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EVALUATION OF ANTI-INFLAMMATORY, ANTIBACTERIAL AND CYTOTOXIC ACTIVITIES OF *Cordia africana* LEAF AND STEM BARK EXTRACTS

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ABSTRACT

Cordia africana (Boraginaceae) is a tree used in traditional medicine to treat inflammation related conditions and infectious diseases. This study was undertaken with the objectives of establishing the scavenging effect of extracts and fractions of Codia africana on the mediator of inflammation Lipoxygenases (LOX), and some non-biological free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), the [2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) radicals and the Ferric ion reducing antioxidant power (FRAP). Antimicrobial activities, total phenolics/flavonoids and cytotoxicity of extracts of Codia africana were also evaluated. Extracts were obtained by maceration. Anti-inflammatory activity was determined using a LOX-inhibitor screening assay kit according to the manufacturer's instructions. A broth serial micro dilution method was used to determine the minimum inhibitory concentration (MIC) against, Gram-positive and Gram-negative bacteria and Mycobacterium species. The antioxidant activity was determined using free-radical-scavenging assays, and the 3-(4,5-dimethylthiazolyl-2)-2,5- diphenyltetrazolium bromide reduction assay was used for cytotoxicity. Both the extracts of C. africana inhibited LOX enzyme. The most active being the methanol extract of the bark with IC_{50} value of 55 ± 0.9 µg/ml. Both the extracts of C. africana, had excellent to weak antimicrobial activites (MICs ranging from 32 to 1024 µg/ml) against bacteria. All the extracts had significant ($P< 0.05$) free-radical scavenging activity (IC_{50} ranging from 6.79 ± 0.07 to 331.98 ± 0.07 µg/ml). There was a positive correlation between the antioxidant activity and the total flavonoid and total phenolic contents of Cordia africana. The cytotoxicity on Vero cells was low with LC_{50} of 81.79 ± 13.31 and 99.67 ± 16.10 µg/ml. The results support the use of C. africana leaves in traditional medicine to treat inflammation related conditions and infectious diseases.

Keywords: Cordia Africana, Inflammation, antibacterial, antioxidant, total flavonoid.

INTRODUCTION

Cordia africana is a small to medium-sized evergreen tree, 4-15 (30) m high, heavily branched with a spreading, umbrella-shaped or rounded crown. Bole typically curved or crooked. It is widely distributed in eastern and southern Africa. In West Africa, this species is restricted to montane and submontane habitats (Schmidt and Mwaura, 2010). The pharmacological studies carried out with extracts and purified compounds indicates that the plants of *Cordia* species possess analgesic, anti-inflammatory, antimicrobial, antiviral and antifertility activities. Various compounds like flavonoids, triterpenes, tannins, alkaloids and fatty acids possessing wide range of bioactivities were isolated from different plant parts of *Cordia* species (Thirupathi *et al.*, 2008). Based on these reports it is clearly indicated that the plants of *Cordia* genus possess potential therapeutic actions. *Cordia africana* is used traditionally to treat stomach ache, toothache, wound and cough (Reta, 2013).

It is well known that reactive oxygen species (ROS), such as superoxide anion (O2•-), hydroxyl radicals (OH•), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2), play a major role in the development of oxidative stress that can lead to many illnesses including cardiovascular diseases, diabetes, inflammation, degenerative diseases, cancer, anemia, and ischemia (Cai *et al.*, 2004). Plant based antioxidant compounds play a defensive role by preventing the generation of free radicals and hence are extremely beneficial to alleviate the diseases caused by oxidative stress (Akinmoladun *et al.*, 2010; Özen *et al.*, 2010). Many investigations revealed that phenolics and flavonoids content (Cai *et al.*, 2004; Hendra *et al.*, 2011) contribute to the antioxidant activities of plants. The anti-inflammatory properties of flavonoids have been extensively studied and beneficial effects have been demonstrated in many animal models (Talhouk *et al.*, 2007).

15-Lipoxygenase (15-LOX) belongs to the class of iron containing Lipoxygenases that catalyse the incorporation of dioxygen into unsaturated fatty acid (Feussner and Wasternack, 2002). Lipoxygenases are the key enzymes in the biosynthesis of leukotrienes that play an important role In several inflammatory diseases (Funk, 2006). Inflammation is one of the manifestations of oxidative stress, and the pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins, are all induced by oxidative stress (Mehta *et al*., 2006). Inhibition of LOX may influence the inflammation processes and thus be of interest for modulation of the lipoxygenase pathway. Therefore, inhibitors of oxidative stress and LOX have been considered as therapeutically useful in the treatment of many related diseases (Sommer, 2005). The objectives of this work therefore is to establish the scavenging effect of extracts and fractions of *Codia africana* on the mediator of inflammation lipoxygenases (LOX), and some non-biological free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), the [2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) radicals and the Ferric ion reducing antioxidant power (FRAP). Antimicrobial activities, total phenolics/flavonoids and cytotoxicity of extracts of *Codia africana* were also evaluated.

MATERIALS AND METHODS

Plant material and extraction

Cordia africana was collected in January, 2014 in Bomo village, Zaria, Nigeria. The plant was identified and authenticated by a taxonomist, in the Herbarium section, Department of Biological Sciences, Ahmadu Bello University, Zaria as compared by a voucher specimen No. 900161. The collected plant material was dried at room temperature and ground using MacSalab Model 200 grinder. The powder obtained (100g) was extracted successively with methanol (250ml), and hexane (250ml) using soxhlet extractor. It was then concentrated under reduced pressure using a rotary evaporator to obtain the crude extract. The crude extracts were kept at 4 ^{0}C prior to use.

Chemicals

Sodium carbonate was obtained from Holpro Analytic, South Africa. Gentamicin was purchased from Virbac, South Africa. Fetal calf serum (FCS) and minimum essential medium (MEM with L-glutamine) was provided by Highveld Biological, Johannesburg, South Africa. Phosphate buffered saline (PBS) and trypsin were purchased from White head Scientific, South Africa. Doxorubicin was obtained from Pfizer.Quercetin, 2,20-azino-bis (3-ethylben-zothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphe-nyl-1-picrylhydrazyl (DPPH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), p-iodonitro tetrazolium violet (INT), Folin–Ciocalteu reagent, gallic acid, 2,5,7,8-tetramethylchroman carboxylic acid (Trolox) and potassium persulfate were purchased from Sigma-Aldrich St.Louis, MO, USA. Sodium dodecyl sulfate, potassium ferric cyanide, iron(II) sulphate from Glycine max were provided by Sigma, Germany. Tris (hydroxy- methyl) amino methane was purchased from Sigma, Switzerland. Ferric chloride and linoleic acid were purchased from Merck, Darmstadt and Schuchardt, Germany respectively, Xylenol orange was obtained from Searle Company, England. LOX-inhibitor screening assay kit (Catalog No. ab133087) was obtained from abcam, UK.

Lipoxygenase inhibition assay

The anti-inflammatory activities of extract from the bark of *Cordia africana* were evaluated for LOX inhibitory activity using a LOX-inhibitor screening assay kit (Catalog No. ab133087, abcam, UK) according to the manufacturer's instructions. This assay detects and measures the hydroperoxides produced in the lipoxygenation reaction using a purified Lipoxygenases. Briefly, the stock solution of the extract was dissolved in methanol and serially diluted two-fold to concentration ranges between 5 to 0.08 mg mg/mL for both extracts and the standard reference (aspirin) and then introduced in a 96 well microtitre plate. The wells include the blank, 15-LOX standard, 100 % initial activity and the inhibitor wells. One hundred microliter of assay buffer, 90 µL of 15-LOX enzyme and 10 µL of Assay Buffer, 90 µL of LOX enzyme and 10 µL solvent and 90 µL of LOX enzyme and 10 µL of extract were added in the wells respectively. The reaction was initiated by addition of 10 µL of substrate (arachidonic acid) to all the wells and incubated at room temperature for 5 min on a shaker. After the 5 min incubation, 10 µL of chromogen was added to each well to stop enzyme catalysis and developed the reaction. The microtiter plate was covered and further incubated at room temperature for 5 min on a shaker. The cover was removed and the absorbance was immediately read at the wavelength of 500 nm using a micro plate reader. Percentage inhibition was calculated using the following equation.

Percentage Inhibition =

$$\left\{\frac{1A - Inhibitor}{IA}\right\} \times 100\ \%$$

Where IA is initial activity

The concentration at which there was 50 % enzyme inhibition (IC_{50}) was determined by graphing the percent inhibition or percent initial activity against the extract concentration.

ANTIOXIDANT ACTIVITY

[2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical assay

The ABTS radical scavenging capacity of the samples was measured with modifications of the 96-well microtitreplate method described by Re et al.(1999). Trolox and ascorbic acid were used as positive controls methanol as negative control and extract without ABTS as blank.

The percentage of ABTS•+ inhibition was calculated using the formula: Scavenging capacity (%) = Radical scavenging capacity

$$= 100 - \left(\frac{Absample - Abblank}{Abcontrol}\right) \times 100$$

Where Ab_{sample} is the absorbance of the extract with DPPH, Ab_{blank} is the absorbance of the extract without DPPH and $Ab_{control}$ is absorbance of methanol and DPPH. The IC_{50} values were calculated from the graph plotted as inhibition percentage against the concentration.

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH radical-scavenging activity was determined using the method proposed by Brand-Williams *et al*. (1995). Ascorbic acid and Trolox were used as positive controls, methanol as negative control and extract without DPPH as blank. Results were expressed as percentage reduction of the initial DPPH absorption in relation to the control. The concentration of extract that reduced the DPPH color by 50% (IC_{50}) was determined as for ABTS •+.

Ferric reducing antioxidant power (FRAP) assay

The FRAP of samples was determined by direct reduction of potassium ferricyanide ($K_3Fe(CN)_6$) to potassium ferrocyanide ($K_4Fe(CN)_6$) (electron transfer process from the antioxidant). The increase in absorbance from the formation of Pearl's Prussian blue complex following the addition of excess ferricion was measured as described by Berker *et al*. (2007) with some modification. The reaction medium (210 mL) containing 40 mL of the test samples or positive controls (Trolox and ascorbic acid; concentration range between: 15.62 and 2000 mg/mL) and 100 mL of 1.0 M hydrochloric acid; 20 mL of 1% (w/v) of SDS; 30 mL of 1% (w/v) of potassium ferricyanide, was incubated for 20 min at 50 ^{0}C, then cooled to room temperature. Finally, 20 mL of 0.1% (w/v) of ferric chloride was added. The absorbance at 750 nm was read and reagent blank absorbance was taken by preparing the reaction medium the same way without the addition of ferric chloride. The Trolox Equivalent Antioxidant capacity (TEAC) was calculated by dividing the slope of each sample (slope obtained from the line of best fit of the absorbance against concentration using the linear regression curve) by that of trolox.

Total phenolic content (TPC) determination

The total phenolic content of extracts was determined colorimetrically using a 96-well microplate Folin–Ciocalteu assay developed by Zhang *et al*. (2006). The total phenolic content was calculated from the lineare quation of a standard curve prepared with galli cacid, and expressed as gallic acid equivalent (GAE) per g of extract.

Total flavonoids content (TFC) determination

Total flavonoid content was determined using the method of Ordonez *et al*. (2006). Avolume of 0.5mL of 2% $AlCl_3$ ethanol solution was added to 0.5mL of sample solution (1mg/mL). After one hour at room temperature, the absorbance was measured at 420 nm. Ayellow color is indicative of the presence of flavonoids. Total flavonoid content was calculated and expressed as mg quarcetin equivalent/g of crude extract using a standard curve prepared with quercetin.

ANTIMYCOBACTERIAL ACTIVITY ASSAY

Microbial culture

Mycobacterium smegmatis (ATCC1441), *Mycobacterium aurum* (NCTC 10437) and Mycobacterium fortuitum (ATCC6841) were cultured as described by McGaw *et al* (2008). They were maintained on Middle brook 7H10 agar slants, supplemented with glycerol or tween 20. Inocula suspensions were prepared by mixing a few microbial colonies with sterile distilled water. The suspension was diluted with sterile water to render a concentration of cells equal to standard Mc Farland1 standard solution (approximately 4 × 10^7 cfu/mL), and then diluted with freshly prepared Middle brook 7H9 broth supplemented with 10% oleic albumin dextrose catalase (OADC) to obtain a final inoculum density of approximately 4 × 10^5 cfu/mL.

Four Gram-positive bacteria, *Bacillius* cereus (ATCC 14579), *Staphylococcus aureus* (ATCC 29213) and Enterococcus faecalis (ATCC 29212), two Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 25922) *Salmonella typhimurium* (ATCC 700720). Bacterial culture was taken from 24 h fresh agar culture plates and inoculated in fresh Mueller-Hinton broth (MHB) (Fluka, Switzerland), prior to conducting the assay. The turbidity of the microbial suspension was adjusted to a McFarland standard 0.5 equivalent to concentrations of 1–5 × 10^8 cfu/ml. The microbial suspensions were further diluted (1:100) in media to obtain a final inoculum of approximately 1.5 × 10^6 cfu/ml.

Determination of minimum inhibitory and bactericidal concentration (MIC)

The broth microdilution technique using 96-well micro-plates, as described by Eloff (1998) was used to obtain the MIC and MBC values of *Cordia africana* samples. Extracts (100 mL) at an initial concentration of 10 mg/mL were serially diluted, two-fold in 96-well microtitre plates, with equal volumes of Middle brook 7H9 broth. Then, 100 mL of inocula were added to each well to give a final concentration range of 2.5–0.019 mg/mL. The plates were incubated overnight for Mycobacterium smegmatis, *Bacillius* cereus, *Staphylococcus aureus*, Enterococcus faecalis, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and 3 days for Mycobacterium aurum and Mycobacterium fortuitum at 37 ^{0}C. To indicate bacterial growth, 40 mL of 0.2 mg/mL INT was added to each well after incubation and the plates incubated further at 37 ^{0}C for 1h. The MIC was defined as the lowest concentration that inhibited the color change of INT (yellow to purple). The experiment was performed in triplicate.

CYTOTOXIC TEST

The cytotoxicity of the extracts (dissolved in acetone) against Vero monkey kidney cells was assessed by the MTT reduction assay as previously described by Mosmann (1983) with slight modifications. Cells were seeded at a density of 1 × 10^5 cells/ml (100 µl) in96-well microtitre plates and incubated at 37 ^{0}C and 5% CO_2 in a humidified environment.

After 24 hrs incubation, extracts (100 ml) at varying final concentrations were added to the wells containing cells. Doxorubicin was used as a positive reference. A suitable blank control with equivalent concentrations of acetone was also included and the plates were further incubated for 48 h in a CO_2 incubator. Thereafter, the medium in each well was aspirated from the cells, which were then washed with PBS, and finally fresh medium (200 ml) was added to each well. Then, 30 ml of MTT (5mg/ml in phosphate buffered saline (PBS)) was added to each well and the plates were incubated at 37 ^{0}C for 4 h. The medium was aspirated from the wells and dimethyl sulphoxide (DMSO) was added to solubilize the formed formazan crystals. The absorbance was measured on aBioTek Synergy microplate reader at 570 nm. The percentage of cell growth inhibition was calculated based on a comparison with untreated cells. The selectivity index values were calculated by dividing cytotoxicity LC_{50} values by the MIC values in the same units (SI = LC_{50}/MIC).

STATISTICAL ANALYSIS

All experiments were conducted in triplicate and values expressed as mean ± standard deviation. Statistical analysis was performed using statistical package for social sciences (SPSS). One way analysis of variance (ANOVA) was used to compare means and results were compared using Fisher's least significant difference (LSD) at a 5% significance level.

RESULTS

DPPH, ABTS, FRAP, total phenolics (TPC) flavonoids content (TFC)

Table 1 shows DPPH, ABTS, FRAP, total phenolics (TPC) flavonoids content (TFC) of *C. Africana*. The free radical scavenging ability has been determined by using several different assays (Table1). There was a very good correlation between DPPH and ABTS values ($R^2$0. .826) and between TPC and TFC (R^2 0.956) of the different extracts. There was significant ($P<0.05$) antioxidant activity in both extracts though lower than the standards (ascorbic acid and trolox) in all cases. Results varied depending on the method used. In both the DPPH and ABTS assays, the methanol extract of the bark of *C. africana* had the highest antioxidant activity with IC_{50} values of 6.79 µg/mL and 12.42 µg/mL respectively. The trend for FRAP activities of the extracts tested, did not markedly differ from their DPPH and ABTS scavenging activities; TEAC values obtained were 12.93 from the hexane extract of the leave and 93.84 from the methanol extract of the bark. Results in Table1 also indicate the TPC and TFC of the extracts analysed as milligram of gallic acid equivalent per gram of extract and milligram quercetin equivalent per gram of extract respectively. The methanol extract (bark) had the highest phenolic and flavonoid content (43.71 mgGAE/g and 3.91 mgQE/g respectively). The hexane extract of the leave of *C. africana* with the lowest antioxidant activity in the DPPH and ABTS assays also had the lowest phenolic and flavonoid contents (2.56 mgGAE/g and 2.75 mgQE/g respectively). Results in Table2 indicate the Pearson's correlation between the total phenolic and total flavonoids content and antioxidant activity, a statistically significant relationship was observed between TPC, TFC and FRAP.

Table 1: Antioxidant activity, total phenolic and total flavonoid contents of extracts from hexane leave and methanol bark of *Cordia africana*

Extract	ABTS IC_{50} (µg/ml)	DPPH IC_{50} (µg/ml)	FRAB (TEAC)	TPC (mg GAE/g)	TFC (mg QE/g)
CHL	331.98±0.07^a	315.86±0.07^a	12.93±0.07^b	2.56±0.07^i	2.75±0.07^i
CMB	12.42±0.07^b	6.79±0.07^d	93.84±0.07^c	43.71±0.07^j	3.91±0.07^k
TRO	7.24±0.07^e	3.26±0.07^f	1.00±0.00^g	nd	nd
Ascorbic acid	3.97±0.07^f	1.41±0.18^g	2.92±0.04^h	nd	nd

Values with different letters are significantly different at $p<0.05$.

Table 2: Coefficient of correlation r^2 and Pearson's correlation coefficients of antioxidant activity (DPPH, FRAP, ABTS), total polyphenol content (TPC) and total flavonoid (TFC) of extracts from hexane leave and methanol bark of *Cordia africana*

		ABTS	DPPH	FRAP	TPC	TFC
r^2	ABTS	1	.826	-.889	-.644	-.326
p			.022	.007	.118	.475
r^2	DPPH		1	-.828	-.654	-.371
p				.022	.111	.413
r^2	FRAP			1	.676	.348
p					.095	.444
r^2	TPC				1	-.026
p						.956
r^2	TFC					1

There is a significant correlation between pairs of variables with $p < 0.05$.

15-Lipoxygenase inhibitory activity

The results presented in Fig. 1 show that both the extracts investigated had a certain level of 15-lipoxygenase inhibitory effect. The methanol extract of the bark had the highest inhibitory activity of 70.45% of 15-lipoxygenase inhibition. Table 3 shows the IC_{50} values. The methanol extract of the bark had the highest IC_{50} value of 55 ± 0.98 µg/mL.

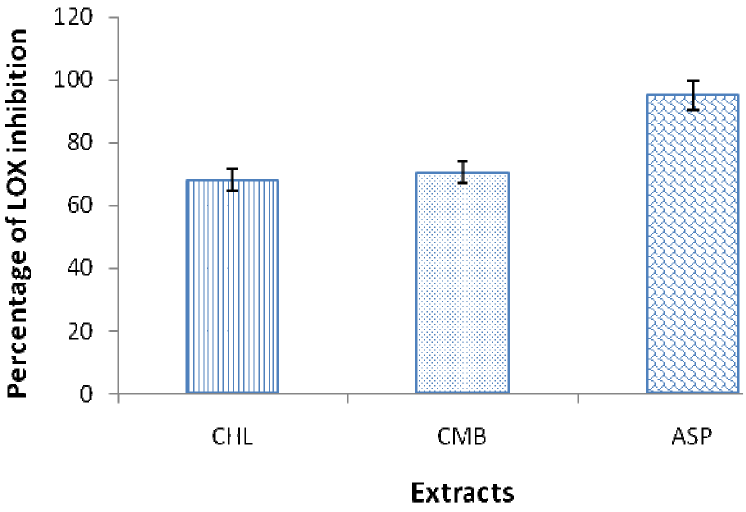

Figure 1: 15-Lipoxygenase inhibitory activity of extracts from hexane leave and methanol bark of *Cordia africana*. Extracts were tested at (5 mg/ml).

Table 3: IC_{50} of extracts from hexane leave and methanol bark of *Cordia africana*

Extracts	**IC_{50} (µg/ml)**
Asp	19 ± 0.9[a]
CMB	55 ± 0.98[b]
CHL	105 ± 1.3[c]

ASP: aspirin, CMB: *Cordia africana* methanol bark, CHL: *Cordia africana* Hexane leave. Values with different letters are significantly different at $p<0.05$.

Antimycobacterial activity

The MIC values of extracts of *C. africana* against three fast growing Mycobacterium species strains are shown in Table 4. In general there were not major differences in the activity of the extracts. The MIC values range between 256 µg/mL to 1024 µg/mL. The methanol extract of the bark of *C. africana* was active against *M. smegmatis* with MIC value of 256 µg/mL. Both the methanol extract of the bark and hexane extract of the leave were active against *M. fortuitum* with MIC values of 512 µg/mL in both cases. The hexane extract of the leave was active against *M. smegmatis,* and *M.aurum* with MIC value of 1024 µg/mL in both cases. However, the methanol extract of the bark had no activity against *M. aurum* with MIC value of >1024 µg/mL. Taking into account the cut-off of the antimicrobial activity of plant extracts of 0.1mg/mL (Eloff, 2004; Kuete and Efferth, 2010), the antimycobacterial activity of the extracts of *C. africana* obtained in this study varied from significant to inactive.

Table 4: Minimum inhibitory concentration (MIC in mg/mL) of extracts from hexane leave and methanol bark of *Cordia Africana* against fast growing mycobacterial strains.

Extracts	**MIC (µg/ml)**		
	Ms	***Mf***	***Ma***
CHL	*1024*	*512*	*1024*
CMB	256	512	>1024
Ciprofloxacin	4	8	8
Rifampicin	8	2	4

CMB: *Cordia africana* methanol bark, CHL: *Cordia africana* Hexane leave, *M. smegmatis, M. fortuitum, M.aurum*

The MIC values of extracts of *C. africana* against six bacterial species are shown in Table 5.

Table 5: Minimal inhibitory concentration (MIC in mg/mL) of extracts from hexane leave and methanol bark of *Cordia Africana* against six bacterial strains

Extracts	MIC (µg/ml)					
	Sa	***Ef***	***Bc***	***Pa***	***Ec***	***St***
CHL	*512*	*256*	*512*	*256*	*512*	*32*
CMB	*1024*	*>1024*	*>1024*	*1024*	*512*	*128*
Cipro.	*4*	*16*	*8*	*16*	*8*	*2*

Cipro: ciproxacine, CMB: *Cordia africana* methanol bark, CHL: *Cordia africana* Hexane leave*, S. aureus, E. faecalis, B. cereus, P. aeruginosa, E. coli, S. typhimurium*

Cytotoxic activity

Over the past decade a number of in vitro methods have been evaluated with the aim of replacing the mouse bioassay for toxicity testing.Cell culture-based toxicity tests are of interest, having the potential to detect more general cytotoxicity end points. In the present study, the toxicity of two extracts of *C. africana* was evaluated on Vero monkey cells by the MTT assay. The LC_{50} values obtained was 81.79 and 99.67 µg/mL (Table 6).

Table 6: Cytotoxicity of extracts from of extracts from hexane leave and methanol bark of *Cordia africana* on Vero monkey kidney cells and their selectivity index (SI) against six bacterial strains

Extract	**LC_{50} (µg/ml)**	**Selectivity index (LC_{50}/MIC)**					
		Sa	***Ef***	***Bc***	***Pa***	***Ec***	***St***
CHL	81.79±13.31	0.16	0.32	0.16	0.32	0.16	2.56
CMB	99.67±16.10	0.10	0.10	0.10	0.10	0.19	0.78
DOXO	3.48±0.45	nd	nd	nd	nd	nd	nd

CMB: *Cordia africana* methanol bark, CHL: *Cordia africana* Hexane, Doxo: Doxorubicin, *S. aureus, E. faecalis, B. cereus, P. aeruginosa, E. coli, S. typhimurium*

Table 7: Cytotoxicity of extracts from of extracts from hexane leave and methanol bark of *Cordia africana* on Vero monkey kidney cells and their selectivity index (SI) against mycobacterial strains

Extract		**Selectivity index (LC_{50}/MIC)**		
	LC_{50} (µg/ml)	***Ms***	***Mf***	***Ma***
CHL	81.79±13.31	*0.079*	*0.159*	*0.079*
CMB	99.67±16.10	0.389	0.194	0.097
DOXO	3.48±0.45	nd	nd	Nd

CMB: *Cordia africana* methanol bark, CHL: *Cordia africana* Hexane, Doxo: Doxorubicin,
M. smegmatis, M. fortuitum, M.aurum

DISSCUSSION

DPPH, ABTS, FRAP, total phenolics (TPC) flavonoids content (TFC)

It has been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds (Table 2) (Velioglu *et al.*,1998). The FRAP and phenolic content of the methanol fruit extract of *C. africana* was previously evaluated and similar results to our findings have been reported (Tewolde-Berhan *et al.*, 2013). The FRAP from the bark extract is higher (93.84) comparable to that found from the bark extract in a similar species *Cordia dichotoma* bark, with 22.8 mg mL-1 TE on a dry weight basis (Ganjare *et al.*, 2011). The average total phenol values are lower than that reported by (Tewolde-Berhan *et al.*, 2013). This could be attributed the part of the plant used and the solvent used in the extraction protocol.

15-Lipoxygenase inhibitory activity

The observed variability in the degree of inhibition of 15-LOX (Fig 1) by the extracts could be attributed to the differences in their phytochemical composition. The in vitro Lipoxygenase effect of *C. africana* is reported for the first time in this study. The Lipoxygenase products constitute an important class of inflammatory mediators in various inflammatory diseases (Carter *et al.*,1991), therefore, inhibition of the biosynthesis of inflammatory mediators by blocking the activities of these enzymes would be important for the treatment of many inflammatory disease states (Benrezzouk et al.,1999). It is noteworthy that, the methanol extract of bark of *C. africana* had the highest TPC with good antioxidant activity, a finding which is consistent with Handoussa et al.(2013) who found a relationship between the anti-inflammatory activity and the presence of polyphenols. Antioxidants are also known to inhibit plant Lipoxygenases (Lin et al., 2001). Studies have implicated oxygen free radicals in the process of inflammation and phenolic compounds may block the cascade process of arachidonic acid metabolism by inhibiting lipoxygenase activity, and may serve as ascavenger of reactive free radicals which are produced during arachidonic acid metabolism (Trouillas *et al.*,2003).

Antimycobacterial activity

The antimycobacterial activity of *Cordia sinensis* has been previously reported (Mariita *et al*, 2010). To the best of our knowledge this is the first report on the antimycobacterial activity of *C. african* against fast growing Mycobacterium species. It has been reported that, activity against the fast growing Mycobacteriumaurum is highly predictive of activity against Mycobacterium tuberculosis, as the two species have similar drug sensitivity profiles (Chung *et al*., 1995). Therefore, the significant activity obtained with the methanol extract of the bark of *C. africana* against *Mycobacterium aurum* in this study may be of interest for further screening against pathogenic Mycobacterium species.

On the bases of criteria of MIC values previously reported by some authors (Eloff, 2004; Kuete and Efferth, 2010), both extracts of *C. africana* had significant to weak antimicrobial activities, with MIC values ranging between 32 µg/mL to 1024 µg/mL. Both the hexane extract of the leave and the methanol extract of the bark of *C. africana* had significant antibacterial activity against *S. typhimurium* with MIC values of 32 µg/mL and 128 µg/mL respectively. The hexane extract of the leave had significant activity against P*. aeruginosa* and *E. faecalis* with MIC values of 256 µg/mL in both cases and a moderate activity against *S. aureous* and E. coli with MIC values of 512 µg/mL in both cases. With the exception of *E. coli* with moderate activity of MIC value of 512 µg/mL, the methanol extract of the bark of *C. africana* had low activity against *S. aureous* and P*. aeruginosa* with MIC values of 1024 µg/mL in both cases. However, both *E. faecalis* and *E. coli* were resistant to the methanol extract of the bark with MIC values of more than 1024 µg/mL in both cases. The antimycobacterial activity of *Cordia dichotoma* has been previously reported (Kuppasta and Nayak, 2003; Sharker *et al* 2009). To the best of our knowledge this is the first report on the antimycobacterial activity of *C. africana.*

Cytotoxic activity

According to the National Cancer Institute (United States) plant screening program, a crude extract is generally considered to have *in vitro* cytotoxic activity if the LC_{50} is <20 µg/mL (Boik, 2001). On the basis of this threshold, all the extracts tested in our study can be considered as safe. This result provides a support on the safety of their traditional use. The methanol extract of bark of *C. africana* had the highest selectivity index (SI) of 2.56 with *S. typhimurium*. In general SI (also called Therapeutic Index) is a measure of potential efficacy versus adverse effects. The higher the selectivity index for a crude extract, the more likely it is that the activity is not due to a general metabolic toxin. An SI >1 for a crude extract increases the likelihood that its toxic and antibacterial compounds are different (Cho-Ngwa *et al*., 2010). For most of the extract, the SI values were less than 1 due to their poor antimycobacterial activity effect.

CONCLUSION

In conclusion, *C. africana* extracts investigated have a certain level of 15-lipoxygenase inhibitory and antioxidant activity. The cytotoxicity activity shows that the extract are generally not toxic to vero cells, thus substantiating their safety. This study provides a scientific support for some of the traditional uses. Although it is dangerous to extrapolate from *in vitro* to *in vivo* results, the pharmacological activities observed in this study suggests that extracts of this plant species may be effective in human health.

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