Chemistry Africa (2021) 4:503–512
https://doi.org/10.1007/s42250-021-00249-5

ORIGINAL ARTICLE

Phenolic Content, Antioxidant Properties and Antimicrobial Activities of the Extracts from *Funtumia africana* and *Funtumia elastica*

Theophilus Fadjare Frempong[1] · Mercy Badu[1] · Vivian Etsiapa Boamah[2] · Isaac Kingsley Amponsah[3] · Blessed Agbemade[1] · Ransford Appianin Boateng[1] · Nathaniel Owusu Boadi[1]

Received: 27 January 2021 / Accepted: 27 April 2021 / Published online: 6 May 2021

Abstract
Funtumia africana and *Funtumia elastica* belong to a group of invaluable plants used in managing a wide range of diseases in West Africa. This study aimed at assessing the antioxidant and antimicrobial activities of extracts of the stem bark of *F. africana* and *F. elastica*. The dried, milled stem barks of *F. africana* and *F. elastica* were extracted with water and 70% ethanol by cold maceration. Total antioxidant capacity (TAC), total phenolic content (TPC), total flavonoids, and tannins were determined using standard methods. The extracts were tested against bacterial and fungal pathogens, namely; *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi,* and *Candida albicans*. The zone of inhibition, minimum inhibitory concentration (MIC), activity index, and the total antimicrobial activity of the extracts on the organisms were evaluated. Preliminary phytochemical screening revealed the presence of flavonoids, reducing sugars, anthocyanins, terpenoids, cardiac glycosides, quinones, tannins, and saponins. The ethanolic extract of *F. elastica* had the highest phenolic flavonoid contents and total antioxidant capacity. A positive correlation was observed between TPC with TAC ($R=0.893$, $p<0.05$) and TFC with TAC ($R=0.613$, $p<0.05$). The extracts were most active against *E. coli* and *C. albicans* and least active against *B. subtilis* and *P. aeruginosa*. The data obtained from the study indicated that the barks of the two plants possessed both antioxidant and antimicrobial properties.

Keywords Total phenols · Antioxidants · Oxidative stress · Extracts · Natural products

1 Introduction

Recent studies have shown the importance of natural products in managing many degenerative diseases such as cancer, arteriosclerosis, diabetes, coronary diseases, and arthritis. The World Health Organization (WHO), has also reported that over 80% of the world's population uses traditional medicine to address their primary healthcare needs [1].

✉ Mercy Badu
mbadu0@gmail.com

[1] Department of Chemistry, Faculty of Physical and Computational Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

[2] Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences (FPPS), KNUST, Kumasi, Ghana

[3] Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences (FPPS), KNUST, Kumasi, Ghana

The *Funtumia* species belongs to the family Apocynaceae, consisting of about 424 genera and 4,600 species distributed worldwide, with well documented anecdotal uses, especially in West Africa [2]. Different plants from this genus are used extensively in folklore medicine to mitigate diverse disease conditions [3]. Funtumia species elaborate a chemically diverse group of metabolites with well-documented antimicrobial, antidiabetic, anticancer, and antioxidant properties. *Funtumia africana* (Benth) Stapf and *Funtumia elastica* (Preuss) Stapf, with common names "False" rubber tree and West African silk-rubber tree respectively [4], are native to Ghana and other parts of West Africa. In Ghana, *F. africana* and *F. elastica* are locally called funtum with *F. africana* described as the "male" and *F. elastic*a, the "female" [5]. Different parts of these two plants have been used in preparations, decoctions, infusions and sometimes used in their raw state for the treatment of diseases such as urinary tract infections, jaundice, chronic wounds, whooping cough, asthma, blennorrhoea, painful menstruation, fungal infections and haemorrhoids [4]. Extracts from different parts of *F. africana* and *F. elastica* have been reported to contain mixtures of

complex secondary metabolites with good pharmacological properties [6–9]. Research has shown that many natural products contain secondary metabolites with specific antioxidant properties. The content of natural product antioxidants is dependent on the extraction technique and the type of solvent used. Secondary metabolites, such as tannins, phenols, flavonoids, and saponins which are considered major secondary metabolites in plants, usually accumulate in most parts of the plant, including the leaves, stem bark, roots, and fruits.

Phenolic compounds exhibit a wide range of biochemical properties that have contributed to the interest in studying many plant medicines. They are reported to have pharmacological properties, such as radical scavenging property leading to anti-inflammatory, anti-aging, and antimicrobial activities [10]. They can be classified as alkali-soluble or alkali-insoluble phenolic compounds [11]. Most phenolic compounds are soluble in water, methanol, ethanol, and ethyl acetate [12]. To effectively utilize the bioactive compounds from plants, it is critical to consider the extraction process because of the complex nature of the compounds. During the extraction process, some important parameters to be considered are the type of solvent, solid –to-solid ratio, time of extraction, temperature, particle size of the sample, and the number of extractions. For example, during an extraction process, the nature of the target bioactive compound is critical to inform the choice of solvent. According to studies conducted, the extraction of phenolic compounds is widely affected by the solvent's polarity [13–15]. Low polarity solvents such as ethyl acetate are less efficient than water and ethanol, which are high polar solvents [16]. Also, in polyphenols' extraction, many studies have discussed the effectiveness of boiling water and ethanol on the extraction yield [17–19]. In this study, the stem bark of *F. africana* and *F. elastica* were extracted with water and 70% ethanol/water mixture as solvents and analyzed for their antioxidant and antimicrobial activities.

In Ghana and other parts of West Africa, most of the preparations and concoctions from the stem barks of plants including *F. africana* and *F. elastica* are made by boiling in water or soaked in some kind of alcoholic beverage. Even though there are few literature reports on the extraction of these compounds following these traditional methods, there are several accounts on the effectiveness of such preparations by indigenes in towns and villages where these trees are prevalent. This study, therefore, seeks to assess the antioxidant and antimicrobial activities of the water and 70% ethanolic extracts from *F. africana* and *F. elastica.*

2 Materials and Methods

2.1 Reagents

All chemicals and reagents used were of analytical grade. Folin-Ciocalteu, catechin, gallic acid, quercetin, ascorbic acid, vanillin, aluminium (III) chloride hexahydrate, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT], ethanol, hexane, chloroform, ciprofloxacin, ketoconazole, disodium hydrogen phosphate, ammonium molybdate, sulphuric acid, sodium carbonate, sodium hydroxide, aluminium chloride, sodium nitrite, and hydrochloric acid were all obtained from Sigma-Aldrich. Nutrient agar and nutrient broth (Oxoid, United Kingdom) were purchased from suppliers.

2.2 Sample Preparation and Extraction

Fresh barks of *F. africana* and *F. elastica* were harvested and authenticated by a botanist at the Department of Herbal Medicine, KNUST. They were cleaned, chopped into smaller pieces, and air-dried on plastic sheets for one week. The dried samples were milled into a fine powder and stored until ready for use. The powders' extraction was by cold maceration using water and 70% ethanol/water mixture for 72 h. The extracts were then lyophilized using a freeze dryer (Powerdry LL3000, Thermo Electron Corporation, Czech Republic) and the concentrates obtained stored at 4 °C in airtight containers until required.

2.3 Determination of Antimicrobial Activity

2.3.1 Bacteria and Fungus Strains

*Escherichia coli (*ATCC 25,922), *Staphylococcus aureus* (ATCC 25,923), *Salmonella typhi* (clinical strain), *Pseudomonas aeruginosa* (ATCC 4853), *Bacillus subtilis (*NCTC 10,073*), and Candida albicans (*clinical strain) were used. All the isolates were obtained from the Pharmaceutical Microbiology Laboratory, Department of Pharmaceutics, KNUST.

2.3.2 Agar well diffusion method

The agar well diffusion method described by Ashraf et al. [20] was used to evaluate the antimicrobial activities of the extracts. A volume of 20 mL of the molten nutrient agar stabilized at 45 °C for 15 min was inoculated with 0.1 mL of each of the selected organisms (1×10^6 CFU/mL) and then poured into Petri dishes. The seeded agar plates were then allowed to set, divided into six sectors, and holes made using a 10 mm diameter cork borer. Each sector was labelled with the corresponding concentration of extract (40, 20, 10, 5 mm) after which the extract was aseptically transferred into the wells. DMSO (10%) was used as the negative control, whereas Ciprofloxacin (50 µg/mL) for bacteria and Ketoconazole (1 mg/mL) for fungi were employed as reference

drugs. The plates were allowed to diffuse on the bench for 30 min, after which they were incubated at 37 °C for 24 h. Zones of growth inhibition were measured (mm), and the measurement was done across the radius of the cup and the mean zones of inhibitions calculated. The experiment was performed in duplicate. The activity index for each extract against the organisms tested was calculated as:

$$Actvity\ Index = \frac{zone\ of\ inhibition\ of\ the\ extracts\ at\ a\ particular\ concentration}{zone\ of\ inhibition\ of\ the\ standard\ as\ in\ control}$$

2.4 Microdilution method

The minimum inhibitory concentration was determined using the method described by EUCAST, [21] with some modifications. In brief, serial dilutions of each extract from 40 to 0.3125 mg/mL were prepared using 10% DMSO. The assay was performed in a 96-well microtitre plate. A total volume of 250 µL was put in each well, made up of 125µL of double strength nutrient broth, 100 µL of extract, and 25 µL of inoculum (bacterial or fungal suspension).

Ciprofloxacin and Ketoconazole were used as reference drugs for bacterial and fungal, respectively. 10% DMSO served as a negative control. The microplates were incubated at 37 °C for 24 h of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added to the wells and incubated at 37 °C for 30 min to detect organism growth or vice-versa.

Growth was detected by the presence of purple colour in the well. The lowest concentration, which showed no growth after the addition of MTT in the well, was recorded as the MIC. All tests were performed in triplicates. The total activity of the extracts in mL/g was calculated as shown below [22].

$$Total\ activity(TA) = \frac{mg\ of\ extract\ of\ dried\ plant\ part}{MIC\ of\ the\ extract}$$

2.5 Determination of Total Phenolic Contents

The total phenol content of the plant extracts was measured spectrophotometrically using the Folin-Ciocalteu method previously reported [23]. Briefly, an amount of 0.5 mL of each solution of the different extracts (31.25–1000 µg/mL) was measured into test tubes, and 2.5 mL of Folin-Ciocalteu reagent added to each. Two (2) mL of aqueous sodium carbonate solution (75 mg/mL) was added to each and kept on a water bath at 50 °C for 10 min. The absorbance was read at 760 nm using a microplate reader (Synergy H1 ™ Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan). The gallic acid solutions were taken through the same procedure and used to plot a calibration curve of absorbance against concentration (µg/mL). The total phenolic content was expressed as mg gallic acid equivalent (GAE) per gram of extract.

2.6 Determination of Total Flavonoid Contents

Each extract's total flavonoid content was assessed by the colourimetric assay, as described by Pękal and Pyrzynska [24]. An aliquot of 1 mL of the extract solution (31.25–1000 µg/mL) was mixed with 0.3 mL $NaNO_2$ (5%$^{w}/_{v}$) and 0.5 mL 10% ($^{w}/_{v}$) $AlCl_3$ solution. The reaction was allowed to stand for 6 min, and 0.5 mL of 1 M NaOH solution was then added to quench the reaction. The mixture was incubated for 30 min at room temperature and then transferred into a 96-well microtitre plate. The absorbance was read at 506 nm using a microplate reader (Synergy H1 ™ Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan). Quercetin (3.125–100 µg/mL), used as standard, was taken through the same procedure. The total flavonoid content was expressed as mg quercetin equivalent (QE) per gram of extract.

2.7 Determination of Condensed Tannin Content

The condensed tannin content was determined by the vanillin/HCl method described by Ahmed et al. [25]. The cleaned, capped test tubes were thoroughly wrapped with aluminium foil. An amount of 0.5 mL of the extract's solutions (31.25–1000 µg/mL) was pipetted into the tube. 3.0 mL of vanillin reagent was then added and mixed thoroughly by shaking. A volume of 1.5 mL of concentrated HCl was added and mixed thoroughly. The resulting mixture was allowed to stand for 15 min at 25 °C. Blank consisted of solvent, vanillin, and HCl. The samples and the blank were transferred into a 96-well microtitre plate, and absorbance was read at 500 nm using a microplate reader (Synergy H1™ Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan). Catechin standard was used to prepare the calibration curve. The total tannin content was expressed as mg catechin equivalent (CE) per gram of extract.

2.8 Total Antioxidant Capacity (Phosphomolybdate assay)

This spectrophotometric assay is based on reducing molybdenum from Mo^{6+} to Mo^{5+} by the extracts and subsequent formation of a green phosphate-molybdate (Mo^{5+}) complex at acidic pH. Modification of phosphomolybdate assay as previously described by Sarpong et al., 2016 [26] was employed. The reagent solution was prepared by adding

Ammonium molybdate (4 mM), disodium hydrogen phosphate (28 mM), and sulphuric acid (6 mM). 1 mL each of the different concentrations of the extract was measured into test tubes. A volume of 3 mL of the reagent solution was added to each and incubated at 95 °C for 90 min. The ascorbic acid solutions were also taken through the same procedure and transferred into a 96-well microtitre plate, and absorbance measured at 695 nm using the microplate reader.

2.9 Phytochemical Screening

The presence of secondary metabolites such as alkaloids, flavonoids, tannins, and saponins was determined using standard methods as discussed previously by Tiwari [27].

3 Results

3.1 Yield of Extract

In this study, aqueous and ethanolic extracts were prepared from the stem bark of *F. africana* and *F. elastica*. The 70% hydro-ethanolic extracts gave the highest yield (Table 1).

3.2 Antimicrobial Activity

Different isolated clinical and typed strains of microorganisms (*S. aureus*, *B. subtilis, S. typhi, E. coli,/P. aeruginosa,* and *C. albicans*) were used for the study. Extracts of both *F. africana* and *F. elastica* suppressed the test organisms' microbial growth considerably (Table 2). The activity index (AI) of the plant extracts against the tested microorganism was also documented. The results revealed that at 40 mg/mL, FEE was more potent against *S. aureus* and *C. albicans* by showing zones of inhibition 26.00 ± 1.41 mm with AI of 0.667 and 27.50 ± 0.71 mm with AI (1.250) against *C. albicans*. FEA also exhibited the largest zone of inhibition against *B. subtilis* (26.50 ± 2.12 mm, AI: 0.929). *S. aureus, S. typhi, E. coli,* and *B. subtilis* were susceptible to FAA only at a concentration of 40 mg/ml from the results but at the same concentration, FAA was inactive against *P. aeruginosa, and C. albicans*. FAE was active against three bacteria (*S. typhi, E. coli, S. aureus*) and *C. albicans* but showed no inhibition zone against *P. aeruginosa and B. subtilis* (Table SM1)**.** The minimum inhibitory concentration and total activity expressed in (mL/g) were also recorded (Table 3 and Table 4).

Table 1 Percentage yield of extracts of *F. africana and F. elastica*

Plant Specie	Distilled water %w/w	Ethanol (70%$^{v}/_{v}$) %w/w
F. africana	1.21	3.59
F. elastica	0.84	4.09

3.3 Total Phenolic, Total Flavonoid and Condensed Tannin Contents and Total Antioxidant Capacity

The total phenolic content (TPC), total flavonoid content (TFC), and condensed tannin content (CTC) were determined and quantified (Tables 5 and 6).

3.4 Correlation between the Polyphenolic compounds and Antioxidant Capacities

To further understand the relationship between the phenolic compounds and the antioxidant properties of extracts of *F. africana* and *F. elastica*, a comparative analysis of the study data was conducted using the Pearson correlation (Table SM3). Figure 1a–f shows a graphical correlation between the parameters.

3.5 Antimicrobial Activity (MIC) and its Bivariate Correlation to Total Phenolic, Total Flavonoid, Total Antioxidant Capacity

Strong negative correlation ranges from − 0.800 to − 1.000, moderate correlation ranges from -0.500 to -0.799, and low correlation from − 0.100 to − 0.499 (Table SM4).

3.6 Phytochemical Analysis

Phytochemical analysis was done on the dried powdered stem bark of *F. africana* and *F. elastica* and their extracts to establish the presence of some essential bioactive compounds. The phytochemical screening results revealed the presence of tannins, glycosides, cardiac glycosides, proteins, saponins, flavonoids, quinones, and triterpenoids in all the extracts (Table 6). *F. elastica* also showed the presence of anthocyanins and steroids.

4 Discussion

The present studies sought to harness the antimicrobial and antioxidant potential of *F. africana* and *F. elastica*. In the antimicrobial susceptibility test, the ethanolic stem bark extract of the two plants showed higher activities than the aqueous extracts. The aqueous extract of *F. elastica* (FEA) gave a more significant zone of inhibitions even at lower concentrations than *F. africana* aqueous (FAA) against *S. aureus, B subtilis, E coli*, *S typhi,* and *C. albicans*. At 10 mg/mL, *S. aureus, S. typhi, E. coli,* and *C. albicans* were highly susceptible to *F. africana* ethanolic extract (FAE) but showed no activity against *P. aeruginosa*

Table 2 Antimicrobial activity of the various stem bark extracts of *F. africana* and *F. elastica* by agar diffusion method

Mean Zones of Inhibition(mm)						
Bacteria						Fungi
Extract (mg/mL)	S. aureus (ATCC 25,923)	B. subtilis (NCTC 10,073)	E. coli (ATCC 25,922)	S. typhi (clinical strain)	P. aeruginosa (ATCC 4853)	C. albicans (clinical strain)
FAA						
40	20.50 ± 0.71^{a}	14.50 ± 0.71^{b}	19.50 ± 0.71^{a}	12.50 ± 0.71^{b}	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00
FAE						
40	24.00 ± 1.41^{a}	0.00	25.50 ± 0.71^{a}	24.00 ± 1.41^{a}	0.00	26.00 ± 1.41^{a}
20	21.00 ± 1.41^{a}	0.00	21.00 ± 1.41^{a}	20.50 ± 0.71^{a}	0.00	22.00 ± 2.83^{a}
10	13.50 ± 2.12^{a}	0.00	14.50 ± 0.71^{a}	15.50 ± 0.71^{a}	0.00	15.00 ± 4.24^{a}
5	0.00	0.00	0.00	0.00	0.00	0.00
FEA						
40	22.50 ± 0.71^{a}	26.50 ± 2.12^{a}	20.00 ± 0.0^{a}	22.50 ± 3.54^{a}	27.00 ± 1.41^{a}	25.00 ± 0.00^{a}
20	15.00 ± 0.00^{b}	19.00 ± 1.41^{ab}	16.00 ± 1.4^{b}	19.50 ± 0.71^{ab}	21.50 ± 2.12^{a}	16.00 ± 1.41^{b}
10	0.00	0.00	11.50 ± 0.71^{a}	13.50 ± 2.12^{a}	0.00	11.00 ± 0.00^{a}
5	0.00	0.00	0.00	0.00	0.00	0.00
FEE						
40	26.0 ± 1.41^{a}	20.0 ± 0.00^{b}	24.0 ± 1.41^{ab}	26.00 ± 2.83^{a}	20.00 ± 0.00^{b}	27.50 ± 0.71^{a}
20	19.0 ± 1.41^{b}	0.00	21.0 ± 1.41^{ab}	24.50 ± 0.71^{a}	0.00	24.50 ± 0.71^{a}
10	17.5 ± 0.71^{a}	0.00	15.00 ± 0.00^{a}	17.50 ± 3.54^{a}	0.00	18.50 ± 2.12^{a}
5	0.00	0.00	12.50 ± 0.71^{b}	15.00 ± 0.00^{a}	0.00	0.00
DMSO	0.00	0.00	0.00	0.00	0.00	0.00
Cipro(µg/mL)						
50	39.00 ± 1.41^{ab}	28.50 ± 2.12^{de}	32.00 ± 2.83^{cd}	43.00 ± 0.00^{a}	36.00 ± 1.41^{bc}	NT
Keto (µg/mL)						
1000	NT	NT	NT	NT	NT	22.00 ± 0.00^{e}

Mean zones of growth inhibition were mean (mm) of zones of inhibition, mean ± SD, $n = 2$ replicates, the diameter of well/cup = 10 mm, reference antimicrobial agents: Cipro, ciprofloxacin (50 µg/mL), Keto, ketoconazole (1 mg/mL). NT: not treated; The DMSO (alone) used as solvent did not show any antimicrobial activity against the test organisms. One-way ANOVA followed by multiple Tukey's HSD comparison test. The different superscript letters in the same row indicated a significant difference ($p < 0.05$). Double superscript denotes no significant difference ($p > 0.05$) between means bearing any of the letters

FAA Funtumia africana aqueous, *FAE Funtumia africana* ethanolic, *FEA Funtumia elastica* aqueous, *FEE Funtumia elastica* ethanolic

Table 3 Minimum Inhibitory Concentration (MIC) (mg/mL) of the various stem bark extracts of *F. africana* and *F. elastica*

Bacteria						Fungi
Extract/MIC (mg /mL)	S. aureus (ATCC 25,923)	B. subtilis (NCTC 10,073)	E. coli (ATCC 25,922)	S. typhi (clinical strain)	P. aeruginosa (ATCC 4853)	C. albicans (clinical strain)
FAA	5	40	2.5	5	40	5
FAE	2.5	40	2.5	2.5	2.5	2.5
FEA	2.5	5	5	5	2.5	2.5
FEE	5	2.5	2.5	2.5	2.5	2.5
Cipro	0.04	0.0025	0.0025	0.0025	0.08	ND
Keto	ND	ND	ND	ND	ND	0.0025

The tests were done in triplicates ($n = 3$). The DMSO (alone) used as solvent did not exhibit any antimicrobial activity against the test organisms. Reference antimicrobial agents: Cipro as ciprofloxacin, keto as ketoconazole. nd: Not determined

Table 4 Total activity of the bark extracts of *F. africana* and *F. elastica* against the tested pathogens

Bacteria							Fungi
Extract	Quantity of extract in mg per g of plant material	S. aureus (ATCC 25,923)	B. subtilis (NCTC 10,073)	E. coli (ATCC 25,922)	S. typhi (clinical strain)	P. aeruginosa (ATCC 4853)	C. albicans (clinical strain)
FAA	12.15	2.43	0.304	4.86	2.43	0.304	2.43
FAE	35.9	14.36	0.897	14.36	14.36	14.36	14.36
FEA	8.4	3.36	1.68	1.68	3.36	3.36	3.36
FEE	40.92	8.184	16.37	16.37	16.37	16.37	16.37

Total activity (ml/g) = mg of extract per gram dried plant part/ MIC

Table 5 Total phenolic, total flavonoid, condensed tannin content, and total antioxidant capacity of different extracts of *F. africana* and *F. elastica*

Extracts	TPC (mg GAE/g of dried extract	TFC (mg QE/g of dried extract	CTC (mg CE/g of dried extract)	TAC (mg AAE/g of dried extract)
FAA	92.09 ± 5.578^{b}	20.86 ± 7.05^{b}	25.90 ± 3.540^{b}	122.1 ± 4.730^{a}
FAE	65.81 ± 1.019^{c}	29.88 ± 2.032^{b}	30.33 ± 3.846^{b}	99.25 ± 37.52^{ab}
FEA	55.63 ± 1.926^{d}	20.48 ± 0.614^{bc}	16.16 ± 2.960^{b}	113.0 ± 5.377^{ab}
FEE	134.30 ± 1.659^{a}	78.01 ± 5.057^{a}	45.92 ± 2.126^{a}	124.5 ± 6.638^{a}

Values are represented as mean ± standard deviation ($n = 3$); Statistical significance: Means in columns that do not share the same superscript letter are significantly different. ($p < 0.05$). (mg GAE)/g = milligram gallic acid equivalent per gram of dried extract; (mg QE)/g = milligram of quercetin equivalent per gram of dried extract; (mg CE)/g = milligram of catechin equivalent per gram of dried extract; (mg AAE)/g = milligram of ascorbic acid equivalent per gram of dried extract

Table 6 Phytochemical analysis of *Funtumia africana* and *Funtumia elastica* extracts in aqueous and ethanolic solvent systems

Metabolites	*FA(PS)*	*FE(PS)*	*FAA*	*FEA*	*FAE*	*FEE*
Alkaloids	−	−	−	−	−	−
Tannins	+++	+++	++	++	+++	+++
Anthocyanins	−	+	−	+	−	−
Flavonoids	+++	+++	++	++	++	+++
Saponins	+++	+++	++	++	+++	+++
Glycosides	+	+	+	+	+	+
Cardiac Glycosides	+++	+++	++	++	++	++
Triterpenoids	+	+	+	+	+	+
Steroids	+	+	−	+	−	+
Protein	+	+	+	+	+	+
Reducing Sugars	+++	+++	++	++	++	++
General quinones	++	++	++	++	++	++
Anthraquinones	−	−	−	−	−	−

Key: F.A(PS): *Funtumia africana* powdered sample, F.E.(PS): *Funtumia elastica* powdered sample. *FAA Funtumia africana* aqueous, *FAE Funtumia africana* ethanolic, *FEA Funtumia elastica* aqueous, *FEE Funtumia elastica* ethanolic: +++ abundantly present, ++ moderately present, + present, − absent

and *B. subtilis* (Table 2). *F. elastica* ethanolic (FEE) at a lower concentration (5 mg/mL) showed considerable activity against *E. coli* and *S. typhi*. The activity index (AI) which describes the effect of polar and non-polar solvent extracts of *F. africana* and *F. elastica* with standard antibiotics was shown in Table SM1. The activity index values are beneficial in the quantitative assessment of the antimicrobial properties of the polar and non-polar extracts compared to the standards [28]. The activity index (AI) value of more than 1 indicates a strong effect

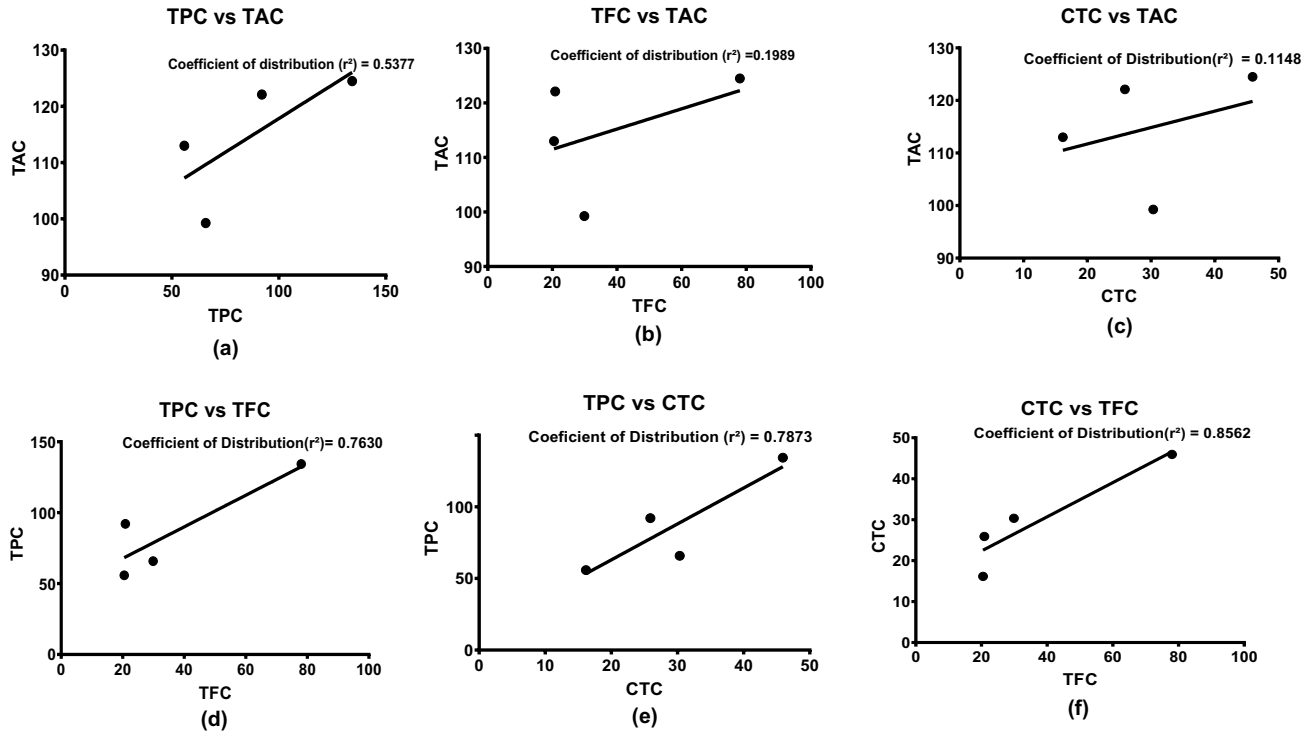

Fig. 1 Graphical correlation between **a** TAC and TPC **b** TAC and TFC **c** TAC and CTC **d** TPC and TFC **e** TPC and CTC **d** CTC and TFC of the tested extracts of both *F. africana* and *F. elastica*

of the plant extract and vice versa. The extracts exhibited a higher activity index against the *E. coli, S. typhi,* and *C. albicans,* which indicated a greater isolates susceptibility. *B. subtilis* and *P. aeruginosa* were found to be the least susceptible to the extracts, particularly FAA and FAE with MIC of 40,000 µg/mL. *B. subtilis*, like many Gram-positive pathogenic bacteria, use ribosomal protection proteins that enable them to gain resistance to clinically important antibiotics. *S. aureus*, *S. typhi, E. coli,* and *C. albicans* were more susceptible to the extracts with MIC range of 2500 to 40,000 µg/mL (Table 4).

Studies conducted on *F. elastica* [29] reported an MIC range of 125 to 1550 µg/mL and 125 to 1750 µg/mL for the ethanolic extract of *F. elastica* leaves and stem bark, respectively against bacterial and fungal test organisms. Also, reports show that the crude ethanolic extracts of the leaves and stem bark of *F. elastica* exhibit higher potency against *Streptococcus pneumoniae, Staphylococcus aureus, Salmonella typhi, Escherichia coli and Candida albicans* [30]. A recent study by Ouattara and co-workers [31] also demonstrated the effectiveness of hydroethanolic extract of *F. elastica* leaves against *Shigella* EEG and S*higella* 1055 that cause gastroenteritis. Crude acetone extract of *F. africana* leaves has been reported to exhibit MICs as low as 80 µg/ml against bacteria and fungi [32]. Kouadio et al., [33] evaluated the antibacterial activities of the crude water and hydroethanolic extracts of the leaves of *F. africana* against methicillin-resistant *S. aureus* with their MICs ranging from 0.046 to 1.56 mg/mL, which demonstrated antibacterial effect against the tested strain. Amos-tautua and his team also reported an MIC of 1.50 µg/ml crude methanolic extract of *F. elastica* leaves against *B. subtilis* and *P. aeruginosa*, MIC of 1.75 µg/ml against *S. aureus* and *E. coli* [34].

Comparing the extracts' antimicrobial activities, the ethanolic extract of *F. elastica* (FEE) showed the highest antimicrobial activity with lower MIC against the isolates, followed by FEA then FAE (Table 3). The antimicrobial activity of the extracts could be attributed to the phenolic compounds in the extracts. High total activity (Table 4) was observed in FEE, which resulted from the high phenolic composition. The total activity expressed as mL/g has several pharmacological tendencies in aiding plant species' selection and evaluating the potency of isolated bioactive compounds in extracts [22].

Different studies have shown the role of phenolic compounds in antimicrobial activity.

Alves et al. [35] reported an MIC of 1 mg/mL for 2,4-dihydroxybenzoic, protocatechuic, vanillic and p-coumaric acids isolated from mushrooms against *E. coli*. In another study, 35 diverse classes of polyphenols (cinnamic

or benzoic acids, flavonoids, stilbenes, coumarins, naphtoquinones) were screened against 6 pathogenic bacterial strains (*Staphylococcus aureus, Bacillus subtilis*, *Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella enteritidis*) and the most affected strains were the *B. subtilis* and *S. enteritidis*. Butyl gallate from the benzoic acid was found to be the most potent, inhibiting all the tested isolates except *P. aeruginosa* [36]. Additionally, it has been reported that the antibacterial activity of ethanolic extract of *Secondatia floribunda* A DC (Apocynaceae) stalk' inner bark gave an MIC of 512 µg/ml against all the tested organisms except *S. aureus* ATCC 12,692 and *E. coli* ATCC 25,922. The antibacterial activity of the plant was attributed to the substantial levels of the polyphenols that was characterized using HPLC–DAD and ATR-FTIR [37].

In brief, our findings revealed that the antimicrobial activities of the water and hydroethanolic extracts of *F. africana* and *F. elastica* could be linked to their diverse polyphenolic content.

Polyphenols have been known to demonstrate a wide range of antimicrobial activities, even against several multidrug resistant organisms [38]. The bacterial growth inhibitory mechanisms of polyphenols include the destabilization of the cytoplasmic membrane, changes in bacterial plasma membrane permeability, the inhibition of extracellular microbial enzymes [39], the alteration of microbial metabolism and a loss of the substrates needed for the growth of the microbes [40].

Polyphenolic compounds have been known to alter the surface electron acceptors for gram-positive and gram-negative bacteria, thereby changing the bacteria's polarity [41]. The antimicrobial properties of phenolic compounds could be attributed to their chemical structures about the substitution position in the aromatic ring and the saturated chain length [42].

The relationship between the polyphenolic content and MIC of the extracts (Table SM4) against the various isolates gave a correlation that confirms a similar relationship reported between TPC, antimicrobial and antioxidant activity [43] of some medicinal plants. TPC was expressed as the mg GAE/ g of dried extract, TFC was expressed as the mg QE/g of dried extract, and CTC expressed as the mg CE/ g of dried extract. Compared to *F. africana*, the hydro-ethanolic extract of *F. elastica* showed the highest TPC, TFC, CTC, and TAC (Table 5). However, for the aqueous extracts, *F. africana* afforded a higher content of total phenols, flavonoids, and tannins than the specie elastica. This highlights the importance of choosing the right solvent in preparations involving these two species. The total antioxidant capacity (TAC) of the extracts were determined, quantified, and the results expressed as mg of ascorbic acid per gram of extract (mg AAE /g of dried extract). The results obtained for the total antioxidant capacity revealed a positive correlation with the total phenolic content.

According to Zlatić and co-workers, many diseases have been linked with high levels of free radicals in the cells of the particular organ from which the disease has occurred [44]. Consumption of plant products rich in antioxidants tends to minimize the risk of having some of these diseases [45]. According to the results obtained from the study, the ethanolic extract of *F. elastica* (FEE) gave the highest TAC value (124.5 ± 6.638 mg AAE/g, $p < 0.05$). This could be attributed to the high content of phenols, flavonoids, and tannins present. These compounds are essential and have been historically characterized on account of their antioxidant and free radical scavenging effects [46]. The relationship between polyphenols and their antioxidant activity has previously been reported for different plant species [47]. Comparing the total antioxidant capacity with the polyphenolic compounds, the study revealed that the total phenolic content and the total antioxidant capacity positively correlated ($R = 0.893$, $p < 0.05$). The total flavonoid content and the extracts' total antioxidant capacity also showed a positive correlation ($R = 0.613$, $p < 0.05$). This trend indicates that an increase in phenols and flavonoid content contributes significantly to the increase in the total antioxidant capacity. Results obtained so far agree with the argument that polyphenols may have a direct link to antioxidant activity, which prevents poor disease progression and leads to a good prognosis [47]. The results in Table SM3 showed a strong positive correlation between the total phenolic content and the total antioxidant capacity ($R = 0.733$) for all extracts.

Various studies report on the biological activities of *F. africana* extracts including antibacterial [33], antifungal, anti-inflammatory activities, and cytotoxicity [32]. Adediwura et al. reported on the larvicidal effect and in vivo anti-inflammatory properties of the methanolic extracts [48]. The extracts of *F. elastica* were found to contain flavonoids, steroids, quinones, anthocyanins, tannins, proteins, terpenoids, saponins, cardiac glycosides, and reducing sugars (Table 6). The plant extracts are known to possess antiplasmodial [49], antioxidant [31], anti-inflammatory [29], anti-asthmatic [50], wound healing [51], and anti-gastroenteritis [31].

Our present results agree in part with the findings of Adekunle and Ikumapayi, 2006 who reported the presence of anthocyanins, betacyanin, flavonoids, steroids, and tannins in the stem bark of *F. elastica*. They did not detect the presence of alkaloids which is consistent with our findings [52]. However, Zirihi et al. (2005) reported steroidal alkaloids in the stem barks of *F. elastica* [50]. This difference in the results may be due to variances in the geographical location of the species.

It is reported that the presence of anthocyanins in *F. elastica* is linked with critical pharmacological activities

of the plant, including its anti-inflammatory, antidiabetic and anticancer properties [52, 53].

5 Conclusion

Data obtained from the studies show that the stem bark of *F. africana* and *F. elastica* exhibited superior antioxidant and antimicrobial properties and can therefore be exploited for lead drug compounds. The ethanol/water mixture gave the highest antimicrobial and antioxidant properties. Comparing the two plants, it can be concluded that *F. elastica* extracts contain high potent antioxidants, hence a high antimicrobial activity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42250-021-00249-5.

Acknowledgements The authors are thankful to Dr. Francis Osei Amoako-Andoh (Research Scientist) and Miss Sylvia Kafui Archer (Laboratory Technician) at Biochemistry Laboratory, CSIR-Crops Research Institute, Fumesua for aiding towards this research. The authors also acknowledge Mr. Francis Amankwaah (Laboratory Technician) at the Pharmaceutical Microbiology Section, Department of Pharmaceutics, KNUST for his immense support.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. However, the authors acknowledge the OWSD Early Career Fellowship programme for providing research support for the laboratory where this study was conducted.

Data Availability All data generated or analyzed in this research work are included in the preparation of this manuscript and the supplementary material and will be available upon request from the corresponding author.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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