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Phytochemical screening, antioxidant and antimicrobial potential of stem barks of *Coula edulis* Baill. *Pseudospondias longifolia* Engl. and *Carapa klaineana* Pierre. from Gabon

Cédric Sima Obiang[1,2*], Joseph-Privat Ondo[1,2], Guy-Roger Ndong Atome[1,2], Louis-Clément Obame Engonga[1,2], Joel-Fleury Djoba Siawaya[3], Edouard Nsi Emvo[1]

[1]*Laboratory of Research in Biochemistry, University of Sciences and Technology of Masuku, P. O. Box 943 Franceville, Gabon*

[2]*Laboratory of Natural Substances and Organometallic Synthesis, University of Sciences and Technology of Masuku, P. O. Box 943 Franceville, Gabon*

[3]*National Laboratory of Public Health, P. O. Box 10736 Libreville, Gabon*

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ABSTRACT

Objective: To evaluate the phytochemical constituents, antioxidant and antimicrobial potential of water-acetone, water-ethanol and water extracts of *Coula edulis* (*C. edulis*), *Pseudospondia longifolia* (*P. longifolia*) and *Carapa klaineana* (*C. klaineana*).
Methods: Presence of total phenols, flavonoids, tannins and proanthocyanidins was evaluated to estimate the effects of plants on microbial diseases. Water-acetone, water-ethanol and water extracts were examined for antioxidant activities. All plant extracts were evaluated against six reference strains, eleven clinical isolates and two fungal strains.
Results: The contents of total phenols [(12 857.56 ± 1.00) mg gallic acid equivalent/100 g], flavonoids [(1 634.13 ± 1.88) mg quercetin equivalent/100 g], tannins [(2 672.00 ± 1.59) mg tannic acid equivalent/100 g] and proanthocyanidins [(395.11 ± 0.83) mg apple procyanidin equivalent/100 g] were highest in the water-acetone and water-ethanol extracts from *C. edulis* in comparison with *P. longifolia* and *C. klaineana*. The water-acetone, water-ethanol and water extracts from *C. edulis* presented the highest antimicrobial activities against *Neisseria gonorrhoeae*, *Enterococcus faecalis* CIP 103907, *Pseudomonas aeruginosa* and *Salmonella* sp. The tested microorganisms showed sensitivity to all extracts of *P. longifolia* and *C. klaineana* with the exception of *Shigella dysenteriae* CIP 5451, *Pseudomonas aeruginosa* and *Salmonella typhi*.
Conclusions: Our results suggest that *C. edulis* extracts contain greater antioxidant and antimicrobial properties than *P. longifolia* and *C. klaineana* extracts.

1. Introduction

Over 80% of the population in the world use traditional medicine and the recent studies are based on research of antioxidants from plant[1]. These antioxidants reduce chronic diseases, such as atherosclerosis, cancer, aging and neurodegenerative disease[2].

Despite several antimicrobial agents, it is important to find new agents because microbial infections, especially bacterial ones cause many diseases such as diarrhea, skin infections (paronychia, abscesses, *etc.*) and salmonellosis[3]. The bacterial strains resistant to antibiotics cause difficulties for the treatment of microbial infections[4].

Coula edulis Baill. (Olacaceae) (*C. edulis*) is a tree with irregular and circumvented stem; it is locally known as "Ewômœ" in Gabon. Its stem barks are employed to treat the ulcers, and they are also applied in form of decoction for diarrhea and oral infections[5,6]. *Pseudospondias longifolia* Engl. (Anacardiaceae) (*P. longifolia*) is a small rather common tree; the bark decoction of *P. longifolia* is used for years in the traditional medicine for the treatment of tooth aches and sexual infections. The stem bark, mixed with red wood (*Pterocarpus soyauxii*), cures the wounds. *Carapa klaineana* Pierre (Meliaceae) (*C. klaineana*), a tree with twisted trunk, is known locally with the name of "Engang". The

*Corresponding author: Cédric Sima Obiang, P. O. Box 913 USTM Franceville, Gabon.
Tel: (+241) 07883962
Fax: (+241) 671660
E-mails: cedricsima@gmail.com, cedricsima@yahoo.fr
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plant is found at the edge of the rivers. Stem bark is used against the intestinal worms and the teeth problem[6].

Several scientific studies on antioxidant and antimicrobial activities of extracts from dry leaves of *C. edulis* have been previously reported[7]. The aim of this study was to evaluate and compare the antimicrobial and antioxidant potential of various plant extracts from stem bark of *C. edulis*, *P. longifolia* and *C. klaineana*.

2. Materials and methods

2.1. Plant materials

The stem barks of *C. edulis*, *P. longifolia* and *C. klaineana* were selected according to their traditional uses. The plant samples were collected in Oyem (Northern Gabon) in July 2014. Identification of the species was carried out at the National Herbarium of Institute of Traditional Pharmacopeia and Medicine, Libreville (Gabon).

2.2. Processing of the plant material

The plant samples were freeze-dried, powdered, kept at ambient temperature, and protected from light. Each sample (20 g) were mixed with 250 mL of suitable solvents [water (100%); water-acetone (30:70, v/v); water-ethanol (30:70, v/v)]. The water extracts were boiled for 60 min. All the extracts were filtered and concentrated. The concentrates were lyophilized and stored in sterile vials at 4 °C.

2.3. Chemicals

Butylated hydroxyanisole (BHA), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), ethanol, ferric chloride, H_2SO_4, HCl, benzene, NH_4OH, sodium chloride, ethanol, Folin–Ciocalteu reagent, Na_2CO_3, gallic acid and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.4. Preliminary phytochemical screening

Each extract was tested for the presence of flavonoids, coumarins, tannins, total phenolic, saponosides, triterpenoids, alkaloids and anthracenosids according to the methods described by Culei[8].

2.5. Quantitative analysis of phytochemicals

2.5.1. Total phenolic contents

Folin–Ciocalteu method was used to measure the total phenolic content[3]. Absorbance was measured at 735 nm. All experiments were carried out in triplicate and phenolic compounds were expressed as mg gallic acid equivalent (GAE)/100 g of extract.

2.5.2. Total flavonoid content

Aluminium trichloride method was used to determine the flavonoid content and absorbance was measured after 10 min at 435 nm. The flavonoid content was expressed as mg quercetin equivalent (QE)/100 g of extract[9].

2.5.3. Determination of the total tannins content

Method of Obame *et al.* was employed to determine the tannin content[10]. Absorbance was measured at 525 nm and tannic acid was used as standard.

2.5.4. Determination of the total proanthocyanidins

Proanthocyanidins was determined by using HCl-butanol assay[10]. Absorbance was read at 550 nm and apple procyanidin was applied as standard.

2.6. Antioxidant activity index (AAI)

AAI based on DPPH was estimated by the method of Scherer and Godoy[11]. A range of concentration from 0.78 to 100 µg/mL was prepared for each extract. Ascorbic acid (vitamin C) and BHA were used as controls. Each sample was prepared in triplicate. Absorbance was measured at 517 nm. Percentage inhibition was obtained by the following formula:

%Radical scavenger activity = [(Absorbance of DPPH – Absorbance of sample)/ Absorbance of DPPH] × 100

The concentration of extracts reducing 50% of DPPH (IC_{50}) was determined from the curve of the percentage inhibition versus concentration of the extract. AAI was calculated using the following formula:

AAI = Final concentration of DPPH/IC_{50}

According to criteria of Scherer and Godoy[11], the extracts of plants show weak antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI is between 0.5 and 1.0, strong antioxidant activity when AAI is between 1.0 and 2.0, and very strong when AAI > 2.0.

2.7. ABTS scavenging activity

ABTS assay is based on the ability of an antioxidant to stabilize $ABTS^{•+}$ radical transforming it into $ABTS^{+}$[12]. Mixture of ABTS solution (7 mmol/L) and potassium persulfate (2.4 mmol/L) was incubated for 12 h in the dark at room temperature until ABTS radical complex was formed ($ABTS^{•+}$). To 60 µL of extract, 2.94 mL of $ABTS^{•+}$ solution was added. The mixture was incubated at 37 °C for 20 min and protected from light. Ascorbic acid (vitamin C) and BHA were used as references. After incubation the absorbance was measured by a spectrophotometer at 734 nm. The percentage inhibition was calculated by the following method:

Percentage inhibition = $[(At_0 - At_{20})/At_0] \times 100$

where, At_0 is the absorbance of $ABTS^{+}$ radical + ethanol, At_{20} is the absorbance of $ABTS^{+}$ radical + sample extract or standard.

2.8. Test microorganisms

The test microorganisms used in this investigation included bacteria *Escherichia coli* CIP 105182 (*E. coli* CIP 105182), *Listeria innocua* LMG 135668 BHI (*L. innocua* LMG 135668 BHI), *Staphylococcus aureus* ATCC 25293 BHI (*S. aureus* ATCC 25293 BHI), *Enterococcus*

faecalis CIP 103907 (*E. faecalis* CIP 103907), *Bacillus cereus* LMG 13569 BHI (*B. cereus* LMG 13569 BHI), *Staphylococcus camorum* LMG 13567 BHI, *Shigella dysenteriae* CIP 5451 (*S. dysenteriae* CIP 5451), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella enterica* (*S. enterica*), *Salmonella typhi* (*S. typhi*), *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *Eschirichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter baumannii* (*A. baumannii*), *Enterobacter aerogenes* (*E. aerogenes*), *Salmonella* spp. and *Neisseria meningitidis* (*N. meningitidis*). The fungal strains were *Candida albicans* ATCC 10231 (*C. albicans* ATTC 10231) and *Candida albicans* ATCC 90028 (*C. albicans* ATCC 90028).

2.9. Positive and negative control

Gentamicin (10 µg/mL) and tetracycline (30 µg/mL) were used as positive control for the tested bacterial strains. Sterilized distilled water and dimethyl sulfoxide were used as negative control.

2.10. Antibacterial susceptibility testing

Disc diffusion method was used to study susceptibility of bacteria against plant extracts[13]. Bacteria were grown in Muller Hinton broth (Liofilchem, Italy) for 18 to 24 h. Each culture was then suspended in a sodium chloride solution (NaCl, 0.9%) to reach turbidity equivalent to that of the 0.5 MacFarland standard[14]. Extracts were diluted in dimethyl sulfoxide to 100 mg/mL. Previously each extract (10 µL) was loaded onto each filter paper disc (Whatman No. 1). Muller Hinton agar was suspended in distilled water, heated until complete dissolution and was autoclaved at 121 °C and then poured into Petri dishes. The discs were placed on cultures and antimicrobial activity was estimated after incubation at 37 °C for 24 h, by measuring the diameter of inhibition zone.

2.11. Determination of the relative percentage inhibition (RPI)

RPI of plant extracts with respect to positive control was calculated by using the following formula[15]:

$$RPI = 100 \times (X - Y)/(Z - Y)$$

where, X is the total area of inhibition of the test extract, Y is the total area of inhibition of the solvent and Z is the total area of inhibition of the standard drug.

2.12. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC and MBC were determined by microdilution method with Muller Hinton broth[13,16]. Briefly, nutrient broth (100 µL/wells) was distributed into wells of a microplate (Nunc). One hundred microliters of extracts were added to first row of wells and twofold dilution was added into other wells. Ninety microliters of nutrient broth and 10 µL of inocula were added into wells. A range of concentration of extract from 0.0049 to 5 mg/mL was prepared in a total volume of 200 µL to each extract. The plates were slightly shaken and incubated at 37 °C for 24 h; inhibition was assessed by observing the absence of turbidity in the wells. Wells without extract were used as negative control.

To determine the MBC, 100 µL from each well demonstrating no visible growth were collected and seeded in Petri dishes containing Muller Hinton agar. The dishes were incubated at 37 °C for 24 to 48 h and the number of colonies was counted[16].

2.13. Antifungal sensitivity test

Antifungal activity of extracts was evaluated by the diffusion and microdilution methods as described above with some modifications[16]. Culture media for this study were potato dextrose broth and the potato dextrose agar.

2.14. Statistical analysis

Experimental results were expressed as mean ± SD. All measurements were replicated three times. The IC_{50} values were calculated using linear regression analysis from the graph of scavenging percentage against extract concentration.

3. Results

Table 1 presents the results of the preliminary phytochemical screening. These results showed that tannin gallic, total phenolic, antracenosids and triterpenoids were present in the stem bark crude extracts of *C. edulis*, *P. longifolia* and *C. klaineana*. However, all the crude extracts from stem bark samples showed negative result for alkaloids and coumarins. The water, water-ethanol and water-acetone crude extracts from stem bark of *C. edulis* and *P. longifolia* also showed presence of total flavonoids and reducing sugars.

Table 1

Results of the preliminary phytochemical screening.

Chemical groups	*C. edulis*			*P. longifolia*			*C. klaineana*		
	WE	WEE	WAE	WE	WEE	WAE	WE	WEE	WAE
Saponosids	++	-	-	++	-	-	++	-	+
Tannin gallic	+++	+++	+++	++	+++	+++	++	+++	+++
Tannin catechin	++	+	+	++	-	+	+++	+	+
Total phenolic	+++	+++	+++	+++	+++	+++	+++	+++	+++
Total flavonoids	++	++	++	++	++	+++	+	+	+
Reducing sugars	++	++	++	+++	++	++	+	++	++
Alkaloids	-	-	-	+	-	-	-	-	-
Proanthocyanidins	-	++	+	++	+	++	+	++	++
Anthracenosids	+++	+++	+++	++	++	+++	+	++	++
Coumarins	-	-	-	-	+	+	+	-	+
Triterpenoids	++	+++	+++	++	+++	+++	++	+++	++

+++: Very abundant; ++: Abundant; +: Not abundant; -: Not detected. WAE: Water-acetone extract; WEE: Water-ethanol extract; WE: Water extract.

The result of total phenols, total flavonoids, total tannins and total proanthocyanidins contents is shown in Table 2. The total phenolic contents (standard curve equation: $Y = 0.0012X + 0.0004$, $R^2 = 0.9982$) from the water, water-ethanol and water-acetone extracts of the stem barks of *C. edulis*, *P. longifolia* and *C. klaineana* varied from (2527.56 ± 2.92) to (12857.56 ± 1.00) mg GAE/100 g of extract. In this research, the water-ethanol extract of *C. edulis* had the highest phenolic content than other extracts.

Total flavonoid content (standard curve equation: $Y = 0.0032X + 0.0077$, $R^2 = 1$) was determined in comparison with standard quercetin and the results expressed in terms of mg QE/100 g of extract. Total flavonoids were more abundant in water-acetone extract of *C. edulis* [(1634.13 ± 1.88) mg QE/100 g of extract] than other extracts of plants.

Table 2

Total phenolic content, total flavonoid content, total tannins content and total proanthocyanidins content of extracts from *C. edulis*, *P. longifolia* and *C. klaineana*.

Extracts	TPC (mg GAE/100 g of extract)	TFC (mg QE/100 g of extract)	TTC (mg TAE/100 g of extract)	TPRC (mg APE/100 g of extract)
Ce WAE	11727.56 ± 4.58	1634.13 ± 1.88	2672.00 ± 1.59	304.00 ± 3.33
Ce WEE	12857.56 ± 1.00	871.63 ± 2.50	2000.89 ± 2.59	395.11 ± 0.83
Ce WE	10497.00 ± 5.63	1158.29 ± 1.32	1621.63 ± 3.95	137.33 ± 1.67
Pl WAE	8517.56 ± 1.39	1301.21 ± 2.50	1415.70 ± 1.22	1186.22 ± 4.44
Pl WEE	7304.22 ± 1.51	721.21 ± 3.06	1509.04 ± 0.62	972.89 ± 9.17
Pl WE	6429.78 ± 1.28	1159.13 ± 0.83	768.30 ± 3.21	837.33 ± 3.33
Ck WAE	3823.11 ± 1.78	939.96 ± 1.94	723.85 ± 3.15	701.78 ± 3.33
Ck WEE	2857.56 ± 1.05	510.38 ± 1.11	Nd	241.78 ± 1.67
Ck WE	2527.56 ± 2.92	446.21 ± 0.16	77.93 ± 0.56	26.22 ± 0.83

TPC: Total phenolic content; TFC: Total flavonoid content; TTC: Total tannins content; TPRC: Total proanthocyanidins content. Nd: Not determinated; Ce: *C. edulis*; Pl: *P. longifolia*; Ck: *C. klaineana*; WAE: Water-acetone extract; WEE: Water-ethanol extract; WE: Water extract. TAE: Tannic acid equivalent; APE: Apple procyanidins equivalent.

Tannins contents (standard curve equation: $Y = 0.0009X + 0.2088$, $R^2 = 1$) were expressed in terms of mg TAE/100 g of extract. The amount of tannin was the highest in the water-acetone extract of *C. edulis* [(2672.00 ± 1.59) mg TAE/100 g of extract].

Levels of proanthocyanidins were expressed in terms of mg apple procyanidins equivalent/100 g of extract. The expression from the calibration curve of the proanthocyanidins by the HCl–butanol method gave $Y = 0.0006X + 0.0024$ with $R^2 = 0.986$.

The antioxidant activities of the extracts are provided in Table 3. The AAI of the extracts from *C. edulis* ranged from 4.59 to 6.93 and can be compared to AAI of vitamin C and BHA (7.02 and 7.58 respectively) while those of *P. longifolia* and *C. klaineana* ranged from 0.66 to 3.90.

Table 4 showed the scavenging activity of stem bark extracts of *C. edulis*, *P. longifolia* and *C. klaineana* against ABTS radical in a concentration dependent manner. A comparable scavenging activity was observed between the extracts and the standard drugs (vitamin C and BHA). The IC_{50} values of the standard vitamin C and BHA were (5.01 ± 0.55) and (4.26 ± 0.25) µg/mL, respectively while water-acetone [(2.97 ± 0.49) µg/mL], water-ethanol [(2.24 ± 0.10) µg/mL] and water extracts [(2.50 ± 0.09) µg/mL] of *C. edulis* recorded high inhibitory activities compared to the extracts of *P. longifolia* and *C. klaineana*.

Table 3

Antioxidant activity of *C. edulis*, *P. longifolia* and *C. klaineana* extracts by DPPH free radical scavenging method.

Extracts	Regression curve equations	R^2	IC_{50} (µg/mL)	AAI
Ce WAE	$Y = 5.61X + 9.60$	0.901	7.21 ± 0.26	6.93
Ce WEE	$Y = 5.6X + 6.8$	0.900	7.71 ± 0.21	6.48
Ce WE	$Y = 4.37X + 2.33$	0.978	10.90 ± 0.89	4.59
Pl WAE	$Y = 1.65X + 2.80$	0.986	28.56 ± 0.38	1.75
Pl WEE	$Y = 4.02X – 1.48$	0.995	12.81 ± 0.78	3.90
Pl WE	$Y = 1.67X + 22.81$	0.992	16.29 ± 0.52	3.06
Ck WAE	$Y = 3.28X + 1.71$	0.979	14.72 ± 0.69	3.39
Ck WEE	$Y = 1.16X + 14.30$	0.984	30.75 ± 0.46	1.62
Ck WE	$Y = 0.66X + 0.42$	0.992	75.12 ± 0.56	0.66
Vit C	$Y = 6.76X + 2.03$	0.985	7.12 ± 0.60	7.02
BHA	$Y = 3.32X + 28.12$	0.950	6.59 ± 0.30	7.58

Ce: *C. edulis*; Pl: *P. longifolia*; Ck: *C. klaineana*; WAE: Water-acetone extract; WEE; Water-ethanol extract; WE: Water extract. Vit C: Vitamin C.

Table 4

Analysis of ABTS radical scavanging activity of stem bark extracts of *C. edulis*, *P. longifolia* and *C. klaineana*.

Extracts	Regression curve equations	R^2	IC_{50} (µg/mL)
Ce WAE	$Y = 7.43X + 27.93$	0.995	2.97 ± 0.49
Ce WEE	$Y = 21.74X + 1.17$	0.996	2.24 ± 0.10
Ce WE	$Y = 8.18X + 29.61$	0.999	2.50 ± 0.09
Pl WAE	$Y = 2.83X + 14.47$	0.985	12.56 ± 0.08
Pl WEE	$Y = 22.39X + 0.49$	0.999	2.21 ± 0.59
Pl WE	$Y = 6.63X – 0.13$	0.999	7.56 ± 0.25
Ck WAE	$Y = 5.82X + 0.19$	0.950	8.55 ± 0.10
Ck WEE	$Y = 3.55X + 2.92$	0.995	13.24 ± 0.70
Ck WE	$Y = 2.04X – 0.29$	0.994	24.63 ± 0.54
Vit C	$Y = 6.76X + 2.03$	0.989	5.01 ± 0.55
BHA	$Y = 3.32X + 28.12$	0.950	4.26 ± 0.25

Ce: *C. edulis*; Pl: *P. longifolia*; Ck: *C. klaineana*; WAE: Water-acetone extract; WEE; Water-ethanol extract; WE: Water extract. Vit C: Vitamin C.

The standard drug (gentamicin) was used to determine the RPI of *C. edulis*, *P. longifolia* and *C. klaineana* stem bark extracts in different solvents. Water-acetone bark extracts of *C. edulis* exhibited the maximum RPI (73.53%, 92.31%, 113.33%, 100.00%, 62.50%, 100.00%, 63.30%, 90.00% and 62.50%) against *E. coli* CIP 105182, *L. innocua* LMG 135668 BHI, *S. aureus* ATCC 25293 BHI, *E. aerogenes*, *S. dysenteriae* CIP 5451, *N. meningitidis*, *E. faecalis* CIP 103907, *P. aeruginosa* and *S. typhi*, respectively. Water-ethanol stem bark extract of *C. edulis* also showed maximum RPI (92.31%, 50.00%, 77.78%, 106.25% and 72.00%) against *B. cereus* LMG 13569 BHI, *S. enterica*, *K. pneumonia*, *A. baumannii* and *Salmonella* spp. respectively while all stem bark extracts of *P. longifolia* and *C. klaineana* showed minimum relative inhibition against *E. faecalis* CIP 103907, *S. dysenteriae* CIP 5451, *S. enterica*, *K. pneumonia* and *Salmonella* spp. (Figure 1).

The antimicrobial activities of extracts varied according to the species tested. Of the three plants tested, at least one extract produced a zone of inhibition greater than 10 mm against at least one species. The most active extracts were the water-ethanol, water-acetone and water extracts from *C. edulis*. In the *C. klaineana*

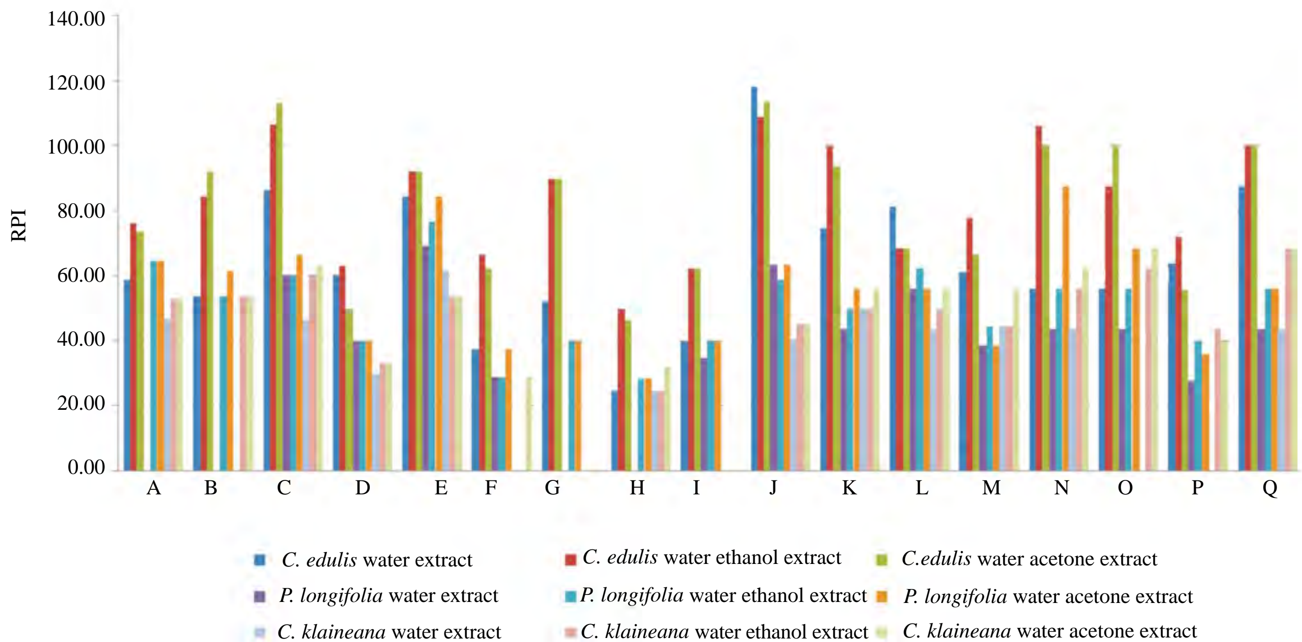

Figure 1. Determination of RPI of water, water ethanol and water acetone extracts from stem bark of *C. edulis*, *P. longifolia* and *C. klaineana* compared to standard antibiotics.
A: *E. coli* CIP 105182; B: *L. innocua* LMG 135668 BHI; C: *S. aureus* ATCC 25293 BHI; D: *E. faecalis* CIP 103907; E: *B. cereus* LMG 13569 BHI; F: *S. dysenteria* CIP 5451; G: *P. aerugenosa*; H: *S. enterica*; I: *S. typhi*; J: *N. gonorrhea*; K: *E. coli*; L: *S. aureus*; M: *K. pneumoniae*; N: *A. baumannii*; O: *E. aerogenes*; P: *Salmonella* spp.; Q: *N. meningitidis*.

Table 5
Inhibition zone diameters (mm) produced by the extracts from *C. edulis*, *P. longifolia* and *C. klaineana*.

Bacteria strains	Extracts									Standards	
	Ce WE	Ce WEE	Ce WAE	Pl WE	Pl WEE	Pl WAE	Ck WE	Ck WEE	Ck WAE	Genta	Tetra
Bacteria reference strains											
E. coli CIP 105182	10.0 ± 1.0	13.0 ± 1.0	12.5 ± 0.0	Nd	11.0 ± 0.0	11.0 ± 0.5	8.0 ± 1.0	9.0 ± 0.5	9.0 ± 0.0	17.0 ± 1.0	Nd
L. innocua LMG 135668 BHI	7.0 ± 1.5	11.0 ± 0.6	12.0 ± 0.5	Nd	7.0 ± 0.0	8.0 ± 1.0	Nd	7.0 ± 0.6	7.0 ± 1.0	13.0 ± 0.0	14.0 ± 0.0
S. aureus ATCC 25293 BHI	13.0 ± 1.0	16.0 ± 0.5	17.0 ± 0.6	9.0 ± 0.5	9.0 ± 0.6	10.0 ± 1.0	7.0 ± 0.3	9.0 ± 1.0	9.5 ± 0.5	15.0 ± 0.3	17.0 ± 0.6
E. faecalis CIP103907	18.0 ± 0.6	18.0 ± 1.0	15.0 ± 1.0	12.0 ± 0.6	12.0 ± 1.0	12.0 ± 0.3	9.0 ± 1.2	10.0 ± 0.0	10.0 ± 0.6	30.0 ± 0.0	19.0 ± 0.0
B. cereus LMG13569 BHI	11.0 ± 0.5	12.0 ± 1.0	12.0 ± 0.7	9.0 ± 0.0	10.0 ± 0.3	11.0 ± 0.9	8.0 ± 0.0	7.0 ± 0.0	7.0 ± 1.0	13.0 ± 0.5	18.0 ± 0.6
S. dysenteriae CIP 5451	9.0 ± 0.5	16.0 ± 1.0	15.0 ± 0.9	7.0 ± 0.6	7.0 ± 0.0	9.0 ± 0.3	Nd	Nd	Nd	24.0 ± 0.5	16.0 ± 0.0
Clinical isolates											
P. aeruginosa	10.5 ± 1.0	18.0 ± 0.5	18.0 ± 1.0	Nd	8.0 ± 0.5	8.0 ± 0.0	Nd	Nd	Nd	20.0 ± 0.0	21.0 ± 1.0
S. enterica	7.0 ± 0.0	14.0 ± 1.5	13.0 ± 1.1	Nd	8.0 ± 0.0	8.0 ± 1.0	7.0 ± 0.6	7.0 ± 0.5	9.0 ± 0.0	28.0 ± 1.0	16.0 ± 0.3
S. typhi	8.0 ± 0.5	12.5 ± 0.0	12.5 ± 1.0	7.0 ± 0.6	8.0 ± 1.0	8.0 ± 0.5	Nd	Nd	Nd	20.0 ± 0.5	15.0 ± 0.5
N. gonorrhoeae	26.0 ± 1.0	24.0 ± 1.0	25.0 ± 0.0	14.0 ± 1.5	13.0 ± 1.6	14.0 ± 1.0	9.0 ± 1.3	10.0 ± 1.0	10.0 ± 1.0	22.0 ± 1.2	10.0 ± 1.0
E. coli	12.0 ± 0.5	16.0 ± 0.9	15.0 ± 1.0	7.0 ± 1.0	8.0 ± 0.0	9.0 ± 1.0	8.0 ± 0.6	8.0 ± 1.0	9.0 ± 1.0	16.0 ± 1.0	9.0 ±1.0
S. aureus	13.0 ± 1.0	11.0 ± 1.0	11.0 ± 0.0	9.0 ± 1.0	10.0 ± 1.0	9.0 ± 0.0	7.0 ± 1.0	8.0 ± 0.5	9.0 ± 1.0	16.0 ± 1.0	8.0 ±1.0
K. pneumonia	11.0 ± 1.2	14.0 ± 1.0	12.0 ± 1.0	7.0 ± 0.6	8.0 ± 1.0	7.0 ± 0.0	8.0 ± 1.0	8.0 ± 1.0	10.0 ± 0.5	18.0 ± 1.0	Nd
A. baumannii	9.0 ± 1.0	17.0 ± 1.1	16.0 ± 0.3	7.0 ± 0.3	9.0 ± 1.0	14.0 ± 1.0	7.0 ± 1.0	9.0 ± 0.0	10.0 ± 1.3	16.0 ± 0.5	10.0 ± 2.0
E. aerogenes	9.0 ± 0.0	14.0 ± 1.0	16.0 ± 0.9	7.0 ± 0.0	9.0 ± 1.0	11.0 ± 1.0	Nd	10.0 ± 0.0	11.0 ± 0.0	16.0 ± 0.0	10.0 ± 0.6
Salmonella spp.	16.0 ± 0.9	18.0 ± 1.6	14.0 ± 2.0	7.0 ± 0.0	10.0 ± 1.0	9.0 ± 1.0	Nd	11.0 ± 1.0	10.0 ± 1.0	25.0 ± 0.0	14.0 ± 1.5
N. meningitidis	14.0 ± 1.0	16.0 ± 0.0	16.0 ± 1.0	7.0 ± 0.0	9.0 ± 1.0	9.0 ± 1.0	7.0 ± 1.0	11.0 ± 0.3	11.0 ± 1.0	16.0 ± 0.0	Nd
Fungi											
C. albicans ATCC 10231	10.0 ± 0.0	13.5 ± 1.0	13.0 ± 0.5	Nd	8.0 ± 1.0	10.0 ± 1.0	Nd	8.0 ± 0.6	8.0 ± 0.3	Nd	Nd
C. albicans ATCC 90028	9.0 ± 1.0	12.0 ± 0.0	12.5 ± 1.0	Nd	10.0 ± 0.3	10.0 ± 0.0	Nd	9.0 ± 0.5	8.0 ± 1.0	Nd	Nd

Nd: Not determinated; Genta: Gentamicin (10 µg/mL); Tetra: Tetracycline (30 µg/mL); Ce: *C. edulis*; Pl: *P. longifolia*; Ck: *C. klaineana*; AE: Water-acetone extract; WEE: Water-ethanol extract; WE: Water extract.

extracts, no inhibition zone was found against *S. dysenteriae* CIP 5451, *P. aeruginosa* and *S. typhi* (Table 5).

Results showed in Table 6 revealed the MIC and MBC of water-acetone, water-ethanol and water extracts of *C. edulis*, *P. longifolia* and *C. klaineana*. The lowest MIC (0.625 mg/mL) was recorded with the water-acetone and water-ethanol extracts of *C. edulis* on *E.*

Table 6

MIC and MBC or fungicidal concentration obtained by microdilution method. mg/mL.

Bacteria strains	Ce WE		Ce WEE		Ce WAE		Pl WE		Pl WEE		Pl WAE		Ck WE		Ck WEE		Ck WAE	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacteria reference strains																		
E. coli CIP 105182	1.25	2.5	1.250	2.50	1.250	2.50	Nd	Nd	1.25	2.5	1.25	2.5	> 5	> 5	5.0	> 5	5.0	>5
L. innocua LMG 135668 BHI	1.25	2.5	1.250	2.50	1.250	2.50	Nd	Nd	5.00	> 5.0	5.00	> 5.0	Nd	Nd	> 5.0	> 5	> 5.0	> 5
S. aureus ATCC 25293 BHI	2.50	2.5	1.250	2.50	1.250	5.00	5.00	> 5	2.50	2.5	1.25	5.0	> 5	> 5	5.0	>5	5.0	> 5
E. faecalis CIP 103907	1.25	2.5	0.625	5.00	0.625	5.00	1.25	5	2.50	> 5.0	1.25	5.0	5	> 5	2.5	5	2.5	5
B. cereus LMG 13569 BHI	1.25	2.5	0.625	1.25	1.250	2.50	5.00	> 5	2.50	5.0	2.50	5.0	> 5	> 5	5.0	> 5	5.0	> 5
S. dysenteriae CIP 5451	1.25	5.0	0.625	1.25	0.625	1.25	> 5.00	> 5	5.00	> 5.0	5.00	> 5.0	Nd	Nd	Nd	Nd	> 5.0	> 5
Clinical isolates																		
P. aeruginosa	1.25	5.0	0.625	1.25	0.625	1.25	Nd	Nd	2.50	2.5	1.25	5.0	Nd	Nd	Nd	Nd	Nd	Nd
S. enterica	2.50	5.0	1.250	5.00	1.250	5.00	Nd	Nd	5.00	> 5.0	5.00	> 5.0	> 5	> 5	> 5.0	> 5	5.0	> 5
S. typhi	1.25	2.5	0.625	1.25	1.250	2.50	5.00	> 5	> 5.00	> 5.0	5.00	> 5.0	Nd	Nd	Nd	Nd	Nd	Nd
N. gonorrhoeae	2.50	2.5	2.500	2.50	1.250	2.50	2.50	5	2.50	5.0	2.50	5.0	> 5	> 5	5.0	> 5	2.5	5
E. coli	2.50	5.0	2.500	5.00	2.500	5.00	5.00	> 5	5.00	> 5.0	5.00	> 5.0	> 5	> 5	5.0	> 5	5.0	> 5
S. aureus	2.50	5.0	2.500	5.00	2.500	5.00	5.00	> 5	2.50	5.0	5.00	> 5.0	> 5	> 5	> 5.0	> 5	5.0	> 5
K. pneumonia	2.50	5.0	2.500	5.00	2.500	5.00	5.00	> 5	5.00	> 5.0	5.00	> 5.0	> 5	> 5	5.0	> 5	5.0	> 5
A. baumannii	1.25	5.0	1.250	2.50	1.250	2.50	5.00	> 5	2.50	5.0	2.50	5.0	> 5	> 5	5.0	> 5	5.0	> 5
E. aerogenes	2.50	5.0	1.250	2.50	1.250	5.00	5.00	> 5	5.00	> 5.0	2.50	5.0	> 5	> 5	2.5	5	2.5	5
Salmonella sp.	2.50	5.0	1.250	5.00	1.250	5.00	5.00	> 5	2.50	5.0	2.50	> 5.0	> 5	> 5	2.5	5	2.5	5
N. meningitidis	1.25	2.5	1.250	2.50	1.250	2.50	5.00	5	2.50	5.0	2.50	5.0	> 5	> 5	2.5	5	2.5	5
Fungi	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C. albicans ATCC 10231	1.25	5.0	0.625	1.25	0.625	5.00	Nd	Nd	2.50	> 5.0	2.50	5.0	Nd	Nd	5.0	> 5	5.0	> 5
C. albicans ATCC 90028	2.50	2.5	1.250	1.25	1.250	1.25	Nd	Nd	2.50	5.0	2.50	5.0	Nd	Nd	5.0	> 5	5.0	> 5

Nd: Not determinated; Genta: Gentamicin (10 µg/mL); Tetra: Tetracycline (30 µg/mL); Ce: *C. edulis*; Pl: *P. longifolia*; Ck: *C. klaineana*; AE: Water-acetone extract; WEE: Water-ethanol extract; WE: Water extract.

faecalis CIP 103907, *S. dysenteriae* CIP 5451, *P. aeruginosa* and *C. albicans* ATCC 10231 and the water-ethanol extract of *C. edulis* on *B. cereus* LMG 13569 BHI and *S. typhi*. The lowest MBC (1.25 mg/mL) was observed with the water-acetone and water-ethanol extracts of *C. edulis* on *B. cereus* LMG 13569 BHI, *S. dysenteriae* CIP 5451 and *P. aeruginosa*. The MIC and MBC values were generally higher for the stem bark extracts of *P. longifolia* and *C. klaineana* against the test organisms compared to those of the *C. edulis* extracts.

4. Discussion

Phytochemical compounds are known for their antioxidant, antimicrobial and antifungal activities[17,18]. The presence of these compounds such as tannin, gallic acid, total phenolic, antracenosids, triterpenoids, totals flavonoids and reducing sugars in *C. edulis*, *P. longifolia* and *C. klaineanum* extracts may give credence to its local usage for the treatment of diarrhea, sexual diseases and oral infections. Contents of tannins and flavonoids in this study can justify their pharmacological effects for diarrheal diseases. Phenolic compounds are also reported to have anti-allergenic, anti-inflammatory and anti-thrombotic effects[19]. Therefore, the ethnomedicinal usage of *C. edulis*, *P. logifolia* and *C. klaineanum* extracts might be attributed to the high concentration of phenolic compounds. In addition, other secondary metabolites such as antracenosids, triterpenoid and proanthocyanidins could be held partially responsible for some of these biological activities[10]. The concentration of compounds as shown in this study could contribute synergistically to the significant antioxidant potency of plants and thus may support the local usage for the treatment of radical related diseases.

The principle of antioxidant activity is their interaction to produce oxidative free radicals. The role of DPPH method is that the antioxidants react with the stable free radical. During the free radical reaction, DPPH is converted into α, α-diphenyl-β-picrylhydrazine with colour change. The rate of colour change gradually decreases to indicate the scavenging potentials of the sample antioxidant. The AAI is based on the DPPH radical test. The extracts of *C. edulis* and water-acetone and water-ethanol extracts of *P. longifolia* have a very strong antioxidant activity. Vitamin C and butylated hydroxytoluene are the antioxidant of references. These extracts have a potential antioxidant which would enable them to play a beneficial role in terms of very significant preventive actions for human health[9]. Antioxidant activity of the plant should be at least partially justified by the presence of phenolic and the flavonoids highlighted by the phytochemical study[20]. While the extracts of *C. klaineana* present a weak antioxidant activity.

The scavenging activity of plants extracts was comparable to the standard drugs used in this study. This observation gives an indication of strong antioxidant potential of the extracts which is confirmed with reducing power, DPPH and ABTS radicals[21,22]. It can be inferred that the presence and the quantity of antioxidant compounds in *C. edulis* and *P. longifolia* could justify the observed results. *C. edulis*, *P. longifolia* and *C. klaineana* are used in folk medicine for the treatment of many diseases including bacterial diseases, parasitic diseases and diarrhea[23]. The antimicrobial activity of the crude extracts of plants was estimated using standard conventional methods. The study showed that the different extracts of these plants inhibited the growth of nearly all microorganisms

used in the essay, indicating the presence of antimicrobial compounds in these plants. These antibacterial actions could be related to their chemical components in the crude extracts[24]. Therefore, the conspicuous antimicrobial activity exhibited by water-acetone, water-ethanol and water extracts in the present study may be attributed to the presence of phenolic compounds.

The present antimicrobial study of different crude extracts of *C. edulis*, *P. logifolia* and *C. kaineana* showed that the water-acetone and water-ethanol crude extracts of *C. edulis* showed the highest activity against the employed bacteria. Similarly, the water-acetone and water-ethanol extracts of *C. edulis* showed the highest antioxidant activity. Phytochemical screening showed that the antioxidant and antibacterial activities of the crude extracts of *C. edulis*, *P. longifolia* and *C. klaineana* depend on the presence of phytochemicals such as total phenolic, antracenosids, terpenoids, flavonoids and tannins. The crude extracts of plants could serve as potential sources of new antimicrobial and antioxidant agents. Further research is needed towards isolation and identification of active principles present in the extracts which could be used for pharmaceutical use.

Conflict of interest statement

We declare that we have no conflict of interest.

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