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Phytochemical screening, free radical scavenging and antibacterial activity of *Cassia sieberiana* root bark extracts

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Abstract

Cassia sieberiana is a tropical plant, widely distributed throughout Sudan and Guinea savannah. It is used in traditional medicine for the treatment of malarial, cancer and stomach ache. The study was conducted to screen for phytochemicals, free radical scavenging and antibacterial potentials of the root bark. It was extracted successively by Soxhlet extractor using n-hexane, dichloromethane and methanol. The phytochemical screening of the extracts revealed the presence of saponins, flavonoids, tannins, anthraquinones, phenolics, alkaloids, carbohydrates, steroids/triterpenoids and cardiac glycosides. Qualitatively screening for free radical scavenging compounds using 1,1-Diphenyl-2-PicrylHydrazyl (DPPH) was carried out on TLC. The result showed greater activity in the methanol extract. *In vitro* quantitative determination of free radical scavenging activity using DPPH at 2.5, 5, 10 and 20 mg/mL also showed methanol extract with highest activity (IC_{50} of 0.25 mg/mL). The activity demonstrated may be due the presence of phenolic compounds. The dichloromethane and methanol extracts showed good antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. The zones of inhibition of growth produced by the dichloromethane and methanol extracts on the microorganisms at 15, 20, 25 and 30 mg/mL ranged from 2 - 20 mm. The MIC and MBC revealed that, dichloromethane and methanol extracts were both bateriostatic and bactericidal. Results obtained from this research have provided preliminary evidence for the use of *C. sieberiana* root bark in traditional medicine for treatment of diseases attributed to free radicals and microbial infections.

Keywords: *Cassia sieberiana*, DPPH, Antibacterial, Phytochemical, Phenolic Content,

INTRODUCTION

Medicinal plants are very rich in bioactive compounds. Plants have been use over years throughout human history for treatment of simple and complex human diseases. Plants and their extracts have been evaluated for pharmacological activities [1]. *Cassia sieberiana* is tropical plant that is widely distributed throughout sudan and guinea savanna, from Senegal to Nigeria including Togo [2]. According to [3] the plant can be found in most part of Nigeria. In the north it can be found in Sokoto, Kebbi, Zamfara, Yobe, Bauchi, Borno and Adamawa. In the southern part of the country it is found in Oyo and Onitsha. The plant has been used in traditional medicine by different cultures for treatment of various diseases. Numerous researches have shown the significance pharmacological activities of

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various part of the plant, such as the root, leaves, bark and extracts. The plant seed was found to moderately contain crude protein, crude fibers, potassium and magnesium [4].

The phytochemical screening and DPPH scavenging of *Cassia fistula* seed extract show the presence of phytochemicals with excellent and potent antioxidant activity [5]. An investigation into three species of *Cassia* family namely, *Cassia occidentalis* (Linn.) *Cassia alata* (Linn.) and *Cassia sieberiana* (DC) revealed the presence of alkaloids, flavonoids, saponins and tannins [6]. They also reported antimalarial and anticancer properties of these plants. Also, *Cassia sieberiana can* be used in treatment of fever, jaundice, stomach ache, pile and ulcer [3]. An extensive research has proved that long use of aqueous extract of *Cassia sieberiana* at high dose for long period of time can cause liver damage [3]. This research is aimed at evaluating the phytochemical, free radical scavenging and antibacterial activity of the extracts and fractions of the root bark of the plant.

EXPERIMENTAL

Collection and identification of the plant. Fresh leaves, flowers and root of *Cassia sieberiana* were obtained from the reputable medicinal plant market located at the Sokoto old market. The identity of the plant was established by U.S. Gallah (Consultant Taxonomist) at the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The voucher specimen was prepared and assigned voucher number PCG/UDUS/CAES/0002 and deposited at herbarium for future reference.

Preparation and extraction of plant sample. The root bark of the plant was washed with clean water and air-dried under shade for 14 days. It was ground to powder using wooden pestle and mortar. The powder was kept in moisture free container for further use. The powdered plant (80 g) was extracted successively with 600 mL each of n-hexane, dichloromethane and methanol using Soxhlet extractor for period of 3 hours. The percentage yield of each extract was calculated.

Qualitative phytochemical screening. The n-hexane, dichloromethane and methanol extracts were screened for the presence alkaloids, saponins, steroids, tannins, glycosides and flavonoids using standard procedure as described by [7-9]

Thin Layer Chromatography (TLC). The separation profiles of the n-hexane, dichloromethane and methanol extracts were determined using TLC pre-coated plate (TLC silica Gel 60 F_{254}) by ascending technique. The n-hexane, dichloromethane and methanol extracts were resolved using a solvent mixture of hexane/ethyl acetate (8:2), n-hexane/dichloromethane/methanol (1:1:8) and ethyl acetate/methanol/water (8:1:1) respectively. The chromatograms were observed for separation (spots) first under short λ (254mn) and long λ (366nm). They were subsequently stained with 10% sulphuric acid and heated in an oven at 105^{o}C for 3 minutes to detect the presence of spots. The R_f values were calculated.

Qualitative determination of free radical scavenging compounds using DPPH. Freshly prepared DPPH solution (DPPH powder dissolved in methanol) was sprayed on freshly developed TLC plate of each extract. Formation of yellow or white spot(s) against purple background indicates the presence of free radical scavenging compound(s).

Determination of total phenolic content. The total phenolic content was determined spectrophotometrically using Folin-Ciocalteu reagent. 2ml (0.5g/10 mL) of the methanol and DCM extracts were mixed with 0.2 mL of Folin-Ciocalteu reagent (previously diluted two-fold with distilled water). After 5 minutes

0.6 mL of prepared Na_2CO_3 solution (200mg/10 mL) was added to the mixture and allowed to incubate for 5 minutes at room temperature. The absorbance was measured at 680 nm using a UV-VIS spectrophotometer (PD 3000 UV). Total phenolic amounts were quantified by calibration curve obtained from measuring absorbance of a known concentration of gallic acid standard (2.5 mg/mL to 20 mg/mL). The absorbance values of the test extracts were recorded at each concentration in triplicate.

Determination of *in vitro* free radical scavenging capacity using DPPH. The free radical scavenging potential of the extracts were tested against a solution of DPPH (DPPH in methanol) using a standard method [11]. Antioxidants react with 1,1-diphenyl-2-picryl hydrazyl (DDPH) and convert it to 1,1-diphenyl-2-picryl hydrazine (DPPH + H). The extracts were prepared in different concentrations (2.5, 5, 10 and 20 mg/mL) by dissolving in methanol. The samples of the different concentration were mixed with 3 mL of prepared DPPH. After 5 minutes the absorbance was measured at 517 nm using a UV-Visible spectrophotometer for the samples and blank (prepared without the extract). Ascorbic acid at various concentrations (2.5, 5. 10 and 20 mg/mL) was used as standard. The lower the absorbance of the mixture the higher free radical scavenging activity (Chatatikun *et al*., 2013). Percentage radical scavenging activity was calculated using the following equation.

$$\%RSA = \{(A_{blank} - A_{sample}) / A_{blank}\} \times 100$$

Antibacterial activity

Test organisms. Standard laboratory strains of *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa* were collected from the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto.

Susceptibility test. The cup plate method was used. The nutrient agar was prepared according to the manufacturer's specification and then sterilized at 121^{o}C. It was allowed to cool and then transferred to sterilized Petri-dishes. The Petri-dishes were flooded with various dilutions of the test bacteria and drained using with Pasteur pipette. Wells measuring 4 mm in diameter were bored into the inoculated plates using a cork borer. The wells were filled with 0.1 mL each of 15 mg/mL, 20 mg/mL, 25 mg/mL and 30 mg/mL of dichloromethane and methanol extracts. The plates were allowed to stand for pre-diffusion time for 2 hours and then incubated for 24 hours at 37^{o}C. The zones of inhibition were measured to the nearest millimeter using a metric rule. The readings were taken in triplicate and the mean values recorded. Similarly, the plate of ofloxacin (10 mg/mL) was used as positive control and the zone of inhibition was also recorded.

Determination of Minimum Inhibitory Concentration (MBC). Two-fold serial dilutions were used to determine the MIC. The dichloromethane and methanol extracts (2 mL) each containing 15 mg/mL were added to a test tube and serially diluted to give 10 different concentrations with the least containing 0.07 mg/mL. The *S. aureus* and *E. coli* (0.5 mL) containing 10^5 cfu/mL were added to the test tubes and incubated at temperature of 37^{o}C for 24 hours.

Determination of Minimum Bactericidal concentration (MBC). The MBC was determined from the MIC and the concentrations just before it. The nutrient agar was prepared and plated with the test organisms. The plates were incubated at 37^{o}C for 24hrs.

RESULTS

The results of phytochemical screening of the fractions are presented in Table 1. The results of qualitative screening for free radical scavenging compounds using

TLC are presented in Table 2 and Plates 1a, 1b and 1c. The results for quantitative estimation of total phenolic content are presented in Table 3 and Figure 1. The results of percentage free radical scavenging activity are presented in Table 4 and Figure 2. The results of the inhibitory concentration (IC_{50}) are presented in Table 5. The results of the antibacterial activity tests are presented in Tables 6, 7, 8 and 9.

Table1: Phytochemical Screening of *Cassia sieberiana* root bark

Metabolites	Test/ reagent	Hexane	DCM	Methanol
Alkaloids	Mayer's reagent	-	-	+
	Dragendorff's reagent	-	-	+
	Wagner's reagent	-	-	+
Carbohydrates	Molisch's test	-	+	+
	Fehling's test	-	+	+
Flavonoids	Shinoda's test	-	+	+
	Potassium hydroxide test	-	+	+
Tannins	Ferric chloride test	-	+	+
	Lead acetate test	-	+	+
Steroids /triterpenoids	Salkowski's test	+	+	+
	Libermann-Burchard's test	+	+	+
Cardiac glycoside	Keller-Killiani's test	+	+	+
Saponins	Froth test	-	+	+
Anthraquinones	Borntrager's test	-	+	+

Key: - = Absent + = Present

Table 2: Qualitative free radical scavenging activity on DPPH

Extracts	Inference
n-Hexane	+
Dichloromethane	++
Methanol	+++

Key: + = least active; ++ = Moderately Active; +++ = Highly Active

Table 3: Estimation of total phenolic content in mg (mean±SEM)

Concentration of extract (mg/mL)	Hexane	DCM	Methanol	Gallic acid
2.5	ND	654±0.289	775±1.732	1192±1.732
5	ND	654±0.289	1140±0.404	1187±0.404
10	ND	1067±0.557	1165±0.404	1174±0.557
20	ND	1163±0.557	1161±0.557	1117±0.924

Note: ND- Not Detected; SEM- Standard Error Mean and Mean is the mean of 3 replicates

Table 4: Percentage of free radical scavenging activity of *Cassia sieberiana*

Concentration of extract (mg/mL)	Hexane	DCM	Methanol	Ascorbic acid
2.5	20.90	23.08	46.03	78.08
5	24.36	98.33	61.92	68.72
10	33.59	51.41	56.41	42.69
20	36.15	15	51.54	61.41

Table 5: IC_{50} values of extract

Extracts	IC_{50}
Hexane	0.85
DCM	0.45
Methanol	0.25
Standard (ascorbic acid)	0.09

Table 6: Antibacterial activity of dichloromethane extract

	Zone of inhibition (mm)			
Concentration (mg/mL)	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
15	5	2	-	2
20	8	6	2	4
25	11	9	7	9
30	14	14	11	11

Key: - = No inhibition

Table 7: Antibacterial activity of methanol extract

	Zone of inhibition (mm)			
Concentration (mg/mL)	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
15	7	7	9	8
20	9	11	14	10
25	12	15	19	11
30	14	20	20	13

Table 8: Antibacterial activity of standard antibiotic (ofloxacin)

	Zone of inhibition (mm)			
Ofloxacin	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
(10 mg/mL)	25	30	26	31

Table 9: MIC / MBC of dichloromethane and methanol extracts (mg/mL)

	Dichloromethane		Methanol	
Test organisms	MIC	MBC	MIC	MBC
E. coli	-		1.875	0.235
S. aureus	3.75	3.75	-	

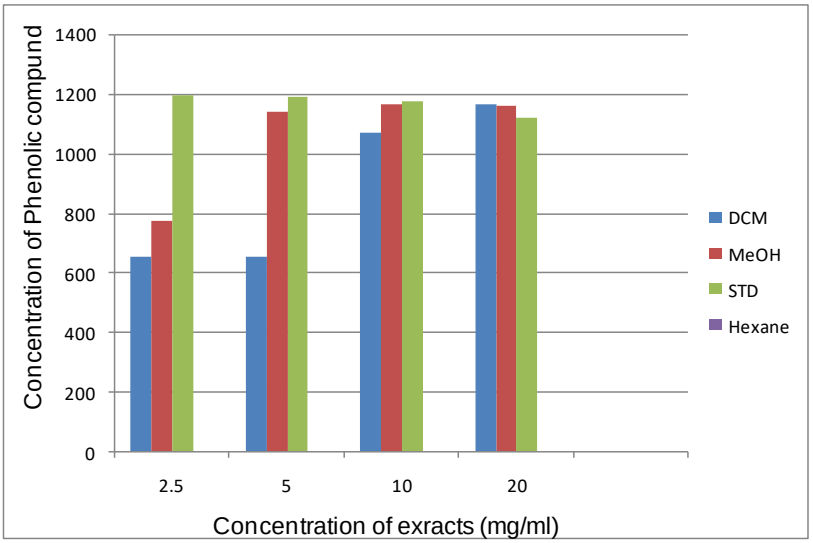

Fig 1: Concentration of Phenolic Compounds Present in Different Extracts

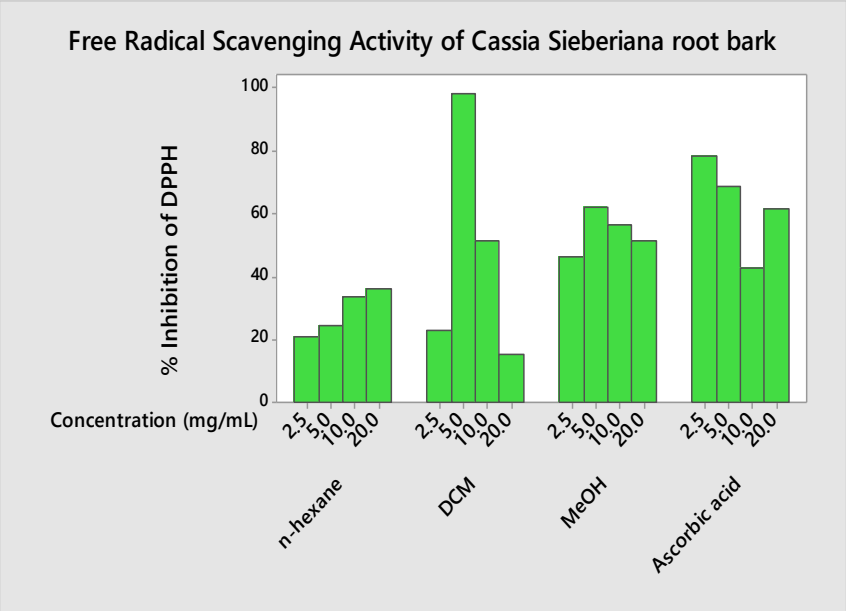

Figure 2: Free radical scavenging activity of different extracts of *Cassia sieberiana* root bark

Plate 1a: n-Hexane extract

Plate 1b: Dichloromethane Extract

Plate 1c: Methanol extract

DISCUSSION

The phytochemical screening of the various extracts of *Cassia sieberiana* root bark, revealed the presence of saponins, flavonoids, tannins, anthraquinones, alkaloids, carbohydrates, steroids/tritepenoids and cardiac glycosides (Table 1). The hexane extract showed the presence of cardiac glycosides and steroids/triterpenoids. On the other hand, the dichloromethane (DCM) extract revealed the presence of the above phytochemicals with alkaloids absent. The methanol extract showed the presences of saponins, flavonoids, tannins, anthraquinones, alkaloids, carbohydrates, steroids/tritepenoids and cardiac glycosides (Table 1). It has been reported that different phytoconstituents have different degrees of solubility in different types of solvents, depending on their polarity [13].

The presence of free radical scavenging compounds was detected in the n-hexane, dichloromethane and methanol. The extracts exhibited different degrees of free radical scavenging activity as observed from the TLC plates (Table 2, Plates 1A, B and C). The hexane extract demonstrated little discolouration (Plate1A). This indicates that it does contain high free radical scavenging compound. It has been reported that Free radical scavenging compounds show yellow or white spot against purple background on TLC plate. The potency of an extract as a free radical scavenger is proportional to the

intensity of the yellow or white spots on the purple background [13]. The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourized as the colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The total phenolic contents of the extracts (Table 3 and Figure 1), Showed that methanol had the highest phenolic content this is followed by DCM. This observation may be attributed to the presence of flavonoids and tannins present in the methanol extract [13]. The *in vitro* free radical scavenging activity of *Cassia sieberiana* root bark extracts (Table 4 and Figure 2), revealed that, at all concentrations (2.5 mg/mL, 5 mg/mL, 10 mg/mL and 20 mg/mL), the methanol extract demonstrated the highest free radical scavenging capacity, followed by DCM and n-hexane extract with the least. The IC_{50} value showed methanol to be more potent the other extracts. Methanol extract had IC_{50} value of 0.25 mg/mL (Table 5). The lower the IC_{50} value the more potent is the extract. The dichloromethane and methanol extracts of *Cassia sieberiana* root bark showed good antibacterial activity on *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* (Tables 6 and 7). The zones of inhibition of growth produced by the dichloromethane extract on the microorganisms at 15, 20, 25 and 30 mg/mL ranged between 2 mm to 14 mm (Table 6). Also, the zones of inhibition of growth of the methanol extract at the same concentration on the microorganisms ranged between 7 mm to 20 mm (Table 7). The antibacterial activity demonstrated by the extracts may be due to the presence tannins, flavonoids and saponins [14]. From the results, it can be deduced that, the methanol extract was more active on the microorganisms than the dichloromethane extract. This may be due to high concentration of tannins, flavonoid and saponins in the methanol extract. Methanol being polar solvent is expected to extract this highly polar compound [12]. From Table 6, it can be observed that the dichloromethane extract was more active on the Gram positive organisms (*S. aureus* and *B. subtilis*) than on negative organisms (*E. coli* and *P. aeruginosa*). This apparent difference in the susceptibilities of the microorganisms to the extracts might be related to the structural differences in the cell envelope compositions of the Gram positive and Gram negative bacteria. The Gram positive cell envelope is simple while that of Gram negative is complex consisting of lipoproteins, outer membrane and lipopolysaccharides [15]. The outer membrane of the Gram negative cell outer envelope blocks the penetration of large molecules and hence the relative resistance of Gram negative bacteria to some antimicrobial agents [15]. It is also observed that, the zone of inhibition of growth produced by the extracts on all the microorganisms is concentration dependent (Tables 6 and 7). For example, from Table 6, the zone of inhibition of growth of the DCM extract on *S. aureus* increases from 5, 8, 11 and 14 mm at 15, 20, 25 and 30 mg/mL respectively. This trend is observed on all the microorganism (Tables 6 and 7). From Table 7, the methanol extract generally inhibited the growth of the microorganisms but *B. subtilis* and *E. coli* are more susceptible. The activity demonstrated by the methanol extract (Table 7) compares favourably with the standard antibiotic (Table 8). The MIC and MBC (Table 9) revealed that, the dichloromethane and methanol extracts are both bateriostatic and bactericidal. The antibacterial activity demonstrated by the extracts may be

attributed to the secondary metabolites present in the plant extracts.

Conclusion. *Cassia sieberiana* root bark extracts contain saponins, alkaloids, simple phenolics, tannins, flavonoids, carbohydrates, cardiac glycosides, polysterol/terpenoids and anthraquinones. The methanol and DCM extracts demonstrated good *in vitro* antioxidant activity and antibacterial on *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa.*

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