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 Aôme,¹,² Louis-Clément Obame Engonga,¹,², Joel-Fleury Djoba Siawaya,³ Edouard Nsi Emvo⁴

¹Laboratory of Research in Biochemistry, University of Sciences and Technology of Masuku, P. O. Box 943 Franceville, Gabon
²Laboratory of Natural Substances and Organometallic Synthesis, University of Sciences and Technology of Masuku, P. O. Box 943 Franceville, Gabon
³National Laboratory of Public Health, P. O. Box 10736 Libreville, Gabon

Keywords: Phytochemical, Antioxidants, Antimicrobial, *Coula edulis*, *Pseudospondias longifolia*, *Carapa klaineana*

Abstract

**Objective:** To evaluate the phytochemical constituents, antioxidant and antimicrobial potential of water-acetone, water-ethanol and water extracts of *Coula edulis* (*C. edulis*), *Pseudospondias 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 ([12857.56 ± 1.00] mg gallic acid equivalent/100 g), flavonoids ([1634.13 ± 1.88] mg quercetin equivalent/100 g), tannins ([2672.00 ± 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 *P. longifolia*. 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 “Engang” 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 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
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, H₂SO₄, HCl, benzene, NH₄OH, sodium chloride, ethanol, Folin–Ciocalteu reagent, Na₂CO₃, 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 anthranecadins 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:

\[ \text{Percentage inhibition} = \frac{(A_{t0} - A_{t20})}{A_{t0}} \times 100 \]

where, At₀ is the absorbance of ABTS radical + ethanol, At₂₀ is the absorbance of ABTS radical + sample extract or standard.

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:

\[ \text{Percentage inhibition} = \frac{(A_{t0} - A_{t20})}{A_{t0}} \times 100 \]

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), Escherichia 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 ATCC 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]:

\[ \text{RPI} = 100 \times \frac{(X - Y)}{(Z - Y)} \]

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 (2.527.56 ± 2.92) to (12.857.56 ± 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 quercitin 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) | TTT (mg TAE/100 g of extract) | TRC (mg APE/100 g of extract) |
|----------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Ce WAE         | 11727.56 ± 4.58               | 1634.13 ± 1.88               | 2672.00 ± 1.59                | 2040.00 ± 3.33               |
| Ce WEE         | 12857.56 ± 1.00               | 871.63 ± 2.50                | 2000.89 ± 2.59                | 1951.11 ± 0.83               |
| Ce WE          | 5107.00 ± 5.63                | 1586.29 ± 1.32               | 1621.63 ± 3.95                | 1373.33 ± 1.67               |
| PI WEE         | 5107.56 ± 1.39                | 1301.21 ± 2.50               | 1415.70 ± 1.22                | 1186.22 ± 4.44               |
| PI WEE         | 7304.22 ± 1.51                | 721.21 ± 3.06                | 1509.04 ± 0.62                | 972.89 ± 9.17                |
| PI WE          | 6429.72 ± 1.78                | 1159.03 ± 0.83               | 768.30 ± 3.21                 | 873.33 ± 3.33                |
| CK WAE         | 3823.11 ± 1.78                | 739.96 ± 1.94                | 723.85 ± 3.15                 | 701.78 ± 3.33                |
| CK WEE         | 2857.56 ± 1.50                | 510.38 ± 1.11                | Nd                             | 241.78 ± 1.67                |
| CK WE          | 2527.56 ± 2.92                | 446.21 ± 0.16                | 77.93 ± 0.16                  | 26.22 ± 0.83                 |

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) | AA1 |
|----------------|-----------------------------|----------|----------------------|-----|
| 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|
| PI WEE         | \( Y = 1.65X + 2.80 \)      | 0.986    | 28.56 ± 0.38         | 1.75|
| PI WEE         | \( Y = 4.02X – 1.48 \)      | 0.995    | 12.81 ± 0.78         | 3.90|
| PI 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          | 0.72|
| BHA            | \( Y = 3.32X + 28.12 \)     | 0.950    | 6.59 ± 0.30          | 0.58|

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 \( S. aureus \) 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 \( S. aureus \). (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 \( C. klaineana \) recorded high inhibitory activities compared to the extracts of \( P. longifolia \) and \( C. klaineana \).
Inhibition zone diameters (mm) produced by the extracts from Table 5.

Table 5

| Bacteria strains        | Extracts          | Standards          |
|-------------------------|------------------|-------------------|
|                         | Ce WE             | Ce WEE            | Ce WAE | PI WE | PI WEE | PI 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 ± 1.0 | 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

|                        | Ce WE | Ce WEE | Ce WAE | PI WE | PI WEE | PI WAE | Ck WE | Ck WEE | Ck WAE | Genta | Tetra |
|------------------------|-------|--------|--------|-------|--------|--------|-------|--------|--------|-------|-------|
| 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    | 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    | 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 ± 1.0 | 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 ± 1.0 | Nd |

Fungi

|                          | Ce WE | Ce WEE | Ce WAE | PI WE | PI WEE | PI WAE | Ck WE | Ck WEE | Ck WAE | Genta | Tetra |
|--------------------------|-------|--------|--------|-------|--------|--------|-------|--------|--------|-------|-------|
| 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 ± 0.5 | 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 determined; 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
M\text{IC} \ and \ M\text{BC} \ of \ fungicidal \ concentration \ obtained \ by \ microdilution \ method, \ mg/mL.

| Bacteria strains                  | Ce WE | C\text{e} \text{WEE} | Ce WE | C\text{e} \text{WEE} | Pi WE | Pi \text{WEE} | Pi WE | Pi \text{WEE} | Ci WE | Ci \text{WEE} | Ci WE | Ci \text{WEE} | Ci WE | Ci \text{WEE} | Ci WE | Ci \text{WEE} | Ci WE | Ci \text{WEE} |
|----------------------------------|--------|-----------------------|--------|-----------------------|-------|---------------|-------|---------------|-------|---------------|-------|---------------|-------|---------------|-------|---------------|-------|---------------|
| E. coli CIP 105182               | 1.25   | 2.5                   | 1.25   | 2.5                   | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             |
| L. innocua DSM 13566 BHI        | 1.25   | 2.5                   | 1.25   | 2.5                   | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             |
| C. edulis P. longifolia         | 1.25   | 2.5                   | 1.25   | 2.5                   | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             |
| C. klaineana C. klaineana       | 1.25   | 2.5                   | 1.25   | 2.5                   | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             |
| C. albicans ATCC 10231          | 1.25   | 2.5                   | 1.25   | 2.5                   | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             | 2.5   | 5             |
| C. albicans ATCC 90028          | 2.50   | 5.0                   | 2.50   | 5.0                   | 2.50  | 5.0           | 2.50  | 5.0           | 2.50  | 5.0           | 2.50  | 5.0           | 2.50  | 5.0           | 2.50  | 5.0           | 2.50  | 5.0           |

Nd: Not determined; Genta: Gentamicin (10 μg/mL); Tetracycline (30 μg/mL); Ce: C. edulis; Pi: P. longifolia; Ci: 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 DSM 13569 BHI and S. typhi. The lowest M\text{BC} (1.25 mg/mL) was observed with the water-acetone and water-ethanol extracts of C. edulis on B. cereus DSM 13569 BHI, S. dysenteriae CIP 5451 and P. aeruginosa. The M\text{IC} and M\text{BC} 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. 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. klaineana 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 pharmaceutical effects for diarrheal diseases. Phenolic compounds are also reported to have anti-allergic, anti-inflammatory and anti-thrombotic effects. Therefore, the ethnomedical usage of C. edulis, P. longifolia and C. klaineana 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. 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 AA1 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.

Phenolic compounds and flavonoids 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. kaineanus* 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. logifolia* and *C. kaineanus* depend on the presence of phytochemicals such as total phenolic, anthracenosids, 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.

**Acknowledgments**

We are very grateful to local informants and healers who shared their knowledge on the use of medicinal plants with us. The authors are very much thankful to the Shell Gabon for the financial support of materials in Laboratory of Research in Biochemistry of USTM (Grant No. SG/CIS/SDM/SA/sa n° 77).

**References**

1. Akoué GN, Obame LC, Ondo JP, Brama I, N’Nang ESO, Tapoyo SY, et al. Phytochemical composition and antiradical activity of *Sakera sinia* Hook. f. medicinal plant from Gabon. *Int J Biomed Biomed* 2013; 3(3): 1-8.
2. Rahman MA, Imran TB, Islam S. Antioxidative, antimicrobial and cytotoxic effects of the phenolics of *Leuca indica* leaf extract. *Saudi J Biol Sci* 2013; 20(3); 213-25.
3. Boulekbache-Makhfouf L, Slimani S, Madani K. Total phenolic content, antioxidant and antibacterial activities of fruits of *Eucalyptus globulus* cultivated in Algeria. *Ind Crops and Prod* 2013; 41: 85-9.
4. Rathee D, Rathee P, Rathee S, Rathee D. Phytochemical screening and antimicrobial activity of *Picrorrhiza kurroa*, an Indian traditional plant used to treat chronic diarrhea. *Arabian J Chem* 2012; doi: 10.1016/j.arabjc.2012.02.009.
5. Raponda-Walker A, Sillians R. *[The useful plants of Gabon]*. Paris: Paul Lechestival; 1961. 614. French.
6. Cédric SO, Louis-Clement OE, Joseph-Privat O, Chekina Z, Edouard NE, Alfred TS. Ethnotherapy study, phytochemical screening and antioxidant activity of *Antoccurun klinaneaun* Pierre and *Anthocleista nobilis* G. Don. medicinal plants from Gabon. *Int J Adv Res* 2015; 3(5): 812-9.
7. Adebayo-Tayo BC, Ajibesin KK. Antimicrobial activities of *Coula edulis*. *Res J Med Plant* 2008; 2(2): 86-91.
8. Culel I. *Methodology for the analysis of vegetable drugs: practical manual on the industrial utilization of medicinal and aromatic plants*. Romania: Center Building; 1982. p. 67-81.
9. Angi-Tahé T, Massala K.K., Engonga LCO, Lebibi J. Phytochemical studies, total phenolic and flavonoids content and evaluation of antiradical activity of the extracts of leaves from *Dischistocaulys sp.* (Acanthacées). *J Pharmacogn Phytomed* 2015; 3(6): 174-8.
10. Obame LCE, Timoléon AB, Privat OJ, Ludovic M, Roger NAG, Fatouma AL, et al. Total phenolic composition, antibacterial and antioxidant activities of *Fagara heitzii* Aubr & Pellegr medicinal plant of Gabon. *Vedic Res Int Phytomed* 2013; 1(3): 85-92.
11. Scherer R, Godoy HT. Antioxidant activity index (AII) by 2,2-diphenyl-1-picrylhydrazyl method. *Food Chem* 2009; 112(3): 654-8.
12. Kumar M, Kumar S, Kaur S. Investigations on DNA protective and antioxidant potential of chloroform and ethylacetate fractions of *Koelreuteria paniculata* Laxm. *Afr J Pharm Pharmacol* 2011; 5(3): 421-7.
13. Mandal M, Paul S, Uddin MR, Mandal MA, Mandal S, Mandal V. In vitro antibacterial potential of *Hydrocotyle javanica* Thunb. *Asian Pac J Trop Dis* 2016; 6(1): 1-6.
14. Weil AM, Al-Blushi AAM, Hossain MA. Evaluation of antioxidant and antimicrobial potential of different leaves crude extracts of *Omani Ficus carica* against food borne pathogenic bacteria. *Asian Pac J Trop Dis* 2015; 5(1): 13-6.
15. Naz R, Bano A, Yasmin H, Ullah S, Farooq U. Antimicrobial potential of the selected plant species against some infectious microbes used. *J Med Plants Res* 2011; 5(21): 5247-53.
16. Naz R, Bano A. Phytochemical screening, antioxidants and antimicrobial potential of *Lantana camara* in different solvents. *Asian Pac J Trop Dis* 2013; 3(6): 480-6.
17. Hossain MA, Nagooru MR. Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant *Corydline terminalis* L. *Kunth. Pharmacogn J* 2011; 3(24): 25-30.
18. Alabri THA, Al Musalami AHS, Hossain MA, Weil AM, Al-Riyami Q. Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. *J King Saud Univ Sci* 2014; 26(3): 237-43.
19. Baydar NG, Baydar H. Phenolic compounds, antiradical activity and antioxidant capacity of oil-bearing rose (*Rosa damascena* Mill.) extracts. *Ind Crops Prod* 2013; 41: 375-80.
20. Igbinosoa OO, Igbinosoa IH, Chigor VN, Uzunigbe E, Oyedemi SO, Odjadjare EE, et al. Polyphenolic contents and antioxidant potential of stem bark extracts from *Jatropha curcas* (Linna). *Int J Mol Sci* 2011; 12(5): 2958-71.
21. Melilda KP, Rathinam X, Marimuthu K, Diwakar A, Ramanathan S, Kathiresan S, et al. A comparative study on the antioxidant activity of methanolic leaf extracts of *Ficus religiosa* L., *Chromolaena odorata* (L.) King & Rabinson, *Cynodon dactylon* (L.) Pers. and *Tidrax procumbens* L. *Asian Pac J Trop Med* 2010; 3(5): 348-50.
22. Mbaebie BO, Edeoga HO, Afolayan AJ. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pac J Trop Biomed* 2012; 2(1): 118-24.
23. Aharayfi FC. Antibacterial, phytochemical and antioxidant activities of *Datura metel*. *Int J PharmTech Res* 2011; 3(1): 974-4304.
24. Sekar D, Kolanjinathan K, Saranjai P, Gajendiran K. Screening of *Phyllanthus amarus*, *Acalypba indica* and *Datura metel* for its antimicrobial activity against selected pathogens. *Int J Pharm Biol Arch* 2012; 3(5): 1231-5.