15-LIPOOXYGENASE, BACTERIAL INHIBITORY AND CYTOTOXIC ACTIVITIES OF *CORDIA AFRICANA* LEAF AND STEM BARK EXTRACTS

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ABSTRACT

This study was undertaken to determine the potential benefits of *Cordia africana* (Boraginaceae) plant used in folkloric medicine to treat inflammation related conditions and infectious diseases. The objectives of this work is to establish the scavenging effect of the extracts and fractions of the plant 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 *C. africana* were also evaluated. Extracts were obtained by maceration with acetone and ethylacetate as solvents. 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. All the extracts of *C. africana* showed strong anti-mycobacterial activity against the bacteria tested. The ethyl acetate extract of the leave of *C. africana* was the most active against *E. coli* and *S. typhimurium* with minimum inhibitory concentrations of 16 µg/mL in both cases as well as the fast growing *Mycobacterium* species (*M. smegmatis*, *M. fortuitum and M. aurum*). All the extracts of *C. africana* inhibited LOX enzyme with the most active being the bark acetone extract with an IC_{50} value of 41± 1.3 µg/mL. All the extracts had significant ($P<0.05$) free-radical scavenging activity (IC_{50} ranging from 7.46±0.17-104.21±3.37 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} between 29.56±3.58 and 98.77±2.04 µg/ml. The data suggest that some of the extracts of *C. africana* could be a rich source of antimicrobial agents. The results further show that there is some merit in the use of the plant in alternative medical practice.

Key words: Antibacteria, antioxidant, *Cordia africana*, anti-inflammation, total flavonoid.
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INTRODUCTION

Lipoxygenases (LOXs) comprise a family of non-heme iron-containing dioxygenases, representing the key enzymes in the biosynthesis of leukotrienes from polyunsaturated fatty acids (PUFA). Leukotrienes have been postulated to play essential role in the pathophysiology of several inflammatory and allergic diseases. The LOXs are classified with respect to their positional specificity of arachidonic acid oxygenation as 5-LOX, 9-LOX, 12-LOX, 15-LOX. The products of LOXs catalysed oxygenation include leukotrienes, lipoxins, hydroperoxy-eicosatetraenoic acids (HPETE), and hydroxyl-eicosatetraenoic acids (HETE) [1]. The common substrates for LOX are linoleic and arachidonic acids. For many *in vitro* studies, soy bean LOX is used due to difficulties in obtaining human LOX for bioassays [2]. During inflammation, arachidonic acid is metabolized via the COX pathway to produce prostaglandins and thromboxane A_2, or via the LOX pathway to produce hydroperoxy-eicosatetraenoic acids and leukotrienes [3]. The LOX pathway is active in leucocytes and many immune-competent cells including mast cells, neutrophils, eosinophils, monocytes and basophils. Upon cell activation, arachidonic acid is cleaved from cell membrane phospholipids by phospholipase A_2 and donated by LOX activating protein to LOX, which then metabolises arachidonic acids in a series of reactions to leukotrienes, a group of inflammatory mediators [4]. Leukotrienes act as phagocyte chemo-attractant, recruiting cells of the innate immune system to sites of inflammation. For instance, in an asthmatic attack, it is the production of leukotrienes by LOX that causes the constriction of bronchioles leading to bronchospasm [5, 6]. Therefore, the selective inhibition of LOX is an important therapeutic strategy for asthma. Inhibitors of the activities of LOX could provide potential therapies to manage many inflammatory and allergic responses. Medicinal plants may therefore be potential sources of inhibitors of COX-2/LOX that may have fewer side effects than NSAIDs [7].

Cordia africana is a small to medium-sized evergreen tree, 4-15 m high, heavily branched with a spreading, umbrella-shaped or rounded crown. The bole is typically curved or crooked [8]. The plant is widely distributed in eastern and southern Africa. In West Africa, this species is restricted to montane and submontane

habitats [8]. A review of the pharmacological studies carried out with extracts and purified compounds indicates that *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 have been isolated from different parts of *Cordia* species [9]. 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 [10].

MATERIALS AND METHODS

Plant material and extraction

Cordia africana was collected in January, (stem bark and leaves) 2014 in Bomo village, Zaria (11°05'N, 7°43'E), Nigeria. The plant was identified and authenticated by the taxonomist, Mal M. Muhammad 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. The powder of the stem bark obtained was extracted with acetone (250 ml), while the powder of the leaves obtained was extracted successively with acetone and ethyl acetate (250 ml each) 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°C until 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 Whitehead Scientific, South Africa. Doxorubicin was obtained from Pfizer. Quercetin, 2,20-azino-bis (3-ethylben-zothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo - liumbromide (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 stem 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 two-fold serially diluted to concentration ranges between 5 to 0.08 mg/mL for both the extract the standard reference (Aspirin) and introduced in a 96-well microtitre plates. In the blank wells, 100 µL of assay buffer was introduced, in the 15-LOX standard wells, 90 µL of 15-LOX enzyme and 10 µL of Assay Buffer were added, in the 100% initial activity wells, 90 µL of LOX enzyme and 10 µL solvent were added, in the inhibitor wells, 90 µL of LOX enzyme and 10 µL of extract were added. 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.

$$\text{Percentage Inhibition} = \left\{\frac{IA - Inhibitor}{IA}\right\} \times 100$$

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-azino-bis (3-ethylben-zothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical assay

The ABTS radical scavenging capacity of the samples was measured with modifications of the 96-well microtitreplate method as described previously [11]. Trolox and ascorbic acid were used as positive controls, methanol as negative control and extract without ABTS as blank. The percentage of $ABTS^{\bullet+}$ inhibition was calculated using the formula:

$$\text{Scavenging capacity (\%)} = \text{Radical scavenging capacity} = 100 - \left(\frac{(Absample - Abblank)}{Abcontrol}\right) \times 100.$$

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.

DPPH assay

The DPPH radical-scavenging activity was determined using the method proposed by Brand-Williams *et al*., [12]. 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 ferric ion was measured as described previously [13] 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); 100 mL of 1.0 M hydrochloric acid; 20 mL of 1% (w/v) of Sodium dodecyl sulfate (SDS); 30 mL of 1% (w/v) of potassium ferricyanide was incubated for 20 min at 50^0C, 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 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*. [14]. The total phenolic content was calculated from the linear equation of a standard curve prepared with gallic acid, 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*. [15]. A volume of 0.5 mL of 2% $AlCl_3$ ethanol solution was added to 0.5 mL of sample solution (1 mg/mL). After one hour at room temperature, the absorbance was measured at 420 nm. A yellow colour 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 microorganism culture

Mycobacterium smegmatis (ATCC1441), *Mycobacterium aurum* (NCTC 10437) and *Mycobacterium fortuitum* (ATCC6841) were cultured as described by McGaw *et al*. [16]. 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.

Three Gram-positive bacteria - *Bacillius cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212), and two Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 25922) *Salmonella typhimurium* (ATCC 700720) were used as test organisms. Bacterial culture was taken from 24 hr 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 $1\text{-}5\times10^8$ 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/MBC)

The broth microdilution technique using 96-well micro-plates, as described by Eloff [17] was used to obtain the MIC and MBC values for *C. 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. Thereafter, 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 37^0C. 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 1 hr. The MIC was defined as the lowest concentration that inhibited the colour change of INT (yellow to purple). The experiment was performed in triplicate.

Cytotoxic activity

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 [18] with slight modifications. Cells were seeded at a density of 1×10^5 cells/ml (100 µl) in 96-well microtitre plates and incubated at 37^0C and 5% CO_2 in a humidified environment. After 24 hr 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 hr 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 (5 mg/ml in PBS) was added to each well and the plates were incubated at 37^0C for 4 hr. The medium was aspirated from the wells and DMSO was added to solubilize the formed formazan crystals. The absorbance was measured on a BioTek Synergy microplate reader at 570 nm. The percentage of cell growth inhibition was calculated based on 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).

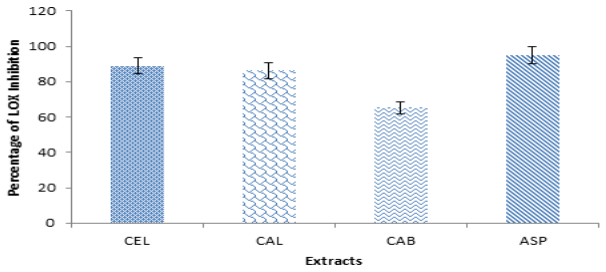
STATISTICAL ANALYSIS

All experiments were conducted in triplicate and values expressed as mean ± standard deviation. Statistical analysis was performed using one way ANOVA and results were compared using Fisher's least significant difference (LSD) at a 5% significance level.

RESULTS

15-Lipoxygenase inhibitory activity

The results presented in Fig.1 show that the extracts investigated had a certain level of 15-lipoxygenase inhibitory effect. The ethyl acetate extract of the leaf had the highest inhibitory activity of 88.81% of 15-lipoxygenase inhibition. Table 1 shows the IC_{50} values. The acetone extract of the bark had the highest IC_{50} value of 41± 1.3 µg/mL.



Key: CAL (*Cordia* acetone leave), CAB (*Cordia* acetone bark) and CEL *Cordia* ethyl acetate bark, ASP (Aspirin).

Fig. 1: 15-Lipoxygenase inhibitory activity of extracts of acetone leave, acetone and ethyl acetate stem bark extracts of *Cordia africana*. Extracts were tested at (5 mg/ml).

Table 2 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 (Table2). 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.

Table 1: IC_{50} of acetone leave, acetone and ethyl acetate stem bark extracts of *Cordia africana*

Extracts	IC_{50} (µg/ml)
ASP	19 ± 0.9^{a}
CEL	56 ± 0.98^{b}
CAL	71 ± 1.3^{c}
CAB	41 ± 1.3^{d}

Values with different letters are significantly different at p<0.05.
Key: CAL (*Cordia* acetone leave), CAB (*Cordia* acetone bark) and CEL *Cordia* ethyl acetate bark, ASP (Aspirin).

There was significant (P<0.05) antioxidant activity in all the extracts. The acetone extract of bark of *C. africana* had the highest antioxidant activity of 7.46±0.17 in the ABTS assay that is comparable with that of the standard antioxidant, Trolox (IC_{50} 7.24±0.07). The same extract had IC_{50} value of 25.40±1.17 in the DPPH assay and 84.25±2.17 trolox equivalent antioxidant capacity. The remaining extracts too had significant antioxidant effects in both the assay techniques (Table2). 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 acetone extract (bark) had the highest phenolic content of 65.48±0.22 and the highest flavonoid content of 40.76±1.24 was obtained from the ethyl acetate extract (leaf). The acetone extracts of the leave and bark of *C. africana* with the highest antioxidant activity in the DPPH and ABTS assays also had the highest phenolic contents (65.48±0.22 mg GAE/g and 22±1.00 mg QE/g respectively).

Results in Table 3 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 2: Antioxidant activity, total phenolic and total flavonoid contents of acetone leave, acetone and ethyl acetate stem bark extracts of *Cordia africana*

Extract	ABTS IC_{50} (µg/ml)	DPPH IC_{50} (µg/ml)	FRAB (TEAC)	TPC (mg GAE/g)	TFC (mg QE/g)
CAL	21.27 ± 0.18^{a}	20.70 ± 0.27^{a}	108.62 ± 0.36^{b}	22 ± 1.00^{i}	27.20 ± 0.49^{i}
CAB	7.46 ± 0.17^{c}	25.40 ± 1.17^{d}	84.25 ± 2.17^{c}	65.48 ± 0.22^{j}	6.74 ± 0.13^{k}
CEL	60.61 ± 2.37^{b}	104.21 ± 3.37^{b}	59.06 ± 1.18^{c}	14.16 ± 0.07^{b}	40.76 ± 1.24^{b}
TRO	7.24 ± 0.07^{c}	3.26 ± 0.07^{d}	1.00 ± 0.00^{g}	Nd	Nd
Ascorbic acid	3.97 ± 0.07^{d}	1.41 ± 0.18^{g}	2.92 ± 0.04^{h}	Nd	Nd

Values with different super scripts on the same vertical column are significantly different at p<0.05.
Key: CAL (*Cordia* acetone leave), CAB (*Cordia* acetone bark) and CEL *Cordia* ethyl acetate bark.

Table 3: 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 acetone leave, acetone and ethyl acetate stem bark extracts of *Cordia africana*

		ABTS	DPPH	FRAP	TPC	TFC
r^2	**ABTS**	1	0.826	-0.889	-0.644	-0.326
P			0.022	0.007	0.118	0.475
r^2	**DPPH**		1	-0.828	-0.654	-0.371
P				0.022	0.111	0.413
r^2	**FRAP**			1	0.676	0.348
P					0.095	0.444
r^2	**TPC**				1	-0.026
P						0.956
r^2	**TFC**					1

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

Antimycobacterial activity

The MBC 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 activities of the extracts. The MIC values range between 64 µg/mL to 512 µg/mL. The ethyl acetate extract of the leaf of *C. africana* was active against *M. smegmatis* with MIC value of 64 µg/mL. The ethyl acetate and acetone extracts of the leaf were active against *M. aurum* with an MIC value 256 µg/mL for each. The acetone extract of the bark was active against *M. smegmatis* and *M. fortuitum* with an MIC value of 256 µg/mL for each. Taking into account, the cut-off of the antimicrobial activity of plant extracts, which was 0.1mg/mL [19, 20], the anti-mycobacterial activity of the extracts of *C. africana* obtained in this study varied from significant to inactive.

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

Table 4: Minimum inhibitory concentration (MIC in mg/mL) of acetone leave, acetone and ethyl acetate stem bark extracts of *Cordia africana* against fast growing mycobacterial strains

Extracts	**MIC (µg/ml)**		
	Ms	***Mf***	***Ma***
CEL	64	128	256
CAL	128	256	256
CAB	256	256	512
Ciprofloxacin	4	8	8
Rifampicin	8	2	4

Key: CAL (*Cordia* acetone leave), CAB (*Cordia* acetone bark) CEL (*Cordia* ethyl acetate bark), *Ms* (*Mycobacterium smegmatis*), *Mf* (*Mycobacterium fortuitum*) and *Ma* (*Mycobacterium aurum*).

Table 5: Minimal inhibitory concentration (MIC in mg/mL) of extracts of acetone leave, acetone and ethyl acetate stem bark extracts of *Cordia africana* against six bacterial strains

Extracts	**MIC (µg/ml)**					
	Sa	***Ef***	***Bc***	***Pa***	***Ec***	***St***
CEL	128	256	128	32	16	16
CAL	256	512	512	64	64	32
CAB	256	128	512	128	256	64
Cipro.	*4*	*16*	*8*	*16*	*8*	*2*

Key: CAL (*Cordia* acetone leave), CAB (*Cordia* acetone bark) CEL (*Cordia* ethyl acetate bark), Cipro (Ciprofloxacin) *Sa* (*Staphylococcus aureus*), *Ef* (*Enterococcus faecalis*) *Bc* (*Bacillius cereus*), *Pa* (*Pseudomonas aeruginosa*), *Ec* (*Escherichia coli*), and *St* (*Salmonella 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 the extracts of *C. africana* was evaluated on Vero monkey cells by the MTT assay. The LC_{50} values obtained was between 29.56 and 98.77 µg/mL (Table 6).

Table 6: Cytotoxicity of extracts from of extracts of acetone leave, acetone and ethyl acetate stem bark extracts 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***
CEL	29.56±3.58	0.23	0.12	0.23	0.92	1.85	1.85
CAL	98.77±2.04	0.39	0.19	0.19	1.54	1.54	3.09
CAB	35.03±2.51	0.14	0.27	0.07	0.27	0.14	0.55
DOXO	3.48±0.45	nd	nd	nd	nd	nd	nd

Key: CAL (*Cordia* acetone leave), CAB (*Cordia* acetone bark) CEL (*Cordia* ethyl acetate bark), Cipro (Ciprofloxacin) *Sa* (*Staphylococcus aureus*), *Ef* (*Enterococcus faecalis*) *Bc* (*Bacillus cereus*), *Pa* (*Pseudomonas aeruginosa*), (*Escherichia coli*), *St* (*Salmonella typhimurium*) and Doxo (Doxorubucin)

Table 7: Cytotoxicity of extracts from of extracts of acetone leave, acetone and ethyl acetate stem bark extracts of *Cordia africana* on Vero monkey kidney cells and their selectivity index (SI) against mycobacterial strains

Extracts		Selectivity index (LC_{50}/MIC)		
	LC_{50} (µg/ml)	***Ms***	***Mf***	***Ma***
CEL	29.56±3.58	0.461	0.230	0.115
CAL	98.77±2.04	0.771	0.385	0.385
CAB	35.03±2.51	0.136	0.136	0.068
DOXO	3.48±0.45	nd	Nd	Nd

Key: CAL (*Cordia* acetone leave), CAB (*Cordia* acetone bark) CEL (*Cordia* ethyl acetate bark), *Ms* (*Mycobacterium smegmatis*), *Mf (Mycobacterium fortuitum*) and *Ma* (*Mycobacterium aurum*).

DISSCUSSION

It has been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds [21]. 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 [22]. The FRAP from the bark extract is higher (93.84) comparable to that found from the bark extract in a similar species *C. dichotoma* bark, with 22.8 mg mL-1 TE on a dry weight basis [23]. The average total phenol values are lower than that reported by other workers [22].

The observed variability in the degree of inhibition of 15-LOX by the extracts could be attributed to the differences in their phytochemical composition. Extracts or compounds from plants inhibiting the pro-inflammatory activities of these enzymes may contain potential leads or templates for the development of potent anti-inflammatory drugs. The lipoxygenase products constitute an important class of inflammatory mediators in various inflammatory diseases [24], 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 [25]. 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*. [26], who found a relationship between the anti-inflammatory activity and the presence of polyphenols. Antioxidants are also known to inhibit plant lipoxygenases [27]. 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 scavengers of reactive free radicals, which are produced during arachidonic acid metabolism [28].

The anti-mycobacterial activity of *Cordia sinensis* has been previously reported [29]. It has been reported that activity against the fast growing *Mycobacterium aurum* is highly predictive of activity against *Mycobacterium tuberculosis*, as the two species have similar drug sensitivity profiles [30]. Therefore, the significant activity obtained with the ethyl acetate extract of the leaf 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 [19, 20], all the extracts of *C. africana* had significant to moderate antimicrobial activities, with MIC values ranging between 16 µg/mL to 512 µg/mL. The antimycobacterial activity of *C. dichotoma* has been previously reported [31, 32].

The ethyl aceate extract of the leave of *C. africana* had significant antibacterial activity against *E. coli* and *S. typhimurium* with MIC values of 16 µg/mL in both cases. The ethyl acetate extract of the leave and the acetone extract of the leave had significant activity against *P. aeruginosa* and *S. typhimurium* with an MIC value of 32 µg/mL for each case and a moderate activity against *P. aeruginosa* and *E. coli* with an MIC value of 64 µg/mL in both cases. All the extracts had moderate MIC values (128-512 µg/mL) against *S. aureus, E. faecalis* and *B. cereus*.

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 [33]. 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 ethyl acetate extract of leave of *C. africana* had the highest selectivity index (SI) of 1.85 with *E. coli* and *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 [34]. For most of the extract, the SI values were less than 1 due to their poor anti-mycobacterial activity effect.

CONCLUSION

The *in vitro* Lipoxygenase effect of *C. africana* is reported for the first time in this study. Our results provide scientific evidence supporting the use of *C. africana* as anti-inflammatory, pain relief and antimicrobial remedies in traditional medicine. The cytotoxicity activity shows that the extracts are generally not toxic to Vero cells, thus substantiating their safety.

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