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RESEARCH ARTICLE

# Antimicrobial and anti-inflammatory properties of *Funtumia elastica*

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## Abstract

**Context:** *Funtumia elastica* (Preuss) Stapf. (Apocynaceae) has a long ethnopharmacological history for uses such as treatment of whooping cough, asthma, blennorhea, painful menstruation, fungal infections, and wounds.

**Objective:** To investigate the antimicrobial and anti-inflammatory properties of ethanol extracts from the leaves and stem bark of *Funtumia elastica* based on its ethnopharmacological uses and also determine the secondary metabolites present in the extracts.

**Materials and methods:** The antimicrobial activities of ethanol leaf and bark extracts of *F. elastica* were determined using the microdilution technique (MIC determination) and agar diffusion method using 10, 25, and 50 mg/mL concentrations against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger* as test organisms. Anti-inflammatory activities of the doses of extracts at 30, 100, and 300 mg/kg per body weight were determined by carrageenan-induced edema in the footpad of 7-day-old chicks and the foot volumes measured at hourly interval post-treatment for 5 h.

**Results:** The MIC ranges of both ethanol leaf and bark extracts against the test organisms were 125 (lowest MIC) to 1550 µg/mL (highest MIC) and 125 (lowest MIC) to 1750 µg/mL (highest MIC), respectively. The ethanol leaf and bark extract of *F. elastica* showed significant anti-inflammatory activity ( $p \leq 0.001$ ) at 30, 100 and 300 mg/kg. Preliminary phytochemical screening revealed that *F. elastica* bark contains hydrolysable tannins, sapogenetic glycosides, steroids and saponins while the leaves contain hydrolysable tannins, flavonoids, starch and alkaloids. Tannin contents of the leaf and stem bark were 2.4 and 1.3% w/w (related to the dried material), respectively.

**Discussion and conclusion:** Both ethanol leaf and bark extracts of *F. elastica* showed antimicrobial and anti-inflammatory activities and these pharmacological properties may be responsible for the ethnomedicinal uses of the leaves and stem bark of the plant.

**Keywords:** Inflammation, bacteria, fungi, antibacterial, antifungal, Apocynaceae

## Introduction

There has been increase in antibiotic resistance and reports of treatment failures with antibiotic therapy in both humans and other animals over the past recent years (Zetola et al., 2005) especially the appearance of methicillin-resistant *Staphylococcus aureus* (MRSA) and multiple drug-resistant strains of *Salmonella typhi*. It has been estimated that in the United States of America alone, at least 18,000 people die every year from drug-resistant infections (Alderman & Hastings, 2003). It is

estimated that about 19,000 people in the United States die every year from MRSA (Klevens et al., 2007) and in 2004, 63% of all reported *Staphylococcus* infections in the United States were caused by MRSA (CDC, 2007). Methicillin was developed as an alternative treatment for the increasing rate of *Staphylococcus* infections already resistant to penicillin. At that time, about 60% of all *Staphylococcus* infections were resistant to penicillin. Hence, traditional antibiotics are not being used for the treatment of methicillin-resistant *Staphylococcus*

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infections (Micet, 2007) but rather “last-resort” intravenous vancomycin and other newer antibiotics are being used or prescribed for the treatment of MRSA infections consequently intensive use of antibiotics often resulted in the development of resistant strains (Tenover, 2006). There is a need for a search for new antibiotics from other sources including medicinal plants.

Inflammation refers to an innate, nonspecific series of highly interrelated series of events that are set into motion in response to foreign invasion, tissue damage or both with the ultimate goal of bringing to the invaded tissue or infected area, phagocytes and plasma proteins that isolate, destroy or inactivate the invaders, remove debris, and prepare for subsequent healing and repair (Sherwood, 2006). Inflammation and infection are frequently accompanied by imbalance in the intestinal microflora. Inflammation is one of the responses of the individual to pathogenic invasion and occurs in most bacterial infections and disorders of the cardiovascular, respiratory, lymphatic, genitourinary, nervous and dermatological systems. A strong inflammatory response may then be mounted to microfloral bacteria, leading to perpetuation of the inflammation and gut barrier dysfunction (Isolauri et al., 2002). For instance, *Helicobacter pylori* resides in the gastric mucus layer of humans and induces a chronic inflammatory response that can result in both peptic ulceration and gastric neoplasms (Baser, 1993) and chronic colonization of the lower respiratory tract by bacterial pathogens induces a chronic inflammatory response with lung damage (Sethi, 2000). Hence, any bioactive agent with antimicrobial and anti-inflammatory properties may be helpful in the above disease conditions. A survey conducted by United Nations Conference on Trade and Development (UNCTAD)-General Agreement on Tariffs and Trade (GATT) showed that 33% of total drugs produced by pharmaceutical industries are derived from plants and microbes, 60% of medicinal products are natural origin (Srinivasan et al., 2001). Some traditional orthodox anti-inflammatory agents have been demonstrated to exhibit antibacterial activity (Annadurai et al., 1998; Dutta et al., 2004) and hence any bioactive agent possessing both properties may be beneficial to patients especially those suffering from acute inflammation and bacterial infections at the same time. Some medicinal and pharmacological values have been observed in different species of Cola (Daels-Rakotoarison et al., 2003; Steinegger & Hansel, 1992).

Medicinal plants have been used in the management of various bacterial infections and inflammatory conditions and some of them have been found to possess both antimicrobial and anti-inflammatory properties. For example, crude extracts of *Dodonaea viscosa* (L.) Jacq. (Sapindaceae), *Rumex nervosus* Vahl. (Polygonaceae) and *Rumex abyssinicus* Jacq. (Polygonaceae) (Getie et al., 2003) and *Mallotus peltatus* (Geist) Muell. Arg. (Euphorbiaceae) leaf extract (Chattopadhyay et al., 2002) have been shown to exhibit both antimicrobial and anti-inflammatory activities and an ethanol extract

of *Desmodium caudatum* (Thunb.) DC. (Leguminosae), traditionally used for the treatment of various forms of pains, has been found to possess analgesic, anti-inflammatory and antipyretic properties (Ke-Jia et al., 2011). *Funtumia elastica* (Preuss) Stapf. (Apocynaceae), known commonly as silk rubber (local Asante-Twi name is “Frumtum/Ofruntum”), is traditionally used to treat whooping cough (Burkill, 1995), inflammatory diseases such as asthma, blennorrhoea, and painful menstruation (Olaniyi, 1989), cutaneous fungal infections, hemorrhoids, syphilis, gonorrhoea (Odugbemi, 2006; Burkill, 1995) and wounds (Adekunle & Ikumapayi, 2006). Some steroidal alkaloids (holarrhetine, conessine, holarrhesine and isoconessimine) have been isolated from the stem bark and conanine group, namely, irehdiamine A and D, irehamine, conkuchine and irehine from leaves of *F. elastica* (Zirihi et al., 2005). This study investigated the antimicrobial and anti-inflammatory activities of ethanol stem bark and leaf extracts of *F. elastica* based on its ethnobotanical uses. Preliminary phytochemical screening for secondary metabolites was also performed.

## Materials and methods

### Plant material and chemicals

Stem bark and leaves of *Funtumia elastica* were collected in July, 2007 from the Bobiri Forest Reserves of the Forestry Research Institute of Ghana (FORIG) near Kubease, Ashanti Region, Ghana, and identified by Dr. A. Asase, Department of Botany, University of Ghana, Ghana. A voucher specimen of the plant (number AA 113) has been deposited at Ghana Herbarium, University of Ghana, Ghana. Unless stated otherwise, all the chemicals were purchased from Sigma (Deisenhofen, Germany).

### Preparation of extracts

The plant materials were air-dried and powdered. Powdered materials (200g) of *Funtumia elastica* were extracted with 70% ethanol using a Soxhlet apparatus. The ethanol extracts obtained were evaporated to dryness under reduced pressure and kept in a desiccator. The yield of the stem extracts were 5% w/w while leaves yielded 19.8% w/w. Quantities of the leaf extract (FLE) and stem bark extract (FBE) were dissolved in normal saline and dimethyl sulfoxide (DMSO) for acute anti-inflammatory and antimicrobial determinations, respectively.

### Preliminary phytochemical screening

Phytochemical screening was conducted on both leaf and stem bark extracts of *F. elastica* to ascertain the presence of starch, tannins, glycosides (sapogenetic, anthracene, and cyanogenetic), flavonoids, steroids and alkaloids (Wagner & Bladt, 1996; Harborne, 1998). The presence of flavonoids, alkaloids and carbohydrates in the extracts were detected by thin-layer chromatography (TLC) as described by Wagner and Bladt (1996). The presence of flavonoids, glycosides, tannins and carbohydrates in the extracts (FLE and FBE) were determined

using a TLC method with mobile phase (EtOAc/HCOOH/H<sub>2</sub>O, 90/5/5) detected with 1% Naturstoff, 1% ferric chloride and thymol-H<sub>2</sub>SO<sub>4</sub> reagents, respectively. The TLC plate (silica gel 60 F<sub>254</sub>) used in the detection of carbohydrates were heated at 105°C for 5 min after spraying with thymol-H<sub>2</sub>SO<sub>4</sub>. The alkaloids were extracted with H<sub>2</sub>SO<sub>4</sub> and made basic (alkaline) with the addition of NH<sub>3</sub> before using a TLC method with mobile phase (EtOAc/HCOOH/H<sub>2</sub>O, 90/5/5) to detect the presence of alkaloids with Dragendorff's reagent. The presence of saponins in the extracts was determined by the frothing method. In addition to the TLC method for the detection of glycosides, the extracts were also added to dilute H<sub>2</sub>SO<sub>4</sub> and boiled for 5 min. They were then filtered, cooled and 20% of NaOH was added to the extracts. Fehling solutions A&B were also added and the result observed.

The tannin content was determined according to the method of Glasl (1983) using pyrogallol (Merck, Darmstadt, Germany, purity 99.5%, HPLC) as reference compound. The powdered plant material, 750 mg (m<sub>1</sub>) was weighed into a round bottom flask with 150 mL of distilled water and heated at 90°C for 30 min and then cooled under running water. The aqueous extract was transferred into a 250 mL narrow necked volumetric flask and the volume made up to 250 mL with distilled water. The extract (30 mL) was centrifuged (3000 × g, 10 min). The clear solution was labeled as the stock solution. The filtrate (5 mL) (stock solution) was pipetted into a 25 mL volumetric flask and then diluted with distilled water to 25 mL (Total Phenol Solution, TPS). The filtrate (10 mL) was added to 100 mg of hide powder for determination of tannic acid, slightly chromated (Merck Darmstadt, Germany), shaken for 60 min, and filtered. The filtrate (5 mL) was diluted to 25 mL with distilled water (Remaining Phenol Solution, RPS). Pyrogallol (0.05 g) (m<sub>2</sub>) was added to 100 mL distilled water and 5 mL of the resultant solution was diluted to 100 mL with distilled water (Reference Solution, RS). One mL Folin-Ciocalteus phenol reagent (Sigma) was added to 2 mL of TPS and 10 mL of distilled water were added. The resultant solution was diluted to 25 mL with 14.06% w/v sodium carbonate solution (Merck, Darmstadt, Germany). The above procedure was repeated for RPS and RS. After 30 min, the absorbances (A) of the solutions were measured at 760 nm after the absorbance of distilled water was zeroed. The percentage of tannin content was determined by the following formula:

$$(\%) = \frac{(62.5 \times (A_{\text{total phenol solution}} - A_{\text{remaining phenol solution}})) \times m_2}{A_{\text{reference solution}} \times m_1}$$

where, A, measured absorbance at 760 nm; m<sub>1</sub>, mass of plant material; m<sub>2</sub>, mass of pyrogallol (reference material). The average of three separate measurements (A) was used in the calculation.

## Determination of antimicrobial activity

### Agar diffusion method

The antimicrobial activities of the extracts (FLE and FBE) and reference drugs (chloramphenicol and clotrimazole) (Sigma, Deisenhofen, Germany) were determined using the agar diffusion method. Nutrient agar (Oxoid Limited, United Kingdom) and Sabouraud agar (Oxoid Limited, United Kingdom) media were used for both determinations of antibacterial and antifungal activities, respectively. The test organisms (1 × 10<sup>6</sup> cfu/mL) (100 µL) (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* NCTC 10073 and clinical fungal agents *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus*) were used to seed nutrient agar and Sabouraud agar plates, respectively. In each of these plates, 4 equidistant wells (8 mm) were cut out using sterile cork borer and wells filled with different concentrations of extracts and reference drugs dissolved in DMSO and allowed to diffuse at room temperature (28–30°C) for 1 h. The zones of growth inhibition measured after 24 h incubation at 37°C (for bacteria) and 3 days at 30°C (for fungi). The activity of solvent alone (DMSO) was determined and was found to exhibit no antimicrobial activity against the test organisms.

### Microdilution method

The MIC of both extracts (FLE and FBE) against the test bacteria was determined using the modified microdilution technique as described by Eloff (1998). Test solutions (100 mg/mL) of both extracts were prepared with DMSO and test solution (25–100 µL) was serially diluted to 100 µg/mL and 100 µL (1 × 10<sup>6</sup> cfu/mL) of the test bacteria grown in nutrient broth (Oxoid Limited, United Kingdom) added to each well in the microplates. The covered microplates were incubated at 37°C for 24 h. To indicate growth, 30 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT, thiazolyl blue] dissolved in water was added to the microplate wells and incubated at 37°C for 30 min. Test fungal agents were cultivated in Sabouraud dextrose broth (Oxoid Limited, United Kingdom) and then incubated for 3 days at 30°C. The MICs of FLE and FBE against the test fungi were determined according to the guidelines described in the National Committee for Clinical Laboratory Standards (1998) for filamentous fungi. The minimum inhibitory concentration of the extract against the test organisms was detected as the minimum concentration of extracts that did not exhibit microbial growth after the addition MTT to the medium and incubation at 37°C for 20 min (Berridge et al., 2005). The growth or nonsurvival of the test organisms with various concentrations of the extracts after the addition of the MTT were compared to the untreated cells (organism without extracts) and the MIC determined as described by Eloff (1998). The above experiments were repeated three times.



### Determination of acute anti-inflammatory activity

The carrageenan-induced inflammatory model in 7-day-old chicks (Roach & Sufka, 2003) was employed and the responsiveness of these chicks to anti-inflammatory drugs was determined.

#### Experimental animals

Day old cockerels *Gallus gallus* (Strain: Shaver 579) purchased from Darko Farms Company Limited, Kumasi, Ghana, were maintained in the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The chicks were housed in stainless steel cages and fed with normal commercial poultry diet (GAFCO, Tema, Ghana), given water *ad libitum* and maintained under laboratory conditions (temperature 28–30°C, relative humidity 60–70%, and normal light-dark cycle). A day before the experiment, the chicks were brought to the laboratory and habituated to experimenter handling and the apparatus to minimize the effect of stress and novelty. The chicks were used on day 7. All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health Services publication No. 83–23, revised 1985). The protocols for the study were approved by the Departmental Ethics Committee.

#### Carrageenan-induced edema model

The initial foot volumes of the chicks were measured using a plethysmometer (IITC Life Science Inc., California, USA) after which 0.01 ml of 2% carrageenan was injected into the plantar of the right foot to induce inflammation. The inflammation produced was measured, the increase in foot volume calculated and those with an increase between 15–40% were selected and put into thirteen groups of five after which they were injected intraperitoneally with either diclofenac (Sigma, purity 98% HPLC) (10, 30 and 100 mg/kg) or dexamethasone (Sigma, purity 98% HPLC) (0.25, 0.5 and 1.0 mg/kg) based on recommended effective human doses per body weight, and FLE (30, 100 and 300 mg/kg), or FBE (30, 100 and 300 mg/kg) given orally based on preliminary investigation and the oral route of administration for the traditional uses of the plant for management of pain. One group, the no treatment group, did not receive any drug (control). Foot volumes were measured again at hourly intervals post-treatment for 5 h. A period of 1 h for oral and 30 min for intraperitoneal route were allowed for absorption of the drugs and extracts before first measurements. The percentage change in foot volume after induction, and treatment of inflammation was calculated and recorded for analysis.

In order to simulate tradition usage of the herbs and the difficulty in administering herbal preparations through the parenteral route, the oral route was chosen. However, for most studies, the parenteral route is

preferred for the reference drugs. The intraperitoneal was therefore chosen for the reference drug because of its closeness to the oral as both routes of administration are subject to absorption and hepatic metabolism. The difference between the two routes will be in the time of absorption which is compensated for by 1 h delay for oral and 30 min for intraperitoneal route before measurements were taken.

#### Statistical analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses.  $p < 0.05$  was considered statistically significant in all analysis. The graphs were plotted using Sigma Plot for Windows Version 11.0 (Systat Software Inc., Germany).

## Results

### Preliminary phytochemical screening

FLE has hydrolysable tannins, flavonoids, alkaloids, steroids, saponins and carbohydrate while FBE contains hydrolysable tannins, sapogenetic glycosides, cyanogenetic glycosides, steroids, and saponins. The tannin content of leaf and stem bark were 2.4 and 1.3% w/w (related to the dried material), respectively (Table 1).

### Antimicrobial activity

Both ethanol extracts (FLE and FBE) were found to be active against the test bacteria (*E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis*) and *C. albicans*. *A. niger*, and *A. flavus* were found to be less susceptible to both extracts (Table 2). The minimum inhibitory concentration ranges of FLE and FBE against the test organisms were 125–1550 and 125–1750 µg/mL, respectively (Table 3).

### Acute anti-inflammatory activity

It was found that all doses of FLE, FBE, dexamethasone and diclofenac caused significant ( $p \leq 0.001$ ) dose-dependent reduction in foot volume of the chicks from the first to the fifth hour post-treatment (Figures 1, 2, 3, and 4, respectively). AUC values reveal that diclofenac reduced the carrageenan-induced inflammation better than dexamethasone. FBE (30, 100 and 300 mg/kg) was found to exhibit higher anti-inflammatory effect than FLE (30, 100 and 300 mg/kg) (Figures 1 and 2) and these effects were comparable to the effects of the reference anti-inflammatory agents.

## Discussion

Both ethanol extracts (FLE and FBE) of *F. elastica* exhibited inhibitory activity against the test organisms, namely, *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *C. albicans*, *A. flavus* and *A. niger* (Table 2), and these test organisms were used to determine the spectrum of activity (Gram negative and positive bacteria and fungal pathogens) of the extracts and also their involvement in various

Table 1. Preliminary phytochemical screening of ethanol leaf (FLE) and stem bark (FBE) extracts of *F. elastica*.

Secondary metabolites	Alkaloids	Saponins	Flavonoids	Steroids	Carbohydrates	Sapogenetic glycosides	Tannins (% w/w)
Extract							
FLE	+	+	+	+	+	-	2.4
FBE	-	+	-	+	+	+	1.3

+, presence of secondary metabolite; -, absence of secondary metabolites.

Table 2. Antimicrobial activity of ethanol leaf extract (FLE) and ethanol stem bark extract (FBE) of *F. elastica* by agar diffusion method.

Extract (mg/mL)	Bacteria			
	<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> NCTC 10073	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853
FLE				
10	0.0	0.0	11.50±0.17	0.0
25	0.0	13.82±0.98	15.91±0.23	0.0
50	13.84±0.13	17.17±0.76	17.20±0.76	12.50±0.87
FBE				
10	0.0	0.0	0.0	0.0
25	0.0	14.15±0.14	16.12±0.42	0.0
50	14.60±0.16	16.50±0.25	17.17±0.51	12.64±0.21
CPC	24.12±0.13	25.16±0.14	27.17±0.15	22.50±0.16
	Fungi			
	<i>C. albicans</i>	<i>A. niger</i>	<i>A. flavus</i>	
FLE				
10	0.0	0.0	0.0	
25	0.0	0.0	11.53±0.13	
50	14.52±0.13	11.65±0.46	11.60±0.17	
FBE				
10	0.0	0.0	0.0	
25	11.56±0.16	0.0	0.0	
50	14.52±0.32	0.0	11.20±0.12	
CTZ	23.12±0.24	17.50±0.12	20.50±0.15	

Mean zones of growth inhibition are mean (mm) of zones of inhibition of three independent experiments, mean ± SD, n = 4 replicates, diameter of well/cup = 8 mm, reference antimicrobial agents: CPC, chloramphenicol (1 mg/mL), CTZ, clotrimazole (1 mg/mL). The DMSO (alone) used as solvent did not show any antimicrobial activity against the test organisms.

Table 3. Minimum inhibitory concentration (MIC) (mg/mL) of ethanol leaf extract (FLE) and ethanol stem bark extract (FBE) of *F. elastica* determined by microdilution method.

Extract/MIC (mg/mL)	<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> NCTC 10073	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>C. albicans</i>	<i>A. niger</i>	<i>A. flavus</i>
FLE	0.125	0.125	0.125	1.55	1.25	1.50	1.50
FBE	0.125	0.250	0.250	1.75	1.50	1.75	1.55
CPC	0.025	0.020	0.025	0.055	nd	nd	nd
CTZ	nd	nd	nd	nd	0.025	0.055	0.075

The experiments were repeated three times. The DMSO (alone) used as solvent did not exhibit any antimicrobial activity against the test organisms. Reference antimicrobial agents: CPC, chloramphenicol, CTZ, clotrimazole. Nd, Not determined.

microbial infections including serious systemic infections and opportunistic infections caused by *C. albicans*. In persons infected with HIV, *S. aureus* is responsible for invasive skin diseases including superficial and follicular lesions such as boils, carbuncles, etc. The MIC range of FLE against both bacterial and fungal test organisms from 125 to 1550 µg/mL and that of FBE was from 125 to 1750 µg/mL (Table 3). This may indicate that the bioactive agent(s) responsible for the antimicrobial activity are present in both the leaves and stem bark.

With the agar diffusion method, concentrations of both FLE and FBE equal or less than 10 mg/mL was found to

exhibit no inhibitory against the test pathogens except *E. coli* and this may be attributed to the fact that the extracts were not fully diffusible into the agar medium (Cos et al., 2006; Eloff, 1998). *P. aeruginosa* was found to be the least susceptible bacterium to extracts with higher MICs for both extracts (Table 3) compared to the other test bacteria and this was not surprising since *P. aeruginosa* has been found to resistant to most antibiotics (Lambert, 2002). *S. aureus*, *B. subtilis* and *E. coli* were more susceptible to both extracts with a MIC range of 125 to 250 µg/mL. Even with the agar diffusion technique, high concentrations of extract (50 mg/mL) exhibited activity against both test

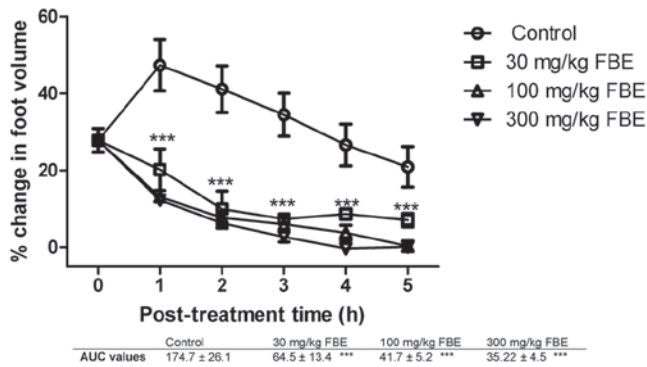


Figure 1. The effects of 30, 100, and 300 mg/kg of ethanolic stem bark extract (FBE) on carrageenan-induced inflammation in chicks. \*\*\*implies  $p < 0.001$  which signifies a significant reduction in foot volume at all dose levels. Data are presented as mean  $\pm$  SEM (n = 5), analyzed by one-way ANOVA followed by Newman-Keuls test for column graphs. Control is the untreated group.

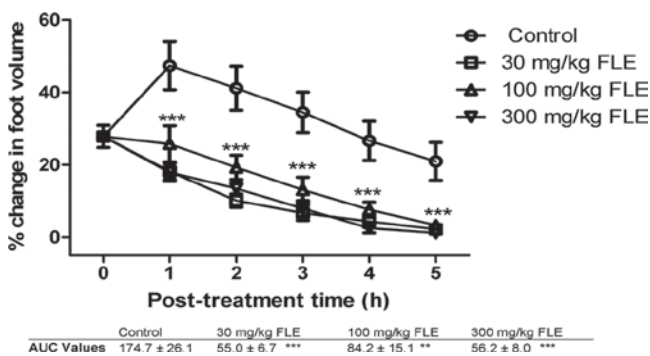


Figure 2. The effects of 30, 100, and 300 mg/kg of ethanolic leaf extract (FLE) on carrageenan-induced inflammation in chicks. \*\*\*implies  $p < 0.001$  which signifies a significant reduction in foot volume at all dose levels. Data are presented as mean  $\pm$  SEM (n = 5), analyzed by one-way ANOVA followed by Newman-Keuls test for column graphs. Control is the untreated group.

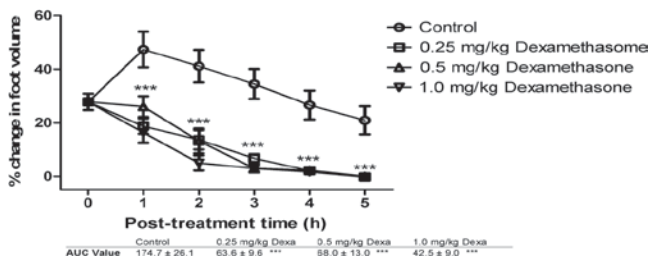


Figure 3. The effects of 0.25, 0.5, and 1.0 mg/kg of dexamethasone on carrageenan-induced inflammation in chicks. \*\*\*implies  $p < 0.001$ , which signifies a significant reduction in foot volume at all dose levels. Data are presented as mean  $\pm$  SEM (n = 5), analyzed by one-way ANOVA followed by Newman-Keuls test for column graphs. Control is the untreated group.

bacteria and fungus. Concentrations equal or less than 25 mg/mL did not show antimicrobial activity against *A. niger* and *A. flavus* except *C. albicans* with a zone of growth inhibition of 3.5 mm (Table 2). This may be attributed to the poor diffusion of the extracts into both solid media.

The activities of the ethanol extracts from the stem bark and leaves against selected bacteria and fungi were found to be almost the same and this may account for

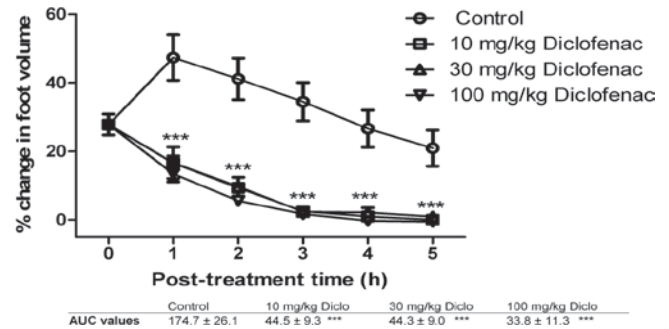


Figure 4. The effects of 10, 30, and 100 mg/kg of diclofenac on carrageenan-induced inflammation in chicks. \*\*\*implies  $p < 0.001$ , which signifies a significant reduction in foot volume at all dose levels. Data are presented as mean  $\pm$  SEM (n = 5), analyzed by one-way ANOVA followed by Newman-Keuls test for column graphs. Control is the untreated group.

the application of extracts from leaves and bark for the treatment of similar infections (Sonibare et al., 2009). The antimicrobial activity exhibited by *F. elastica* extracts may be responsible for its use as agent for the treatment of wounds and venereal diseases such as syphilis and gonorrhoea. In fact, conessine, a steroid alkaloid found in *F. elastica*, could possibly account for its strong antibacterial properties (Zirihi et al., 2005; Sonibare et al., 2009). The MICs of the FLE extract against the test organisms were found to be lower than that of FBE extract. For the agar diffusion method, the FBE extract exhibit relatively higher activity in terms of zones of growth inhibition compared to the FLE extract and this may be due to the inability of the bioactive components of FLE to diffuse into the agar (Cos et al., 2006).

Carrageenan induces acute inflammation mediated by cytokines [interleukin (IL)-1 and tumor necrosis factor (TNF)- $\alpha$  which cause vasodilatation, increased vascular permeability, and fluid exudation], and proinflammatory agents [PGE<sub>2</sub> and PGI<sub>2</sub> generated by the local tissues and blood vessels, PGD<sub>2</sub> released mainly by mast cells, and leukotriene (LT) B<sub>4</sub> a chemotaxin] in the foot of the chick, causing the foot volume to increase.

The level of FLE anti-inflammatory activity was not proportional to increases in the doses of the extract as the least dose of 10 mg/kg body weight was more effective at reducing the edema than high doses of 30 and 100 mg/kg body weight (Figure 2) which may be due to receptor desensitization or the presence of competing or antagonistic compounds in the extract.

The significant reduction in foot volume cause by FBE and FLE is a clear indication of anti-inflammatory effect comparable to dexamethasone and diclofenac. The mechanism of anti-inflammatory effect of FLE and FBE may possibly be similar to that for dexamethasone because the phytochemical screening showed that the extracts contained steroidal components. Dexamethasone is a steroidal anti-inflammatory agent which prevents phospholipase A<sub>2</sub> (PLA<sub>2</sub>) from hydrolyzing arachidonic acid from phospholipids in cell membrane with a resultant reduction in the production of the prostaglandins

(PGE<sub>2</sub>, PGI<sub>2</sub> and PGD<sub>2</sub>) and thromboxanes. Diclofenac is a nonsteroidal anti-inflammatory agent (NSAID) that also reduces the production of prostaglandins and thromboxanes from arachidonic acid by inhibiting cyclooxygenase enzymes (Rang et al., 2007).

Extracts from several medicinal plants have been found to inhibit both acute and chronic phases of inflammation (Abad et al., 1996; Mostafa et al., 2010). The anti-inflammatory property observed for *F. elastica* bark extract could be due to the steroids such as cyclofuntumienol and cycloeculalenol present in the leaves and stem bark (Zirihi et al., 2005). Phytosterols have iscosmodulating effects, normalizing an over-reactive antibody response as well as anti-inflammatory properties.

## Conclusion

Both ethanol extracts of *F. elastica* (FBE and FLE) exhibited significant antimicrobial activity with MIC ranges of 125–1550 µg/mL and 125–1750 µg/mL, respectively, against the test organisms, and anti-inflammatory (30, 100, and 300 mg/kg) activities. This may justify its folkloric use for the treatment of wounds and various forms of pains. Further bioactivity-guided fractionation and isolation of the bioactive compounds responsible for the antimicrobial and anti-inflammatory activities would be performed.

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## Declaration of interest

The authors report no conflicts of interest.

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