of solvent extraction on phytochemical profile and quantification of bioactive compounds in *Ocimum suave* (wild)'', Journal of Current Opinion in Crop Science **6** (2025) 27. https://doi.org/10.62773/jcocs.v6i1.304.

[22] N. N. Mahmoud & M. T. Selim, ''Phytochemical analysis and antimicrobial activity of *Silybum marianum* L. via multi-solvent extraction'', AMB Express **15** (2025) 122. https://doi.org/10.1186/s13568-025-01925-2.

[23] L. Burdejova, B. Tobolkova, M. Polovka & J. Neugebauerova, ''Differentiation of medicinal plants according to solvents, processing, origin, and season by means of multivariate analysis of spectroscopic and liquid chromatography data'', Molecules **28** (2023) 4075. https://doi.org/10.3390/molecules28104075.

[24] M. T. El-Saadony, A. M. Saad, D. M. Mohammed, S. S. Alkafaas, T. A. Abd El-Mageed, M. A. Fahmy, A. E. Ahmed, et al., ''Plant bioactive compounds: extraction, biological activities, immunological, nutritional aspects, food application, and human health benefits–a comprehensive review'', Frontiers in Nutrition **12** (2025) 1659743. https://doi.org/10.3389/fnut.2025.1659743.

[25] A. P. Widiyana & D. N. Illian, ''Phytochemical analysis and total flavonoid content on ethanol and ethyl acetate extract from neem (*Azadirachta indica* Juss.) leaves utilizing UV–Vis spectrophotometric'', Jurnal Farmasi Sains dan Praktis **8** (2022) 60. https://doi.org/10.31603/pharmacy.v8i1.6582.

[26] M. M. Dahiru, A. A. M. Abaka & S. P. Artimas, ''Phytochemical analysis and antibacterial activity of methanol and ethyl acetate extracts of *Detarium microcarpum* Guill. & Perr'', Biology, Medicine, & Natural Product Chemistry **12** (2023) 281. https://doi.org/10.14421/biomedich.2023.121.281-288.

[27] A. Nicolescu, M. Babota, L. Barros, G. Rocchetti, L. Lucini, C. Tanase, A. Mocan, C. I. Bunea & G. Crisan, ''Bioaccessibility and bioactive potential of different phytochemical classes from nutraceuticals and functional

foods'', Frontiers in Nutrition **10** (2023) 1184535. https://doi.org/10.3389/fnut.2023.1184535.

[28] F. Fernandes, C. Delerue-Matos & C. Grosso, ''Unveiling the potential of agrifood by-products: a comprehensive review of phytochemicals, bioactivities and industrial applications'', Waste and Biomass Valorization **16** (2025) 2715. https://doi.org/10.1007/s12649-024-02622-0.

[29] S. Baliyan, R. Mukherjee, A. Priyadarshini, A. Vibhuti, A. Gupta, R. P. Pandey & C.-M. Chang, ''Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*'', Molecules **27** (2022) 1326. https://doi.org/10.3390/molecules27041326.

[30] M. R. Hasan, M. M. Haque, M. A. Hoque, S. Sultana, M. M. Rahman, M. A. A. Shaikh & M. K. U. Sarker, ''Antioxidant activity study and GC–MS profiling of *Camellia sinensis* Linn'', Heliyon **10** (2024) e23514. https://doi.org/10.1016/j.heliyon.2023.e23514.

[31] I. Gulcin, ''Antioxidants: a comprehensive review'', Archives of Toxicology **99** (2025) 1893. https://doi.org/10.1007/s00204-025-03997-2.

[32] A. A. Masresha, D. Marinella, V. Duccio, T. Hagos, T. Frehiwot, D. Eyob & G. G. Solomon, ''Phytochemical profiling, antioxidant and anti-inflammatory potential of methanolic extracts of *Moringa oleifera* (L.) Lam. and *Moringa stenopetala* (Bak.) Cufod. leaves grown in Arba Minch, Ethiopia'', RSC Advances **15** (2025) 43818. https://doi.org/10.1039/D5RA05914C.

[33] S. Sreelatha & P. R. Padma, ''Antioxidant activity of *Moringa oleifera* leaves'', Food and Chemical Toxicology **47** (2009) 2196. https://doi.org/10.1016/j.fct.2009.06.011.

[34] F. Anwar, S. Latif, M. Ashraf & A. H. Gilani, ''*Moringa oleifera*: a food plant with multiple medicinal uses'', Phytotherapy Research **21** (2006) 17. https://doi.org/10.1002/ptr.2023.