

Biological activities and phytochemical screening of *Elaeodendron croceum* (Thunb.) DC. Leaves and Stem barks Extracts

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Received: 28 Sep 2016

Accepted: 08 Dec 2016

Published: 28 Dec 2017

Abstract

From reports *Elaeodendron croceum* has been used traditionally for spiritual cleansing, anti-obesity, anti-inflammatory and for the management of diabetes. The leaves and bark extracts were therefore analyzed for its biological activities.

The antibacterial activities of *E. croceum* leaves and stem barks were evaluated against both Gram positive and gram negative bacteria using agar well diffusion method. The analysis for the phytochemical contents, antioxidant and anti-inflammatory activities of the leaves and barks were determined using standard procedures.

The *S. aureus*, *S. flexneri*, *E. faecalis* and *S. typhimurium* were susceptible to the aqueous leaves and stem barks extracts of *Elaeodendron croceum* while *S. aureus* was susceptible to only the aqueous leaf extract. The antibacterial activity showed MIC ranging from 0.625 – 2.5 mg/ml. The composition of flavonoids (52.72 mg/g), proanthocyanidins (32.66 mg/g), alkaloids (65.44 mg/g) and saponins (68.21 mg/g) were significantly higher in the aqueous leaf extract while phenols (94.13 mg/g) content was higher in the aqueous bark extract. The free radical scavenging activity using ABTS, FRAP and DPPH showed that the extracts possess good free radical scavenging activities with different free radical species that is comparable with ascorbic acid and also showed significant inhibitory activity on protein denaturation as an indication of anti-inflammatory potential.

These findings support the folkloric usage of *Elaeodendron croceum* and indicates that it can be used to manage diseases caused by the tested organisms. The presence of alkaloids, flavonoids, and total phenols and proanthocyanidins, previously suggested to have antioxidant, anti-inflammatory and antibacterial activity indicate that *E. croceum* is a potential source of anti-inflammatory, free radical scavenging and antimicrobial agents.

Keywords: phytochemicals, antioxidants, *Elaeodendron croceum*, Anti-bacteria, anti-inflammatory

Introduction

Traditional medicine has been a historical practice and scientific investigations of medicinal plants are emerging. Plants synthesize these biologically active compounds as a defense against infections or pests that feed on them. Although these compounds are synthesized in small quantities but the diversity of their usage cannot be over emphasized. For instance, the various secondary metabolites such as flavonols, phenolic acids, quinones and tannins have been reported as antibacterial, antifungal, antioxidants and anti-inflammatory agents [1].

In recent years several (oriental) medicinal plants have been reported to have numerous biological activities [2]. They are important in drug development and pharmacological research when

these bioactive compounds are used directly as therapeutic agents or components for drug synthesis [3][4]. Also, the sales of chemicals derived from plants such as those used as pharmaceutical, fragrance, colouring, and flavour ingredients in the world market are more than billions of dollars every year [3][4].

One of the frequently used medicinal plants in the Eastern Cape Province, South Africa is the *Elaeodendron croceum* (Thunb.) DC. belonging to the Celastraceae family distributed worldwide in temperate and tropical zones [5]. *Elaeodendron croceum* has been reported with different names such as *Cassinacrocea*, *Ilexcrocea*, *Crocoxyloncroceum*, *C. papillosa*, *E. capense*, and *E. papillosum* [5][6][7]. *Elaeodendron croceum* has been used traditionally to clean the digestive tract, the bark as a purgative, to burn body fats, to clear a congested chest, curing of symptoms related to tuberculous such as cough, fever, blood in the sputum [8][9]

DOI:10.5138/09750185.1956



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while the roots are used to cause death by traditional healers[10]. The bark has also been reported to be fatal and poisonous to humans, probably due to the presence of alkaloid and poisonous triterpenes[11]. Several compounds isolated from celastraceae suggest this family of plant to be inhibitor of lipid peroxidation, inhibitor of cytokines (TNF – and IL –1 β) that play major role in the development of inflammation and inhibitor of induced nitric oxidesynthesis in the macrophage lineage cells[12][13]. Some of these compounds such as diterpene lactones with the ability to interact with nucleic acids, this is a potential source of mutagenic activity. It will be desirable to identify compounds from *Elaeodendron croceum* extract that has useful pharmacological effects without the interaction with nucleic acids. The present study, therefore, reports the antibacterial activities of *Elaeodendron croceum* leaf and stem bark extracts on some bacteriastrains as a way of validating its relevance in folkloric applications within Eastern Cape Province of South Africa. Hence, the screening for the qualitative andquantitative biologically active compounds with antioxidant and anti-inflammatory activities in the aqueous extracts of the leaf and bark.

Materials and Methods

Drugs and Chemicals

Ascorbic acid and BHT, and 2, 2, Diphenyl-2-picrylhydrazyl (DPPH) were from Sisco Research Laboratories (Pvt. Ltd., Mumbai, India). Folin-Ciocalteu reagent and methanol were obtained from Merck Limited, (Mumbai, India). All other chemicals and reagents used were of analytical grade.

Collection and Preparation of the Plant Extract

Leaves and barks of *E. croceum* were collected in a bush near Alice and identified by Tony Dold at the Rhodes University Herbarium and authenticated with a voucher number of ELE16/ 001 by the Botany Department, University of Fort Hare, and Alice, South Africa. The fresh leaves and barks were oven-dried at 35°C for five days and pulverized into a fine powder using an electric blender. 50 g of the powdered *E. croceum* was extracted in 500 ml of n-hexane and distilled water for 24 h and then filtered.

Determination of percentage total yield

The percentage of total yield from the extractions was calculated using the formula

$$Y = \left(\frac{m_2 - m_1}{m_0} \right) \times 100$$

Where Y is the percentage yield, m2 is the weight of the extract and container, m1 the weight of the container alone, m0 the weight of the initial dried sample

Sterility test for plant extracts

The aqueous extracts of the leaves and stem barks of *E. croceum* were tested for growth or contaminants. The sterility test was carried out by inoculating 1ml of the plant extracts on Mueller Hinton Agar in a petri dish and was incubated at 37 °C for 24 hours. The plates were observed for growth. The absence of visible growth after incubation indicated that they were sterile. The different extracts were then accessed for antibacterial activity.

Collection and maintenance of the test microorganisms

Staphylococcus aureus, *Proteus vulgaris*, *Shigella flexneri* KZN, *Klebsiella pneumoniae* ATCC 4352, *Enterococcus faecalis* ATCC 29212 and *Salmonella typhimurium* ATCC 13311 were the bacteria used for this study. These test microorganisms were collected from the Department of Biochemistry and Microbiology, University of Fort Hare, Eastern Cape Province, South Africa. The agar, Mueller-Hinton dextrose agar (MDA) was prepared as instructed by the manufacturer. The nutrient agar was suspended in deionized water and then boiled until completely dissolved. The solution was autoclaved for 15 min at 121°C. afterwards, the bacteria were kept at 4°C on MDA plates, and the inoculums used during the assays were prepared. This was done by diluting scraped cell mass in sterile normal saline (0.85% sodium chloride solution) and confirmed to be 0.5 McFarland by comparing its turbidity with 0.5 McFarland standard (Scientific Device Laboratory, United States).

Determination of Antibacterial activity Antibacterial Susceptibility Assays

Agar diffusion and microdilution technique was used to assay for the antibacterial activities of the extracts against these test bacteria.

In Vitro Susceptibility Test

Otang *et al.*[14] Agar well diffusion method was used with slight modifications to determine the antibacterial susceptibility test. Briefly, 0.5 McFarland solutions of each bacterial cultures was prepared in sterile normal saline, this was picked and spread over the surface of an agar plate with sterile cotton swab (6"). Four wells were made in individual agar plate with a flamed but cooled cork borer (with diameter of 5 mm) while sterile needle was used to remove all the agar plugs. 50 μ L of the extracts (ECL and ECB) at concentration of 50 mg/mL were added to the first and second wells, Amoxicillin (0.0125 mg/mL) was added to the third well, while sterile normal saline served as the negative control was added to the fourth well. The culture plates were then incubated for 24 h at 37°C. The clear zone around each bored well indicated the activity



of the plant extract against the bacteria which was measured in millimeter.

Minimum Inhibitory Concentration (MIC) Assay

The MIC was determined by modified broth micro dilution method. Briefly, 120 μL of sterile distilled water was added into every well of the 1st (A) row and 8th (H) row and also into each well of the 12th column. Then, 120 μL of nutrient broth (NB) was added into every well of the 2nd row (B). 150 μL of nutrient broth was then added into wells of the 1st column from C1 to G1 and 100 μL into the remaining wells from the 2nd column to the 11th column. Fifty micro liters of the plant extracts (ECL and ECB) was then added into the 3rd and 4th well of the 1st column (C1 and D1), while 50 μL of Amoxicillin and sterile distilled water was added separately into the remaining wells of the 1st column. Then a twofold serial dilution was carried out by mixing the contents in each well of the 1st column (starting from the 3rd row) and transferring 100 μL into the 2nd well of the same row. The procedure was then repeated up to the 11th well of the same row and the last 100 μL was then discarded. With this method different concentrations of the plant extracts from 5 to 0.005 mg/mL had been prepared in the wells from the twofold dilution. Then 20 μL of 0.5 McFarland bacteria suspensions was inoculated into the wells except those that contained sterile distilled water. The absorbance reading with micro plate reader (Synergy Mx Biotek, USA) before and after incubation at 620 nm was used to measure the bacterial growth. The plates were incubated for 24 hat 37°C. MIC was recorded and defined as the lowest concentration of the antibacterial agent that had inhibition on 50% bacterial growth. This was determined by assessing growth by calculating the difference in absorbance between the test wells and the control wells that had the broth and antimicrobial agent alone without the test bacteria.

Phytochemical Screening

Preliminary phytochemical screening

The aqueous extracts of *E. croceum* were subjected to routine phytochemical analysis to identify the presence of various phytochemicals. These were determined using chemical methods and by adopting standard protocols to identify the constituents such as phenols, flavonoids, alkaloids, Tannins and saponins as previously described [15].

Determination of Total Phenolic Content

The method of Azrul *et al.* [16] Was used to determine the total polyphenols. The phenolic constituent (mg/ml) in the extracts was calculated using the equation: $Y = 0.0013x$, $R^2 = 0.984$, where Y the absorbance and x the tannic acid equivalent (mg/ml). The total

phenolic constituent was expressed as tannic acid equivalent (TAE) using the following equation:

$$TP = C \times V_T / W$$

Where,

TP= total phenolic constituent in mg/g of the extracts as TAE, C = tannic acid concentration derived from the calibration curve (mg/ml), V_T = volume of the extract in the reacting solution(ml) and W= weight of the extract (g).

Determination of Flavonoids Content

The flavonoids constituent was determined using the colorimetric method [17]. The total flavonoid content (mg/ml) was calculated using the equation $Y = 0.0029x$ ($R^2 = 0.9657$). Where Y = absorbance and x = catechin equivalent (mg/ml).

The total flavonoids content was expressed as quercetin equivalents (QE)/100g by the following equation:

$$TF = C \times V_T / W.$$

Where TF is the flavonoids constituent in mg/g of the extracts as QAE, C = the concentration of quercetin established from the calibration curve (mg/ml), V_T = the volume of the extract in the reacting solution (ml) and W= the weight of the extract (g).

Determination of Proanthocyanidin

The proanthocyanidins content of the extracts were analyzed as previously described [18]. Total proanthocyanidins content (mg/ml) was calculated using the calibration curve equation $Y = 0.0023x$; $R^2 = 0.9951$, where Y was the absorbance and x is the catechin equivalent (mg/ml). The content of proanthocyanidins in extracts was expressed as catechin equivalents (CE)/100g by the following equation:

$$TP = C \times V_T / W.$$

Where TP is the proanthocyanidins content (mg/g) of the extracts as CE, C is the concentration of quercetin established from the calibration curve (mg/ml), V_T is the volume of the extract in the reacting solution (ml) and W is the weight of the extract (g).

Determination of Alkaloids

The alkaloid content of was analyzed as previously described[19]. The alkaloid content was then calculated from the following formula:

$$\% \text{ alkaloid} = \frac{\text{final weight of sample}}{\text{initial weight of extract}} \times 100.$$

Determination of Saponinscontent

Following the method as described by Okwu *et al.* [20], the saponins content in the plant extracts were determined. The percentage saponins constituent was calculated using the following formula:



% saponins = final weight of sample / initial weight of sample × 100.

Antioxidant determination

DPPH Radical Scavenging Activity Assay

The modified method of Carmona-Jiménez [21] using microtitre plate was used for the determination of DPPH free radical scavenging potential. Briefly, 100 µL of methanol was added into all the wells except second (B) and third (C) rows. Then, 100 µl of different concentrations of the plant extracts (0.05 mg/ml) or standards prepared in Me OH were then added in triplicates from the third row (C) to the seventh row. Different concentrations of the plant extracts and standards from 0.05 to 0.01 mg/ml were prepared. A solution of 0.135 mM DPPH radical in methanol was prepared. 100 µl of this solution was then added into all the wells. The reaction mixture was then vortexed and left in the dark for 30 min at room temperature. The absorbance of the mixture was measured using spectrophotometer at 517 nm. The actual decrease in absorbance was measured against that of the control. The scavenging ability of the plant extract was then calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

Where a control is the absorbance of DPPH + methanol and A sample is the absorbance of DPPH+ sample (or standard).

ABTS (2, 2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) Radical Scavenging Activity Assay

The method described by Thai pong et al.[22] Was used for the determination of ABTS scavenging activity. The percentage inhibition was then calculated as follows:

$$\text{ABTS scavenging activity (\%)} = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

where A_{sample} is the absorbance of ABTS radical + sample (extract/standard) A_{control} is the absorbance of ABTS radical + methanol.

Ferric Reducing Power Assay (FRAP)

The ferric reducing power of the extracts was determined as described by Mamta et al.[23]

Anti-inflammatory Activity

Protein Denaturation Method

The method of Sakat et al. [24] Was used to determine the protein denaturation assay. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\text{Percentage inhibition (\%)} = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

Where A sample is the absorbance of ABTS radical + sample (extract/standard) A_{control} is the absorbance of ABTS radical + methanol.

Statistical analysis

The result obtained were statistically analyzed by ANOVA. The means and standard deviations were computed by one-way NOVA, using IBM SPSS 20.0 computer software package. The level of significance was determined at p<0.05.

Results

Percentage of total yield

Table 1 show the percentage yields of *E. croceum* barks and leaves extracts in hexane and water. The aqueous extracts have the highest percentage yield compared with the hexane. However, the highest yield percentage was from the aqueous leaf extracts.

Table 1: Percentage yield

	Yield (%)	
	Hexane	Water
Barks	1.4	7.94
Leaves	3.6	13.6

Sterility test for plant extract

The sterility test on the aqueous extracts of *E. croceum* showed no visible growth for contaminants as indicated in Table 2.

Table 2: Sterility test for the aqueous extracts of *E. croceum* leaves and stem barks

Extracts	Sterility test
<i>E. croceum</i> leaf extracts	-
<i>E. croceum</i> bark extracts	-

Key (-) no growth

Anti-bacterial analysis

The susceptibility result shows that the aqueous leaf and bark extracts of *E. croceum* inhibit the growth of all the tested bacteria. This is shown by the diameter of the clear zones observed around



the wells cut in the agar plates (**Error! Reference source not found.**3). The two extracts also showed inhibition against *P. vulgaris* and *S. typhi*, which were resistant to amoxicillin. Aqueous leaf extract has higher inhibition on *S. aureus*, *S. flexneri*, *E. faecalis* and *S. typhi* with 15 ± 2.0 , 14.3 ± 1.2 , 15 ± 1.0 , 15.7 ± 0.6 mm but lower at *P. vulgaris* and *K. pneumonia* with inhibition zone diameter of 13.3 ± 0.6 , 13.7 ± 0.6 mm respectively. The aqueous bark extract however, has higher inhibition on *E. faecalis*, *S. typhi* and *S. flexneri* with inhibition zones diameters of 15 ± 1.0 , 15 ± 0.0 , 14.7 ± 1.5 mm respectively but lowest on *S. aureus* with

inhibition zone diameter of 13 ± 0.0 respectively. Comparing the two extracts, aqueous leaf extract has higher inhibition on *S. aureus* and *S. typhi* with inhibition zone diameter of 15 ± 2.0 mm but has lower inhibition on *P. vulgaris* with inhibition zone diameter of 13.3 ± 0.6 mm. The inhibition of the two extracts on *S. flexneri*, *K. pneumonia* and *E. faecalis* were not significantly different. The varying concentrations between 0.04 – 5 mg/ml of the plant extracts were carried out to calculate the minimum inhibitory concentration (MIC) (Table 4).

Table 3: Inhibition zone diameters caused by aqueous extracts of *E. croceum*

Sample	Inhibition zone (mm)					
	<i>Staphylococcus aureus</i>	<i>Shigella flexneri</i>	<i>Proteus vulgaris</i>	<i>Klebsiella pneumoniae</i>	<i>Enterococcus faecalis</i>	<i>Salmonella typhi</i>
ECL	15 ± 2.0^A_a	$14.3 \pm 1.2^A_{ab}$	13.3 ± 0.6^A_b	13.7 ± 0.6^A_b	15 ± 1.0^A_a	15.7 ± 0.6^A_a
ECB	13 ± 0.0^B_a	$14.7 \pm 1.5^A_{bc}$	$13.7 \pm 0.6^B_{ac}$	$13.7 \pm 1.2^A_{ac}$	15 ± 1.0^A_b	15 ± 0.0^B_b
Amoxicillin	26.5 ± 0.71^C_a	14.5 ± 2.12^A_b	0 ± 0.00^C_c	13 ± 1.41^A_d	12.5 ± 0.71^B_d	0 ± 0.00^C_c
Sterile normal saline	0 ± 00^D	0 ± 00^B	0 ± 00^C	0 ± 00^B	0 ± 00^C	0 ± 00^C

ECL: *E. croceum* leaves; ECB: *E. croceum* bark; Data expressed as means \pm SD; n = 3; the values along the same row with different subscripts are significantly different ($P < 0.05$). the values in the same column with different superscript are significantly different ($P < 0.05$). Concentration of positive control is 0.0125 mg/mL.

Table 4: Minimum inhibitory concentrations (MIC) of aqueous leaf and stem bark extracts of *E. croceum* against the tested opportunistic bacteria

Sample	MIC (mg/ml)					
	<i>Staphylococcus aureus</i>	<i>Shigella flexneri</i>	<i>Proteus vulgaris</i>	<i>Klebsiella pneumoniae</i>	<i>Enterococcus faecalis</i>	<i>Salmonella typhi</i>
ECL	0.625	1.25	2.5	5	0.625	>5
ECB	1.25	2.5	2.5	5	1.25	>5
Amoxicillin	0.625	1.25	>5	5	5-2.5	>5

Qualitative phytochemical analysis of *E. Croceum*

Phytochemical screening of the *E. croceum* leaves and stem barks aqueous extracts shows the presence of secondary

metabolites investigated, these are phenols, flavonoids, tannins, saponins and alkaloids (Table 5).

Table 5: showing the qualitative phytochemical screening of *E. croceum* leaves and stem barks

Phytochemical constituents	<i>E. croceum</i> leaf extracts	<i>E. croceum</i> bark extracts
Phenol	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Alkaloids	+	+

Key (+) present

Quantitative phytochemical screening

The results of the phytochemical analysis showed varying amounts of analyzed constituents in all the extracts (Figure 1). The aqueous

Leaves extracts showed significantly higher amount of the phytochemical constituents analyzed than the aqueous bark extracts except for total phenols and proanthocyanidins ($P < 0.05$).



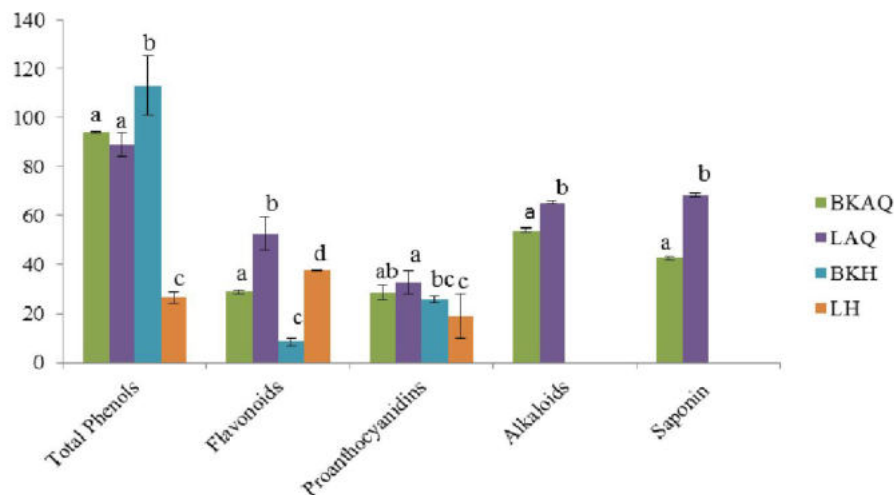


Figure 1: Phytochemical contents of the aqueous and hexane extracts of *E. croceum* Barks and leaves. Bars with different letter superscripts within the same phytochemical content are significantly different ($P < 0.05$). BKAQ: aqueous bark extract, LAQ: aqueous leaf extract, BKH: hexane bark extract, LH: hexane leaf extract

Antioxidant activities

The aqueous leaves extracts showed remarkable free radical scavenging activities compared to the aqueous bark extract. In this study, the antioxidant potentials of the extracts were estimated with their ability to scavenge free DPPH, ABTS radicals and ferric reducing power. The varying concentrations showed increasing scavenging activity with increasing concentration and were used to determine the concentration required to attain 50% DPPH radical scavenging effect (IC_{50}). The aqueous bark extracts revealed higher percentage inhibition of DPPH radical at other concentrations but were not significantly different from the aqueous leaf extract at concentrations of 0.2 and 0.3 mg/ml (Figure 2). The IC_{50} of the DPPH scavenging activities of the sample and standards were in the following order vitamin C < BHT < aqueous

bark extract < aqueous leaf extract (Table 6). The ABTS scavenging activity and IC_{50} of the aqueous leaf and bark extracts were significantly different and lower than the standards in all the concentrations investigated (Figure 3). The aqueous leaf extract showed lower IC_{50} value compared to the aqueous bark extract. The IC_{50} for the ABTS follows the following order ascorbic acid < rutin < BHT < aqueous leaf extract < aqueous bark extract. The ability to reduce Fe^{3+} to Fe^{2+} was also estimated as a measure of antioxidant potentials (Figure 4). The aqueous leaf extract showed significantly higher reducing capacity that was not significantly different from the ascorbic acid at concentrations of 0.03, 0.04 and 0.05 mg/ml. The aqueous leaf extract had the lowest IC_{50} compared to the aqueous bark extract and follow the following order aqueous leaves extract < ascorbic acid < rutin < aqueous bark extract (Table 6).

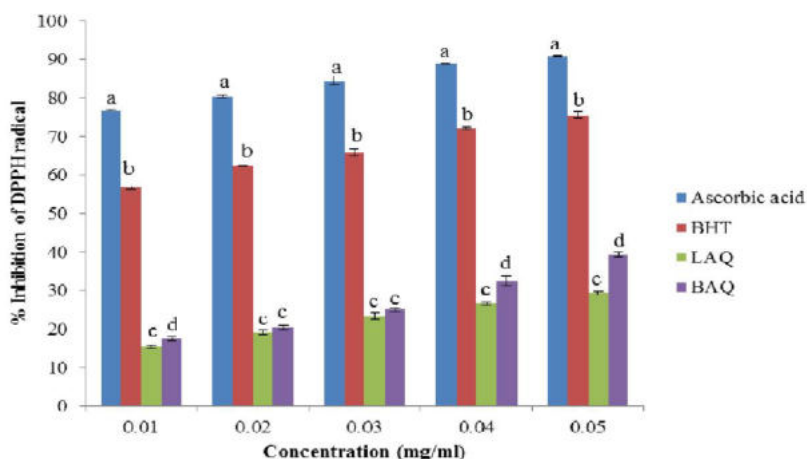


Figure 2: DPPH radical scavenging activity of the aqueous extracts of *E. croceum*. Bars with different letter superscript within the same concentration are significantly different ($P < 0.05$). BAQ: aqueous bark extract, LAQ: aqueous leaf extract.



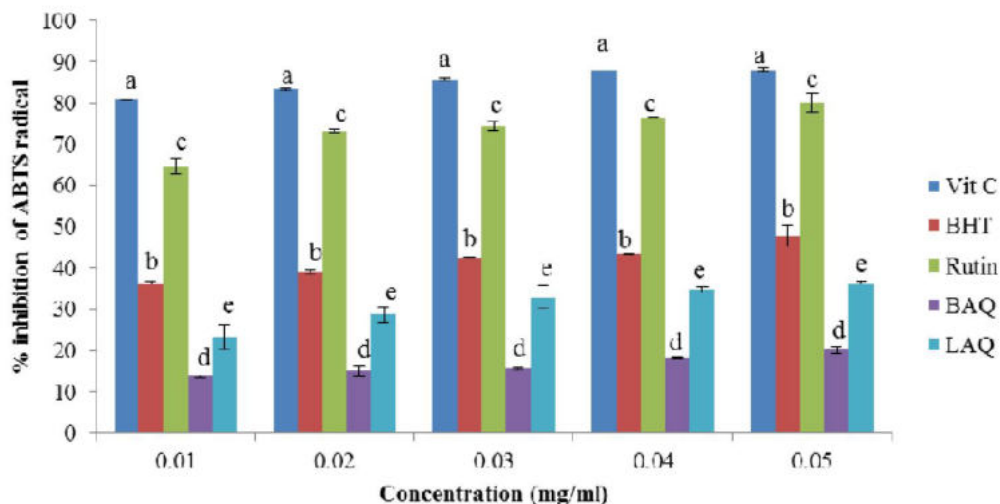


Figure 3: ABTS radical scavenging activity of the aqueous extracts of *E. croceum*. Bars with different letter superscript within the same concentration are significantly different ($P < 0.05$). BAQ: aqueous bark extract, LAQ: aqueous leaf extract

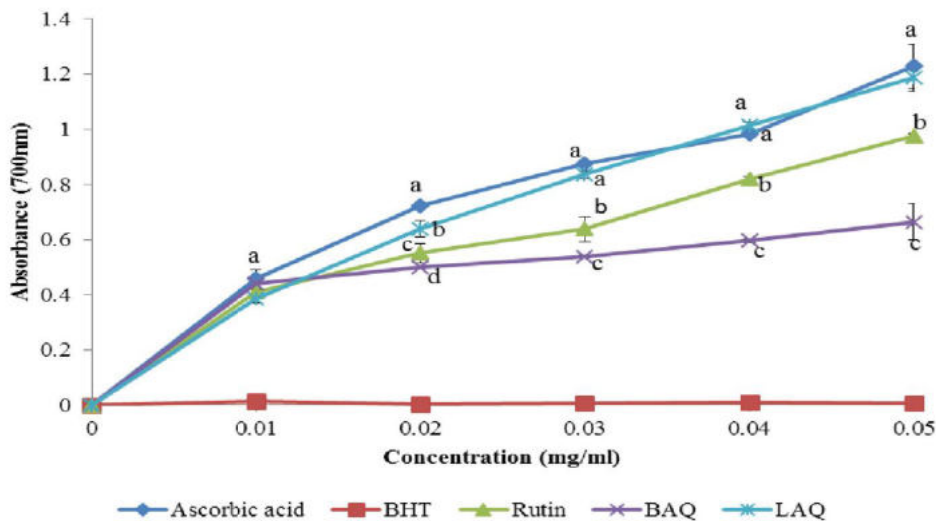


Figure 4: Reducing power of the aqueous extracts of *E. croceum* Barks and leaves. Line points with different letter superscript within the same concentration are significantly different ($P < 0.05$). BAQ: aqueous bark extract, LAQ: aqueous leaf extract

Table 6: Half maximal inhibitory concentrations of *E. croceum* leaf and bark extracts

	FRAP (mg/ml)	DPPH (mg/ml)	ABTS (mg/ml)	Inhibition of protein denaturation(mg/ml)
<i>E. croceum</i> leaf extracts	2.52	0.107	0.088	0.92
<i>E. croceum</i> bark extracts	9.15	0.0714	0.236	1.85
BHT	-	0.026	0.06	-
Ascorbic acid	2.75	0.021	0.021	-
Rutin	3.54	-	0.024	-
Diclofenac	-	-	-	0.31

Key (-) not determined

In Vitro Anti-inflammatory Activity

Both aqueous extracts protected the albumin from denaturation in a dose-dependent manner but were significantly lower from the

standard (Figure 5). The two extracts were not significantly different from each other ($P < 0.05$) but the IC_{50} for the aqueous leaf extract is lower than that of the aqueous bark extract (Table 6).

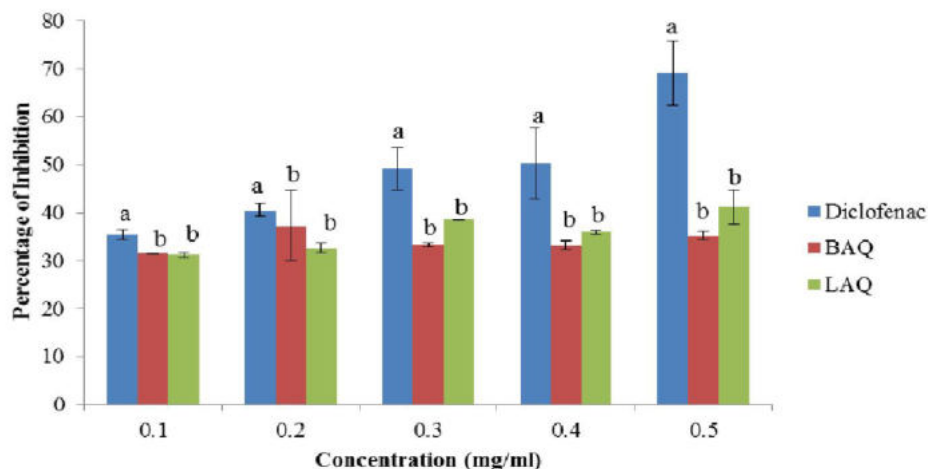


Figure 5: Protein denaturation activity of *E. croceum* aqueous leaf and bark. Bars with different letter superscript within the same concentration are significantly different ($P < 0.05$). BAQ: aqueous bark extract, LAQ: aqueous leaf extract

Discussion

The result of the percentage yield from this study suggests that the aqueous solvent was better for the extraction of *E. croceum*. This explains why only the aqueous solvent investigations were reported in this study. The aqueous leaf and bark extracts of *E. croceum* showed remarkable antibacterial inhibitory properties (Table 3 and Table 4). Both extracts greatly inhibited the growth of both gram-negative and gram-positive bacteria. Notably, when tested against *S. aureus* and *S. flexneri*, aqueous leaf extract showed comparable MIC values with amoxicillin. This suggests that aqueous leaf and bark extracts of *E. croceum* are potential sources of broad-spectrum antibacterial agents. Gram-negative bacteria have been reported to be insensitive to antibacterial agents and have been attributed to the impermeable barrier of the outer membrane of the bacteria and the efficient multidrug efflux pumps that transverse the bacterial membranes [25][26]. The inhibitory activity of the aqueous leaf and bark extracts of *E. croceum* against *P. vulgaris* and *S. typhi* suggests that the bioactive constituents present can weaken these defense mechanisms in gram-negative bacteria. Flavonoids have been reported to exhibit antibacterial activity through the inhibition of bacterial energy metabolism, nucleic acid synthesis, and cytoplasmic membrane functions [27]. One of the isolated flavonoid from *E. croceum* named naringenin has been reported to inhibit CYP3A4 activity in human liver microsomes and the metabolism of the carcinogen, benzo-a-pyrene [5][28][29]. Previously isolated triterpenes from the leaves of *E. croceum* have

been reported to be cytotoxic in different cell lines and has been indicated as a potential source of anticancer agent [5]. The inhibitory activity of the aqueous extract of the bark correlates with earlier report of the toxicity of the bark of *E. croceum* to humans, this may be due to the alkaloids constituents present [11]. Therefore, it is important to isolate and elucidate these compounds and determine their pharmacological properties. Although, it is not clear from this study if these compounds could be acting singly or in combinations to potentiate the plants' potentials. The result of this present study also reveals considerable amount of secondary metabolites. The aqueous extracts showed higher phytochemical contents than the hexane extracts, this may be due to the different extracting capacity of the two solvents. Different model assays were used to investigate the antioxidant capacity of *E. croceum* leaf and bark (Figure 2–4). For instance in DPPH assay, the free radical scavenging activity through proton donating ability was assessed while breaking the free radical chain by reductones present in plant extracts accomplishes the antioxidant ability in FRAP assay. The high phenolic contents of the leaf and bark extracts supports the high antioxidant activities observed. It has been reported that phenolic compounds scavenge free radicals by inhibition of hydroperoxide decomposition into free radicals or by inactivating lipid free radicals [30]. The higher inhibitory percentage of DPPH observed in the bark extract could be as a result of the high phenolic content. It could be said that aside from free radical scavenging activity of aqueous bark extract through proton donating ability in DPPH assay, the antioxidant activity of *E. croceum* aqueous leaf extracts could be accomplished through

reductones, which exert activity by breaking the free radical chain suggested in FRAP assay. Proanthocyanidins are class of oligomeric or polymeric flavan-3-ol units or condensed tannins of the flavonoid family; hence, they have similar mechanism of action as antioxidants as that of flavonoids[31]. Flavonoids and proanthocyanidins exert their antioxidant activities through the hydrogen atom donation, inactivation of metallic ions and suppression of the superoxide-driven Fenton reaction, which is the most common source of reactive oxygen species[31]. Alkaloids are widely distributed secondary metabolites in several plant families as β -carbolinic and have also been reported to possess antioxidant activities. Protein denaturation is caused by inflammatory process and is a well-documented process common in disease pathogenesis and infections. Protection against protein denaturation, which is the main mechanism of action of NSAIDs may play an important role in the anti-rheumatic activity of NSAIDs[32]. Thus, in this study, inhibition of protein denaturation was used as a measure of anti-inflammatory activity. The aqueous extracts of *E. croceum* exhibited minimal protein denaturation inhibitory activity compared to the standard drug. This bioactivity observed in these extracts could be due to the presence of the phytochemical constituents capable of inhibiting the inflammation processes. Polyphenols have been reported to possess anti-inflammatory activities. For instance, flavonoids have been reported to exhibit its anti-inflammatory activities through inhibiting a number of immune system inflammatory mediators [33]. Both alkaloids and proanthocyanidins have also been reported to possess anti-inflammatory activities. Alkaloids block the cyclooxygenase and lipoxygenase metabolic pathways of arachidonic acid metabolism while proanthocyanidins exhibits its anti-inflammatory activities through the inhibition of oxidative stress [34].

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Conclusion

This study demonstrated that aqueous leaf and stem bark extracts of *E. croceum* showed antibacterial activity against antibiotic resistant and sensitive pathogens. The extracts also showed good antioxidant activity which could be due to the phenolic and flavonoid contents. Therefore, the aqueous leaf and bark extracts of *E. croceum* can be used as a natural source of antioxidant and antibacterial compounds supporting the folkloric usage. However, further study is required to investigate the toxicity effect of *E. croceum* and determine its effective dosage using experimental animals. Also, isolation and purification of the bioactive compounds could also be carried out.

Author's contributions

Samuel Odeyemi conceived the idea, Anthony Afolayan supervised the study. Samuel Odeyemi wrote the first draft while both authors gave notable contributions to the design and final writing of the manuscript.

Acknowledgements

The authors appreciate Govan Mbeki Research and Development Centre (GMRDC) for their financial support.

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