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Research Article
Phytochemical, GC-MS Analysis and Antimicrobial Activity of the Methanol Stem Bark Extract of *Cassia siamea* (Fabaceae)

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Abstract

Background and Objectives: *Cassia siamea* widely distributed in the southern and northern parts of Nigeria is extensively used in folkloric medicine for the treatment of urinogenitory disorders, herpes, rhinitis, constipation, diabetes, insomnia, hypertension, asthma, typhoid fever, microbial infections among others. The study was aimed to screen the antimicrobial potency of the methanol stem bark extract of *C. siamea* as well established the phytoconstituents responsible for such activity. **Materials and Methods:** This study was evaluated the phytochemistry, antimicrobial effects as well as the GC-MS analysis of *Cassia siamea* stem bark extracted with absolute methanol using cold maceration method for 72 h. **Results:** The extract showed promising antibacterial activity against all the tested pathogens namely *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumoneae*, *Salmonella typhi*, *Shigella* sp, *Escherichia coli* and *Pseudomonas aeruginosa*. The best activity was recorded for the gram negative strain of *E. coli* with a zone of inhibition (mm) of 16.77±0.22 while the least activity was recorded for the gram positive strain of *S. aureus* with a zone of inhibition (mm) of (11.16±0.12) at the highest investigated concentration of 100 mg mL^{-1}. The GC-MS analysis confirmed the occurrence of a total 23 phytocompounds in the methanol extract. **Conclusion:** The stem bark extract has varying degree of antimicrobial potential ranging from mild to high activity correlated by the presence of selected bioactive compounds.

Key words: *Cassia siamea*, phytochemicals, stem bark extract, bioactive compounds, folkloric medicine

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In recent years, drug resistance to human pathogenic microorganism has been commonly reported from all over the world[1]. This has prompted researchers to focus on producing medicines and products that are naturally capable of either inhibiting the growth of pathogens or kill them[2,3] and at the same time have no or the least toxicity to host cells to complement the existing synthetic antimicrobial drugs that are gradually becoming less potent against pathogenic microorganisms[4]. Medicinal plants have been scientifically proven to be a potent alternative source to challenge these diverse array of diseases hence corroborating the traditional claims on the value of these natural products[5] as further confirmed by the world health organization who reported that over 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies which involve the use of plant extract and their constituents[6]. Phytochemicals present in plants such as terpenoid, flavonoids, steroid, alkaloids and phenolic compounds among others possess impressive pharmaceutical relevance such as analgesics, aesthetic, antibiotics, antiparasitic and anti-inflammatory among others[7].

Cassia siamea belongs to the sub-family Fabaceae (Caesalpinioideae) of family Leguminosae[8]. It is widely distributed in various countries including Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil. It was believed to be introduced to Africa from tropical Asia. The leaves of the plant is used traditionally as vegetables in Thailand[9] as well as ethno-medicinally as laxative, blood cleaning agent, cure for digestive system, urinogenitory disorders, herpes and rhinitis[10], constipation, diabetes, insomnia[11], hypertension, asthma, typhoid fever and diuresis[12], antimalarial agent[13], ringworm and other fungal skin infections[14]. Besides its pharmacological uses, its extract is also recommended for pest and disease control[15-17] as well as used in the treatment of broad spectrum of diseases[18].

It has become necessary to further evaluate the pharmacological potential of *C. siamea* stem bark. Hence, this present study was aimed at determining the phytochemical constituents and antibacterial activity of *C. siamea* methanol stem bark extract against selected clinical isolates of bacteria namely *S. pyogenes*, *S. aureus*, *Klebsiella pneumoneae*, *Salmonella typhi*, *Shigella* sp., *Escherichia coli* and *Pseudomonas aeruginosa* recovered from different samples of infected patients as well as subjecting the extract to GC-MS analysis for the identification of the various components that are responsible for the antimicrobial properties hence could be explored for the development of novel antimicrobial agents.

MATERIALS AND METHODS

Collection and authentication of plant materials: Fresh *Cassia siamea* stem bark used in this study was collected by Mr. Jude Ibeabuchi Ali from a forest in Owerri, Imo State, South-East Nigeria in June, 2018. They were immediately transported fresh to the Department of Pure and Applied Chemistry, Usmanu Danfodiyo University, Sokoto, North-Western Nigeria. Identification and authentication of the plant was done at the herbarium unit in the Department of Plant Biology, Usmanu Danfodiyo University, Sokoto where voucher specimens were deposited for future reference. The stem bark was washed with clean tap water to remove dust and earthy impurities before being air dried for three weeks and pulverized into fine powder using a clean mortar and pestle. The powdered samples were kept in a clean air tight glass container until ready for use.

Preparations of the crude methanol stem bark extract: The powdered sample (500 g) was extracted by cold maceration for 72 h using methanol (BDH Chemicals Ltd, Poole, England). Manual agitation of the flask using a sterile glass rod was performed after every 24 h. After 72 h, the extract was filtered using a clean sterile muslin cloth and then using Whatman filter paper. The filtrate was then concentrated using a rotary evaporator in vacuum at 40°C at reduced pressure. The extract obtained was dried to constant weight under room temperature. The percentage yield with respect to the original mass macerated was determined. The extract was placed in a clean sterile glass bottle before being stored in a refrigerator at 4°C for further use.

Qualitative phytochemical screening: The phytochemical screening of the plant material for various phytochemical constituents such as terpenoids, flavonoids, alkaloids, reducing sugars, steroid, glycoside, phenol, anthraquinones, saponin and tannin was conducted using standard methods as described by Sofowora[19] and Trease and Evans[20].

Preparation of test organisms and inocula preparation: Clinical isolates of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumoneae*, *Salmonella typhi*, *Shigella* sp., *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from Microbiology Laboratory of Usmanu Danfodiyo University Teaching Hospital Sokoto, Sokoto State, Nigeria.

These isolates were subjected to gram staining, microscopic appearance, colony morphology and biochemical tests according to standard protocols[21-23]. The inocula of the

test isolates were prepared using the colony suspension method[24]. Colonies picked from 24 h old cultures grown on nutrient agar were used to make suspensions of the test organisms in saline solution to give an optical density of approximately 0.1 at 600 nm. The suspension was then diluted 1:100 by inoculating 9.9 mL of sterile nutrient broth with 100 µL of the bacterial suspension.

Gas chromatography-mass spectrometry (GC-MS) analysis of methanol extract: The GC-MS analysis was carried out using GC-MS-QP 2010 Plus Shimadzu system and Gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: Column Elite-1 fused silica capillary column (30 m×0.25 mm 1D×µL df, composed of 100% dimethyl polysiloxane). For GC-MS operation, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate of 1 mL min^{-1} and an injection volume of 2 µL. Split ratio 10:1 injector temperature (250°C) and ion-source temperature of 280°C was used. The oven temperature was programmed from 110°C (Isothermal for 2 min) with an increase of 10°C min^{-1} to 200°C then 5-280°C min^{-1}, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV, a scan interval of 0.5 sec and fragments from 40-550 Da. Total GC running time was 60 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The mass spectra of the components were matched with the data available in the National Institute of Standards and Technology (NIST) library Ver. 2.0 year 2008 library where the retention time, chemical name and molecular weight of the components of the extract was revealed.

Screening of antibacterial activities: Antimicrobial activity of the crude extract was determined by agar well diffusion method as described by Kavanagh[25] as modified by Ogbiko *et al.*[26]. Immediately after autoclaving, the media was allowed to cool at 45-50°C. The freshly prepared and cooled media was poured into petri dishes (90 mm in diameter) placed on a level. The agar media was allowed to cool and solidify at room temperature and the plates were incubated at 35°C for 18-20 h before use to confirm sterility. About 0.1 mL of the test inoculum was evenly spread on the surface of the solidified agar media and spread it on plate evenly by using sterile spreader. Four equidistant wells of 8 mm in diameter and 3 mm in depth were then made on the agar plate. About 100 mL of the each plant extract was filled into the wells. As control, absolute methanol was used. The plates were then incubated for 24 h at 37°C. Antimicrobial activity was determined by measuring the diameters of zones of inhibition. The experiment was conducted in triplicate and the average values were recorded. Ciprofloxacin 20 µg mL^{-1} (Micro Lab limited) served as the positive control for the experiment.

Statistical analysis: The data of average zone of inhibition produced by the isolates against the extract used were analyzed using one-way ANOVA from statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means±standard deviation. Significance level for the differences was set at $p<0.05$.

RESULTS

Percentage yield of the crude methanol stem bark extract: About 20.85 g corresponding to a percentage yield of 4.17% was obtained for the extraction of the pulverised stem bark of *C. siamea*.

Phytochemical screening: The preliminary phytochemical screening of the methanol stem bark extract of *C. siamea* showed the presence of important secondary metabolites which is presented in Table 1.

Antibacterial activity of stem bark methanol extract of *C. siamea*: The antibacterial activity of methanol extract of *C. siamea* is presented in Table 2.

The GC-MS profiling of the methanol stem bark extract of *C. siamea* revealed the occurrence of a total of 23 peaks corresponding to the presence of 23 chemical compounds were identified as presented in Fig. 1. These compounds belong to different chemical classes and most of them are reported to exhibit important biological activities. The identified compounds with their peak number, retention time (RT), peak area (%), compound name and chemical formulae are presented in Table 3.

Table 1: Phytochemical constituents of the stem bark extract

Phytochemicals	Stem bark
Alkaloids	+
Flavonoid	+
Glycosides	+
Reducing sugar	+
Saponins	+
Steroids	+
Phenols	-
Terpenoids	-
Anthraquinones	+
Tannin	+

+: Presence, -: Absence

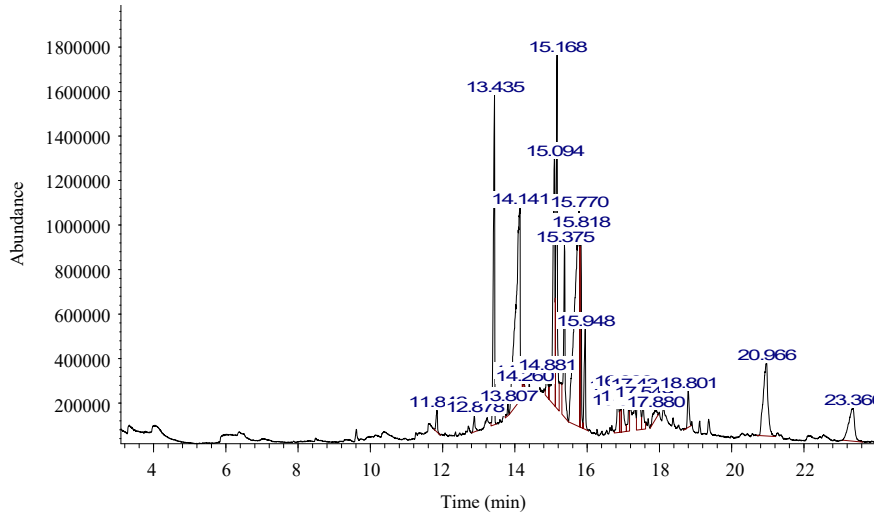

Fig. 1: Gas ion chromatogram of the methanol stem bark extract of *C. siamea*

Table 2: Antibacterial activity of methanol extract of *C. siamea*

	Concentration (mg mL^{-1})/zone of inhibition (mm)				
Isolates	25	50	75	100	CP (20 µg mL^{-1})
S. pyogenes	9.10±0.01^{a}	11.84±0.00^{a}	12.44±0.11^{a}	13.17±0.12^{a}	24
S. aureus	7.24±0.02^{a}	8.81±0.00^{a}	10.86±0.11^{a}	11.16±0.12^{a}	23
K. pneumoneae	10.18±0.10^{a}	11.64±0.12^{a}	13.22±0.15^{a}	14.11±0.13^{b}	20
S. typhi	9.68±0.12^{a}	11.91±0.11^{b}	12.47±0.05^{b}	14.26±0.22^{b}	19
Shigella spp.	10.86±0.32^{a}	12.55±0.15^{a}	14.70±0.32^{b}	16.57±0.37^{b}	21
E. coli	12.98±0.22^{a}	13.00±0.20^{b}	15.98±0.11^{b}	16.77±0.22^{b}	22
P. aeruginosa	10.90±0.12^{a}	12.92±0.26^{a}	14.12±0.14^{b}	15.33±0.13^{b}	20

CP: Ciprofloxacin, values having different superscript on the same row are considered significantly different at p<0.05

Table 3: Compounds identified in the methanol extract of the stem bark of *C. siamea* by GC/MS analysis

Peak No.	RT (min)	Compound name	Area (%)	M.F.	m/z
1	11.846	Tetradecanoic acid	0.581	$C_{14}H_{28}O_2$	228
2	12.878	Pentadecanoic acid	0.499	$C_{15}H_{30}O_2$	242
3	13.435	Hexadecanoic acid, methyl ester	8.084	$C_{17}H_{34}O_2$	270
4	13.807	Dibutyl phthalate	0.258	$C_{16}H_{22}O_4$	278
5	14.141	n-Hexadecanoic acid	16.946	$C_{16}H_{32}O_2$	256
6	14.260	1H-2-Benzopyran-1-one, 3,4-dihydro-3,8-dihydroxy-3-methyl	0.194	$C_{10}H_{10}O_4$	194
7	14.881	α-Methyl mannopyranoside	0.903	$C_{14}H_{28}O_6$	163
8	15.094	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	9.407	$C_{19}H_{34}O_2$	294
9	15.168	9-Octadecenoic acid, methyl ester	9.779	$C_{19}H_{36}O_2$	296
10	15.375	Methyl stearate	4.914	$C_{19}H_{38}O_2$	298
11	15.770	9,12-Octadecadienoic acid (Z,Z)-	20.445	$C_{18}H_{32}O_2$	280
12	15.818	cis-13-Octadecenoic acid	4.983	$C_{18}H_{34}O_2$	282
13	15.948	Octadecanoic acid	2.585	$C_{18}H_{36}O_2$	284
14	16.850	Ethanone, 1-[6-hydroxy-2-(1-methylethenyl)-5-benzofuranyl]-	1.432	$C_{13}H_{12}O_3$	216
15	16.922	1'-Acetonaphthone, 2'-hydroxy-4'-methoxy-	0.661	$C_{13}H_{12}O_3$	216
16	16.983	6-tert-Butyl-4-methylcoumarin	1.849	$C_{14}H_{16}O_2$	216
17	17.155	Methyl 18-methylnonadecanoate	0.711	$C_{21}H_{42}O_2$	326
18	17.434	9,10-Anthracenedione, 1,8-dihydroxy-3-methyl-	2.206	$C_{15}H_{10}O_4$	254
19	17.549	Eicosanoic acid	0.967	$C_{20}H_{40}O_2$	312
20	17.880	8H-Imidazo[4,5-E]-2,1,3-benzothiadiazole, 7-methyl-	0.781	$C_8H_6N_4S$	190
21	18.801	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	1.163	$C_{19}H_{38}O_4$	330
22	20.966	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	6.502	$C_{21}H_{40}O_4$	354
23	23.360	DL-alpha-Tocopherol	3.723	$C_{31}H_{52}O_3$	430

M.F.: Molecular formulae, RT: Retention time, m/z: Mass to charge ratio

DISCUSSION

The result of the phytochemical screening as presented in Table 1 showed the presence of alkaloid, flavonoid, glycosides, reducing sugars, saponins, steroids, anthraquinones and tannins. Phenols and terpenoids were however reported to be absent. The bioactive components present in a plant as revealed by the phytochemical screening (Table 1) are responsible for protecting plant against microbial infections as well as infestations by pests[27]. The presence of alkaloid and reducing sugar in this study contradicts the report made by Usman *et al.*[28] and Nas *et al.*[29] respectively. This could be attributed to the fact that geographical distribution of plants, affect both the morphology and expression of phytochemicals[30,31]. The presence of various phytochemicals in *C. siamea* extracts has also been reported by Alli Smith[32], Mohammed *et al.*[33] and Ahmad-Alizaga and Olayanju[34], who all reported that these active phytochemicals are known for their medicinal as well as physiological actions and as such confer the therapeutic potentials of all medicinal plants. Alkaloids, saponins and tannins found in the stem bark have been reported to inhibit bacterial growth as well as offer protection to plants from fungal infections[35]. The trado-medicinal use reported by *C. siamea* in managing bacterial infections among others may be attributed to the presence of these phytochemical constituents.

The results as presented in Table 2 showed that the zones of inhibition recorded by the different isolates vary with the type of bacterial isolates in a dose dependent for the extract. While the best activity was recorded for the gram negative strain of *E. coli* (16.77 ± 0.22), the least activity was recorded for the gram positive strain of *S. aureus* (11.16 ± 0.12) at the highest investigated concentration of 100 mg mL^{-1}. Thou the research showed a broad activity among the different bacterial strains investigated, their activity was statistically significant when compared with the standard antibiotic ciprofloxacin at 20 µg mL^{-1} where it exhibited near 100% inhibition for all seven bacterial strains investigated. This may be due to the mixtures of bioactive compounds present in the extract compared to the pure compound contained in the standard antibiotic used[36].

The result of the GC-MS as revealed by the chromatogram showed that there are 6 major compounds namely 9, 12-Octadecadienoic acid (Z,Z)-(20.445%), n-Hexadecanoic acid (16.946%), 9-Octadecenoic acid methyl ester (9.779%), 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester (9.407%), hexadecanoic acid, methyl ester (8.084%) and 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (6.502%). Most of these compounds are known to exhibit various pharmacological activities as evidenced in many medicinal plants extracted with methanol[37-39]. Hexadecanoic acid is known to exhibit strong antimicrobial and anti-inflammatory activity[37,40]. Although there is a widespread use of vitamin E as a topical medication with claims for improved wound healing and reduced scar tissue, reviews have repeatedly concluded that there is insufficient evidence to support these claims[41-43]. Coumarin's found in the stem bark extract are promising class of bioactive heterocyclic compounds with a wide range of antimicrobial activities[44]. Thou some of the prominent compounds are yet to be described in detail, more research efforts are required to isolate, characterize and evaluate these compounds from *C. siamea* stem bark to validate their pharmacological importance.

CONCLUSION

It is evident from the results of the antibacterial assay that the extract from *Cassia siamea* stem bark demonstrated fairly well to excellent activity against the seven bacterial pathogens investigated. Their activities however fall far short from that of the standard drug ciprofloxacin employed. The antibacterial activities were confirmed by not only the presence of phytoconstituents like alkaloid, saponin, tannin, flavonoids and anthraquinones but also by the presence of hexadecanoic and coumarin which have established antimicrobial activities. Conclusively, this research provides justification for the therapeutic potentials of *C. siamea* stem bark especially its antibacterial activity as claimed in by traditional medicine practitioners.

SIGNIFICANCE STATEMENT

It scientifically justified the therapeutic potentials of *C. siamea* stem bark especially its antibacterial activity as claimed in by traditional medicine practitioners in Nigeria. This study will help the researcher to uncover the critical areas of phytoconstituents investigation in stem bark of *Cassis siamea* which has not to my knowledge being explored by many researchers. Thus a new theory on the correlation of its antimicrobial activity to the actual phytoconstituents may be arrived at.

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