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PHYTOCHEMICALS AND BIOLOGICAL ACTIVITIES FROM THE CONSTITUENTS OF *CASSIA ANGOLENSIS* GROWING IN TANZANIA

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ABSTRACT

This study was carried out to isolate and characterize constituents from the leaves, stem and roots ethanolic extracts of the *Cassia angolensis* and evaluate the antibacterial and antimalarial activities. Therefore, compounds of flavan-3-ol nature including epiafzelechin (**1**) as well as epicatechin (**2**) were isolated and characterized. Biological activities of isolated compounds were not evaluated due to small amount of sample obtained during isolation. The ethanolic root extract showed the maximum zone of inhibition of 11 mm with an activity index (AI) of 0.45 and Minimum Inhibitory Concentration (MIC) of 0.3 mg/mL against *Staphylococcus aureus* (ATCC 25923). Furthermore, the ethanolic leaves extract showed activity at MIC 0.6 mg/mL against *S. aureus* and 1.25 mg/mL against *Klebsiella pneumoniae* (ATCC 708903), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). The stem ethanolic extract was the least active compared to the rest, showing an activity of 2.5 mg/mL against *E. coli, S. aureus* and *K. pneumonia* and 5.0 mg/mL against *P. aureginosa*. On the other hand, antimalarial tests showed notable significant ($P < 0.05$) parasitaemia (*P. berghei*) suppression at 92.9% and 93.5%, for root and leaves extracts, respectively at 300 mg/kg body weight dosage. The results reported herein, correlates with the local use of the plant for treatment of malaria and other ailments, therefore, revealing the potentiality of the plant in the treatment of both malarial and bacterial infections.

Keywords: Cassia, *Cassia angolensis*, antiplasmodial, antibacterial, *Staphylococcus aureus*

Introduction

Since prehistoric times, the dependence of humans on plants for food, shelter, cosmetics, medicines and others have been reported (Hardy, 2019; 2021) . The use of plants for medicinal purposes has become a familiar practice in various traditional medicine systems (Harshal & D'mello 2011). According to WHO, about 80% of people in developing countries depend on traditional medicineas their source of primary health care (Harshal & D'mello 2011). Worldwide, it is estimated that 80% of people use traditional herbal medicines, and for tens of millions of people, they formulate the first line of defense against diseases (De Luca, *et al* 2012; WHO 2013;, Kor, 2014). Some plants of the genus *Cassia* serve in traditional medicine as well as in nutrition. Various phytochemical studies on these plants have shown the existence of various classes of chemical compounds possessing diverse pharmacological and therapeutic properties (Graz *et al.,* 2011; Alam *et al.,* 2015).

Cassia, a largest genus in Fabaceae family, contains about 500 species (Lodha *et al.,* 2010), some of these are commonly

known for their medicinal properties in various traditional medicine systems (Harshal & D'mello 2011; Shivjeet *et al.,* 2013). The plants of this genus are reported to be used against various skin diseases such as ringworm, eczema, and scabies. Moreover, *Cassia* species have been used against headaches, fever, wounds, scabies, ringworm, jaundice, anorexia, rheumatism, and gastrointestinal problems (Deshpande & Bhalsing 2013; Singh *et al*., 2013). Some plants of the genus have been reported to exhibit anti-inflammatory, antitumor, antiplasmodium, antioxidant, and hypoglycemic activities (Iwalewa *et al*., 1997; Hori *et al*., 2018).

The leaves and seeds of these plants are acrid, laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardiotonic and expectorant (Harshal & D'mello 2011). It is further reported that, these plants are also used for healing leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders (Kamagaté *et al.,* 2014; Singh *et al.,* 2013). A significant antimalarial, anti-inflammatory and hepatoprotective effects have been reported for *Cassia* species (Maitya *et al.,* 1998; Singh *et al.,* 2013). Scientific reports have shown that *C. siamea* is renowned for its potency against malaria infections due to the presence of cassiarins (Kamagaté *et al.,* 2014).

Scientific researches all over the world are gaining speed in evaluating the pharmacological and medicinal properties of plants (Sarker *et al*., 2006). Therefore, different advanced scientific methods have been discovered in these modern days to facilitate the isolation and characterization of pharmacologically active compounds. These methods include the hyphenated methods which may include the combination of separation-separation, separation-identification and identification- identification techniques (Sarker *et al*., 2006). Therefore, including LC-MS, LC-NMR, HPLC-PDA, LC-MS-NMR, GC-MS, allowing fast separation and identification of the natural products (Sarker *et al*., 2006). Other advanced methods in isolation of natural products include hHigh-performance thin-layer chromatography (HPTLC), mMultiflash chromatography (e.g., Biotage®), vacuum liquid chromatography (VLC), cChromatotron, solid-phase extraction (e.g., Sep-Pak®), dDroplet countercurrent chromatography (DCCC), hHigh-performance liquid chromatography (HPLC) (Sarker *et al*., 2006). Therefore, various types of flavonoids, anthraquinones, alkaloids, phenylpropanoids, xanthones, chromones, stilbenes, γ-naphthopyrones, and pentacyclic triterpenes have been reported from the genus Cassia, some of which have displayed diversity of pharmacological activities (Agrawal *et al.,* 2012; Kumar *et al.,* 2013; Vijayalakshmi *et al.,* 2016). Being motivated by this fact, this research was designed to evaluate biological activities of *C. angolensis*. Therefore, this paper reports for the first time the isolation, and evaluation for antibacterial and antiplasmodial activities of constituents from *C. angolensis*.

Experimental

Plant material collection, preparation and extraction

Plant materials (Figure 1) were collected from Amani Nature Reserve forest in Muheza district, Tanga region, Tanzania. The collected plant material was identified by the taxonomist, Mr. F. M. Mbago, and Voucher specimen FMM 3816 was deposited in the Herbarium, Department of Botany, College of Natural and Applied Sciences, University of Dar es Salam. After collection, the plant materials including

roots, stems, and leaves were washed using tap water to remove dust and dirt and allowed to air-dry at room temperature. The air-dried roots, stems, and leaves were separately crushed and milled into fine powder. The extracts were prepared by maceration, where, powdered plant material of 900 g each were macerated with 95% ethanol (2.5 L) for 72 h at room temperature and then filtered. Maceration was repeated three times, and the filtrates were collected and then evaporated to dryness using a vacuum rotary evaporator at 40°C. The dried extracts (40g stem, 38g roots, and 43g leaves) were stored in a closed container, in the refrigerator ready for analysis.

(a) **(b)** **(c)**

Fig. 1: *C. angolensis*: Leaves (a); stem (b); and crushed roots (c).

Spectral analysis

NMR spectra were acquired on a Bruker Avance Neo 500 MHz spectrometer equipped with a TCI cryogenic probe, at Sternhagen Analys. Lab AB, Gothenburg, Sweden. Characterization of the isolated compounds was based on 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC, HMBC) NMR spectra.

Isolation of epicatechin and epiafzelechin from roots and stems ethanolic extracts

The crude stem extract (31 g) was adsorbed onto 35 g of silica gel, and loaded on a silica gel (230−400 mesh) column. The increasing polarity solvent using increasing EtOAc (0−100%) in hexanes mixture was used, in gradient elution, and 50 fractions were obtained. The first 13 fractions contained inseparable fatty acids, while, fraction 35-50 contained highly polar mixtures, thus, could not be purified further. Therefore, fractions 14-27, and 28-34 were involved in further separation. Combined fraction 14-27 were subjected to silica gel filtration using 1:1 EtOAc:Hexanes to obtain 40 fractions; 1-21, 22-34 and 35-40 according to similarities observed on TLC plates. Thus, fractions 22-34 were subjected to sephadex® LH-20 gel chromatography eluted at 100% methanol to obtain 13 mg of epiafzelechin (**1**). Combined fractions 35-40 were also subjected to sephadex® LH-20 gel chromatography eluted at 100% methanol to obtain 18 mg of epicatechin (**2**). Combined fractions 28-34 were run in sephadex® LH-20 silica gel chromatography eluting at 100% methanol, to obtain 7 mg of compound **2**.

40g crude roots ethanolic extract were adsorbed in 45 g of silica gel, and loaded on a silica gel column. Thus, subjected to gradient column chromatography, eluted with increasing EtOAc (0−100%) in hexanes mixtures, to obtain 20 fractions. Fraction 1-7 contained inseparable mixtures, while 16-20 contained polar compounds that did not move on TLC under 100% EtOAc. Fractions 8-15 were combined and subjected to silica gel column chromatography, to give 10 fractions, basing on their similarity on TLC, two major fractions (1-5 and 6-10) were obtained. Fractions 1-5 were subjected to sephadex® LH-20 gel chromatography eluted at 100% methanol to obtain 9 mg of epiafzelechin (**1**) and 14 mg of epicatechin (**2**).

Epiafzelechin (**1**): White solids; yield 22 mg; melting point 234 - 236 ºC; *p*-anisaldehyde stain– yellow to brown; ^{1}H and ^{13}C NMR, see Table 1.

Epicatechin (**2**): Yellow solids; yield 39 mg; melting point 242 - 245 ºC; *p*-anisaldehyde stain– yellow to orange ^{1}H and ^{13}C NMR, see Table 2.

Animal stock and Plasmodium parasite strain for antiplasmodial activity evaluation

The Swiss albino mice of 6-9 weeks with weight range of 21 - 25 g were used in these investigations. The mice were raised at Muhimbili University of Health and Allied Science (MUHAS) at the Institute of Traditional Medicine (ITM) and were acclimatized to the new laboratory of National Institute for Medical Research for a week. Acclimatization included standard feeding of the mice by supplying food and water (*ad libitum*) before being used for the test according to the national and international guidelines for handling of laboratory animals (Institute of Laboratory Animal Resources (US) 1986).

Parasite used for these investigations were chloroquine sensitive *P. berghei* NK 65 strain, obtained from MUHAS at the ITM.

Innoculation of parasites and administration of extracts

The determination of *in vivo* antiplasmodial activity of the ethanol extracts of the roots, stem and leaves of *C. angolensis* was determined at the National Institute of Medical Research (NIMR). This followed 4-day suppressive test protocol according to Tona *et al.,* (2001). On the fifth day, tail blood from each mouse was drawn, and thin blood films were made, and stained with Leishman's stain to reveal parasitized erythrocytes. Therefore, the number of parasitized erythrocytes out of 500 erythrocytes were counted in random fields of microscope, according to Ettebong *et al.,* (2012).

Parasitaemia determination

Thin smears were prepared from tail blood on the 5th day of the experiment and were put on microscopic slides. The examination of parasitaemia under microscope was done after fixing the blood films on the microscopic slides using methanol and was stained with Giemsa. The percentage parasitaemia was calculated using the formula (EQ. 1) described by Okokon *et al.,* (2008).

$$\%\ \text{Parasitaemia} = \frac{\text{Number of infected RBCs}}{\text{Total number of RBCs examined}} \times 100\% \qquad 1$$

The calculation of percentage suppression of parasitaemia was done by comparing the parasitaemia of the negative control with those which received different dose concentrations of the test *C. angolensis* ethanol extracts (EQ. 2).

$$\%\ \text{Suppression} = \frac{A - B}{A} \times 100\% \qquad 2$$

Where:
A = Parasitaemia in negative control
B = Parasitaemia in test group

The one way analysis of variance (ANOVA) and students' *t*-test were used to determine the significance of the mean difference between the experimental and the control group. In this study a p value of < 0.05 was considered significant.

The antiplasmodial activity efficacy of *C. angolensis* extracts was evaluated by using chloroquine sensitive *P. berghei* NK 65 strain. The percentage of parasitaemia suppression in the *P. berghei* infected mice was determined through the use of blood smears from the vein of the tail on day 5 post-infection. The obtained data were analyzed at

95% confidence level and the probability (*p*) value $p < 0.05$ was considered significant. The antiplasmodial activity based on the two references given is considered as very good when the percentage of parasitaemia reduction was equal or greater than 50% at the dose of 100 mg/Kg body weight and good when it was equal or greater than 50 % at doses of 200 - 300 mg/Kg body weight (Munoz *et al.,* 2000).

Antibacterial Assays

Reagents

Dimethyl sulfoxide (DMSO) was purchased from Sigma® (Poole, Dorset, UK), Muller Hinton agar and broth, Sabouraud Dextrose agar and broth from HIMEDIA® (Himedia Laboratories Pvt Ltd, Mumbai, INDIA), *p*-Iodonitrotetrazolium chloride was bought from SIGMA- ALDRICH®, St Louis, USA, Microtitre plates were supplied by KAS medics Tanzania.

Microorganisms

Authentic pure cultures of human pathogenic bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 708903), and *Staphylococcus aureus* (ATCC 25923) were obtained from the Department of Microbiology and Immunology, MUHAS. The pure strains of these species were sub cultured in a Sabouraud dextrose agar and incubated at 35 °C prior to testing.

Preparation of test extracts and control antibiotic susceptibility disks

All the extracts of *C. angolensis* (root, stem and leaves) were tested for their antibacterial activity against the test microorganisms through disk diffusion method protocol according to Balouiri *et al.,* (2016). Therefore, overnight broth cultures were adjusted using a turbidometer to yield approximately 1.0 X 10^8 cfu per ml. Extract-impregnated discs (20 µl) were placed on agar plates and were incubated at 37°C for 24 hours. Pure DMSO (20 µl) was used as a negative control, while gentamycin discs (30 µl) were used as a positive control. Antibacterial activities were then determined by measuring the clear zones of inhibition.

The inhibition zones (IZ) were determined after incubation for 24 hours at 37 ^{O}C as following the procedure described by Cheesbrough, (2006). The activity index (AI) of the test samples was calculated as the ratio of inhibition zone of the test sample to inhibition zone of the standard drug, represented by the following formula (EQ. 3).

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of the test sample (IZTS)}}{\text{Inhibitizone of the standard drug (IZSD)}} \qquad \text{(EQ. 3)}$$

Where:

IZTS is the diameter of the inhibition zone of the test sample at a given concentration.

IZSD is the diameter of the zone of inhibition of a standard drug at a known concentration.

Determinations of Minimum Inhibitory Concentration (MIC)

The MIC was determined by two-fold microdilution tests performed in a sterile flat bottom 96-well polystyrene microtiter plates. The extracts were tested against standard and clinical isolates of bacteria including: *E. coli, P. aureginosa, K. pneumoniae,* and *S. aureus.* The microorganism's *innocula* were prepared from 24 hours grown cultures. The serial dilution method was performed as follows: 50 µL of broth (Sabouraud's dextrose broth) were pipetted into each well of microtiter plates. To each row of the well, 50 µL of the extract solution, fluconazole and solvent were added separately. Each of the test materials were

tested in duplicate. After thorough mixing, 50 µL of the mixture was drawn and transferred to the second well in the row and after mixing well again 50 µL of the mixture was drawn and transferred to the third well in the row. This procedure was repeated until the last well in the last row. Then 50 µL of the mixture was discarded from each last well of the row. One additional row was used as growth control, in which no drug was added instead a blank culture medium was added. The inoculated microtiter plates were incubated at 37 °C for 24 hours. MIC values were determined using tetrazonium salt indicator, iodonitrotetrazolium chloride (INT) which changes color from pink to yellow by viable microbes. In the MIC assay plate, a volume of 20 µL of a 0.2% INT was pipetted into each well and incubated at 37 °C for 2 hours. The lowest concentration at which there was no growth observed was taken as MIC.

Results

Compounds isolated

Ethanolic extracts of both stem and roots yielded two flavan-ol compounds namely, epiafzelechin (**1**) and epicatechin (**2**) as shown in **Figure 2**.

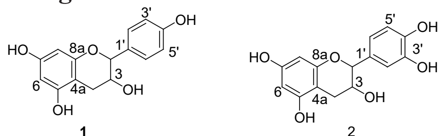


Fig. 2: Structures of the compounds isolated from *C. angolensis* stem and roots ethanolic extracts.

The ^{1}H (**Figure 1**, supporting information) and ^{1}H-^{1}H COSY NMR spectra (**Figure 2**, supporting information) for compound **1** showed an ABX coupling pattern at δ_H 4.22 (td, 4.4, 3.7, 2.0 Hz, H-3), 2.91 (dd, 16.8, 4.7 Hz, H-4eq) and 2.78 (dd, 16.9, 2.9 Hz, H-4ax) of ring C protons in the flavan-3-ol moiety (Hosny & Rosazza 2002, Hori *et al*. 2018). The presence of two aromatic rings were shown by the presence of four *ortho*-coupled protons resonating at 7.35 (d, J = 8.5 Hz, 2H) and 6.82 (d, J = 8.6 Hz, 2H) suggested the presence of an AA BB splitting pattern of *p*-substituted aromatic ring referred to ring B of the flavan-3-ol moiety (Hori *et al*. 2018). The two *meta* coupled protons at 5.96 (d, J = 2.2 Hz, 1H, H-6) and 5.98 (d, J = 2.3 Hz, 1H, H-8) proposed the *meta*-unsubstituted aromatic ring A.

Analysis of the ^{13}C NMR (**Figure 3**, supporting information) and HSQC spectra (**Figure 4**, supporting information) showed six quaternary carbons at δ_C 158.2, 158.1, 157.9, 157.6, 131.8, and 100.2. The connectivity of the A, C rings to B ring of the flavonoid system was shown in HMBC spectrum (**Figure 5**, supporting information). Therefore, the correlation of H-2 at δ_H 4.91 to carbons at δ_C 131.8 and 129.3 (C-1', and C-2' respectively), revealed the connectivity of ring C to ring B of the flavonoid system. The H/H and H/C correlations in the COSY and HMBC, HSQC respectively, were all consistent with the proposed structure. The spectral data (**Table 1**) for compound **1** were consistent to those previously reported for epiafzelechin which was isolated from the root barks of *C. sieberiana* (Kpegba *et al*., 2010) and leaves extracts of *C. alata* (Duong *et al*., 2017) thus, confirming compound **1** to be epiafzelechin.

TABLE 1

*^{1}H, ^{13}C NMR Spectral Data and HMBC Interaction for Epiafzelechin (**1**) 500MHz, (MeOD)*

C/H δ_H	***J*** **(Hz)**	δ_C	**HMBC ($^1H \rightarrow {}^{13}C$)** **Observed**	**Reported**	
2	4.91	s	80.1	78.0	1’, 2', 4
3	4.84	td, 4.4, 3.7, 2.0	67.6	64.8	4
4ax	2.91	dd, 16.8, 4.7	29.5	28.2	3
4eq	2.78	dd, 16.9, 2.9			2, 3
4a	-	-	100.2	98.4	-
5	-	-	157.9	156.2	-
6	5.96	d, 2.2	96.1	94.1	8
7	-	-	158.2	156.5	-
8	5.98	d, 2.3	96.6	95.1	6
8a	-	-	158.1	156.5	-
1'	-	-	131.8	130.0	-
2', 6'	7.35	d, 8.5	129.3	128.2	2', 6', 2
3', 5’	6.82	d, 8.6	115.9	114.4	3', 5'
4'	-	-	157.6	155.7	-

The ^{1}H NMR and COSY spectra (**Table 2**, **Figure 6** and **7**, Supporting Information) of **2** suggested that the compound has two distinct aromatic systems. Ring A contained meta coupled protons resonating at δ_H 5.96 (d, *J* = 2.4 Hz, 1H, H-8) and 5.93 (d, *J* = 2.3 Hz, 1H, H-6). Ring B contained an ABX spin system resonating at δ_H 6.98 (d, *J* = 2.0 Hz, 1H, H-2'), 6.82 (dd, *J* = 8.2, 2.1 Hz, 1H, H-6') and 6.78 (d, *J* = 8.2 Hz, 1H, H-5'). Ring C was revealed by the presence of an ABMX coupling pattern at δ_H 4.86 – 4.82 (m, H-2), 4.20 (ddd, *J* = 4.7, 3.0, 1.5 Hz, H-3), 2.91 (dd, *J* = 16.7, 4.7 Hz, H-4eq), 2.78 (dd, *J* = 16.7, 3.1 Hz, H-4ax), typical of a flavan-3-ol moiety. (Hosny &Rosazza 2002, Hori *et al* 2018).

The analysis of ^{13}C NMR spectrum (**Table 2**, **Figure 8**, supporting Information) showed the presence of 15 carbon atoms. Detailed analysis of the spectrum in conjunction with HSQC spectrum (**Figure 9**, supporting information) revealed the presence of 7 quaternary aromatic carbons. Among these carbon signals, 5 were oxygenated aromatic at δ_C 158.0, 157.1, 157.6, 146.0, 145.8 while, signals at δ_C 132.3 and 100.1 were for non-oxygenated quaternary aromatic carbons. Five methine aromatic carbons at δ_C 115.9, 95.9, 96.4, 115.3, and 119.4 were also observed.

The connectivity of ring C (ABMX system) to ring B (aromatic ABX ring system) was confirmed by the long-range correlation of H-2 at δ_H 4.92 to C-1' at δ_C 132.3 as it is shown in HMBC spectrum (**Figure 10**, supporting Information). The rest of the correlations are as summarized in **Table 2**.

The spectral data for compound **2** were comparable to those previously reported for epicatechin as it is shown in **Table 2**, which was isolated from the root barks of *C. sieberiana* (Kpegba *et al*. 2010), thus, confirming compound **2** to be epicatechin.

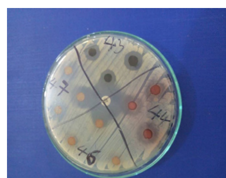
TABLE 2

*^1H, ^{13}C NMR Spectral Data and HMBC Interaction for Epicatechin (**2**) 500MHz, (MeOD)*

C/H	δ_H	J (Hz)	δ_C Observed	δ_C Reported	HMBC ($^1H \rightarrow {}^{13}C$)
2	4.92	s	79.8	79.8	1', 2', 4
3	4.20	ddd, 4.7, 3.0, 1.5	67.4	67.4	4
4ax	2.91	dd, 16.8, 4.6	29.2	29.2	2, 3
4eq	2.81	dd, 16.8, 2.3			5, 8a
4a	-	-	100.1	100.8	-
5	-	-	157.1	157.3	-
6	5.93	d, 2.3	95.9	5.90	-
7	-	-	158.0	158.0	-
8	5.96	d, 2.4	96.4	96.3	6, 8a
8a	-	-	157.6	157.3	-
1'	-	-	132.3	132.3	-
2'	6.99	d, 2.0	115.3	115.3	2, 6'
3'	-	-	146.0	145.9	-
4'	-	-	145.8	145.7	5', 2', 6'
5'	6.78	d, 8.2	115.9	115.8	-
6'	6.82	dd, 8.2, 2.1	119.4	119.3	2', 2

Antibacterial Activity

The antibacterial activity of the CARE, CASE and CALE was carried out against *E. coli, P. aureginosa, K. pneumoniae* and *S. aureus* test organisms. The results revealed a notable activity on CALE and CASE against *S. aureus*, however, CARE showed no significant activity against the tested organisms (Figure 3).



Key:
43: CALE
44: CARE
46: CASE

Fig. 3: Antibacterial activity of CARE, CASE and CALE against *Staphylococcus aureus* The zones of inhibition of the tested *C. angolensis* extracts in the disk diffusion method are indicated in Table 3.

TABLE 3

Zones of inhibition and activity indices for antibacterial activity of C. angolensis extract against the test organisms

Extracts/ Standards	Test organisms							
	E. coli		*P. aureginosa*		*K. pneumonia*		*S. aureus*	
	ZI	AI	ZI	AI	ZI	AI	ZI	AI
CARE	5	0.29	5	0.20	5	0.29	11	0.45
CALE	5	0.29	5	0.20	4	0.20	9	0.38
CASE	4	0.23	5	0.20	4	0.20	5	0.20
DMSO	ND	ND	ND	ND	ND	ND	ND	ND
Gentamicin	17	1	24	1	20	1	24	1

Key: ZI: Zone of inhibition (mm); AI: Activity Index; DMSO: Dimethyl Sulfoxide; ND: Not detected. CARE: *Cassia angolensis* root ethanolic extract; CASE: *Cassia angolensis* stem ethanolic extract; CALE: *Cassia angolensis* leaves ethanolic extract.

The antibacterial activity of CARE and CALE against *S. aureaus* implying that they were moderately active against Gram-positive representative bacteria. On the other hand, the minimum inhibitory concentration (MIC) of the crude extracts from *C. angolensis* against *E. coli, P. aeruginosa, K. pneumonia* and *S. aureus* are shown in Table 4.

TABLE 4

MIC for antibacterial activity of crude extracts from root, stem and leaves of C. angolensis

Test sample	The minimum inhibitory concentration, MIC (mg/mL) Test Organisms			
	P. aureginosa	*E. coli*	*S. aureus*	*K. pneumonia*
CARE	2.5	2.5	0.3	1.2
CASE	5.0	2.5	2.5	2.5
CALE	5.0	2.5	0.6	2.5
Gentamicin (Standard)	2.0×10^{-4}	2.0×10^{-4}	2.0×10^{-4}	2.0×10^{-4}

It is shown that both CARE and CALE exhibited significant antibacterial activity against *S. aureus* with MIC = 0.3 mg/mL and 0.6 mg/mL, respectively. Also, the lowest MIC value of 5.0 mg/mL against *P. aeruginosa* was recorded in CASE and CALE. In addition, high activity was observed for CARE against *K. pneumonia* with an MIC value of 1.2 mg/mL. Overall, CASE exhibited lower antibacterial activity against both *E. coli* and *K. pneumonia* as compared to CALE and CARE. The observed antibacterial activity suggest that the roots extracts could be a good source of antibacterial agents.

Discussion

The compounds isolated from stem and roots of *C. angolesis* ethanolic extracts indicated the plant to contain flavonoid compounds, especially, flavan-3-ol, epiafzelechin (**1**) and epicatechin (**2**). Although the two compounds have not been tested for their biological activities in this study due to inadequate amounts isolated, but, they have been reported to exhibit strong antioxidant activities that plays a crucial role in inflammation and cancer prevention. Therefore, scientific reports have shown that free radical scavenging and extracellular matrix degradation retardation induced by ultraviolet radiation are among health benefits related to using plants rich in catechin type compounds. They have also shown ability to activate collagen synthesis and inhibit production of matrix metalloproteinase enzymes (Kamagaté *et al.,* 2014).

The ethanol extracts of roots and leaves of *C. angolensis* have shown high antiplasmodial activity in mice infected by *P. berghei in vivo*. The observed antiplasmodial activity may be attributed by the presence of either single individual active ingredient or the combination of a group of active compounds (synergism) in the crude extract (Okokon *et al.,* 2008). However, before using leaves and roots extracts for malaria control, further studies to establish their toxicological profile and proper dosage are recommended.

On the other hand, *C. siamea*, a plant belonging to the same genus as *C. angolensis* has been reported to exhibit antiplasmodial activities *in vitro* (Jun *et al.,* 2012). Similarly, previous studies indicated leaves extracts to

exhibit stronger antiplasmodial activities than the roots and stem (Hussian, 1991). Therefore, the expressed antiplasmodial activity of leaves of *C. angolensis* corroborate well with these reports. Although cassiarin A (a potent antimalarial compound in *C. siamea* leaves) was not isolated in this study, it is speculated that the observed antiplasmodial activities of the leaves ethanolic extract might be due to the presence of this compound. This is because of chemotaxonomical resemblances, where plants within the same genus are expected to yield compounds of the same kind with little variations (Wiwied *et al.,* 2009).

The expressed antibacterial activity by *C. angolensis* crude ethanolic extracts is supported by other studies previously reported on *Cassia* species. The ethanolic extracts of flowers of *C. fistula* have been reported to exhibit antibacterial activity against *E. coli* and *K. pneumonia* with MIC of 10 and 5 mg/mL, respectively (Eyyednejad *et al.,* 2014). An inhibition range of 12 - 20 mm was observed in methanol and chloroform leaves extracts of *C. auriculata* against *S. aureus* and *E. coli*, *K. pneumonia* (Murugan *et al.,* 2013). Likewise, methanolic leaves extracts of *C. occedentalis* exhibited an activity with MIC value of 25 mg/mL when tested against *A. niger* and *C. albicans* (Murugan *et al.,* 2013).

Conclusions

The ethanolic extracts of roots and leaves of *C. angolensis* at a dose of 300 g/Kg body weight significantly provided a promising activity against *P. berghei* with effective dose (ED_{50}) 74.3 mg/kg body weight. The evaluation of antibacterial activity of CALE also showed MIC value of 0.3 mg/mL against *S. aureus* and MIC values ranging from 1.2 - 2.5 mg/mL were also observed against *K. pneumonia, P. aureginosa,* and *E. coli*. These results imply that CALE and CARE possess antiplasmodial and antibacterial activity. This corroborate to the traditional use of the plant as a source of antimalarial and antibacterial agents. It is therefore worth noting that, the expressed antibacterial activities could have been accelerated or retarded by the synergism effects, since pure samples were not tested in this study.

Conflic of Interest

The authors declare no conflict of interest, financial or otherwise.

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