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## RESEARCH ARTICLE

# PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *GUIBOURTIA EHIE* AND *SYZYGIUM ROWLANDII* MEDICINAL PLANTS FROM GABON

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**ABSTRACT**

Medicinal plants have been used for centuries as remedies for human diseases and continue to do important services at human being. These plants have served as a very important replacement for the resistant strains. Aqueous, chloroform and methanol extracts of *Guibourtia ehie* and *Syzygium rowlandii* were evaluated for phytochemical analyses, antimicrobial and antioxidant activities. The antimicrobial activity was evaluated by disc diffusion and microdilution assays. Antioxidant Activity Index (AAI) was determined for antioxidant activity evaluation. Phytochemical screening showed also high total phenol, saponins, flavonoids, alkaloids, sterols, triterpenes and proanthocyanidins. The extracts of *Guibourtia ehie* reduce the concentration of DPPH free radical, $IC_{50}$ values are 34.0 ± 0.15 µg/mL and 49.0± 0.19 µg/mL for water and methanol extracts. Oxidation of β-carotene is inhibited by methanolic and aqueous extracts (55.64 ± 0.05% and 68.4 ± 0.05%). The $IC_{50}$ of *S. rowlandii* is 91.5 ± 0.15 mg/mL. The relative antioxidant activity of methanol extracts was 95.4 %. However, water extract of *Guibourtia ehie* and methanol extract of *Syzygium rowlandii* had strong effects, AAI are 2.31 and 8.54, and can be compared to AAI of Vitamin C and BHT (AAI values of 11.32 and 7.85, respectively). The results of susceptibility testing with extracts of *Guibourtia ehie* and *Syzygium rowlandii* show that these plants exhibit the growth of nearly all microorganisms used in the essay. The study confirm the multiple uses of *Guibourtia ehie* and *Syzygium rowlandii* for the treatment of many infectious diseases and place them as candidate for further investigations for traditional drug utilizable as complementary and alternative medicines development and new active compounds.

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## INTRODUCTION

Plants have always been considered as an important source of medicine for human. Phytomedicines have been an integral part of traditional health care system in most parts of the world for thousands of years (Rath *et al*., 2009). Research has shown the potential exploitation of plant products as a source of new bioactive compounds (Gil-Chávez *et al*., 2013). *Guibourtia ehie* or Kévazingo (Ovengkol in Fang) belongs to Cesalpinoides subfamily. The geographic distribution is tropical Africa, this species occurs in Cameroon, Côte d'Ivoire, Gabon, Ghana, Liberia and Nigeria. *G. ehie* is a forest species, preferring closed rain forests and transition forests (WCMC, 1991). It is also supported on large buttresses, often erect and is recognizable by its leaves composed of two sagging leaflets. It is a tree with a cylindrical barrel, straight and slender. The bark is fairly smooth, ash-white, and the leaves are of a single pair of opposite leaflets, with slightly leafy limb, green above and lighter below. The sessile flowers are white, in terminal

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panicles. The dried fruits are indehiscent, papery, in the form of small dark gray pods, a single seed oval, flat, of a brown yellow. This species suffers from high exploitation rates. It is used for the manufacture of quality furniture. *Guibourtia ehie* is used for cleansing wounds and gonorrhea. Wood of this species is a popular substitute for rosewood (Raponda-Walker and Sillans, 1961; Hawthorne, 1995b; White and Abernethy, 1996). *Syzygium* is a large genus of Myrtaceae in Malaysia with about 1000 species, confined to the tropics of Old World. In Congo, *Syzygium rowlandii* usually has smaller flowers compared to specimens of West Africa (Craven, 2001). It occurs in forests, often near water and swamps, usually 500-2000 m altitude in the mountain forest in Eastern RD Congo. *Syzygium rowlandii* is a culturally rich and has extensive local medicinal uses, application as a local anesthetic to be used for diarrheal diseases, sexually transmitted infections: syphilis and Monolia (Kerharo, 1974; Hoffmann, 1988). A decoction of dried bark of *Syzygium rowlandii* added lemon juice gives a black dye. The wood is strong, hard, and resistant to water. It is used in Liberia for the keels of boats. Powdered bark mixed with clay and spices treat rheumatic pains. A bark infusion uses in Congo against coughs, asthma, throat problems, intercostals pain, food poisoning, stomach pain, diarrhea and dysentery. The juice of leaves and bark decoction is used as a lotion to kill parasites and treat scabies, but they also are consumed as a tonic. The root powder is applied to the teeth and toothache cures (Raponda-Walker and Sillans, 1961). The present work reports results of phytochemical analyses, antioxidant and antimicrobial proprieties of *Guibourtia ehie* and *Syzygium rowlandii*, Gabonese medicinal plants. Such a study may help in the contribution of the ongoing search for beneficial uses of these plants to eradicate various resistance infectious diseases.

## MATERIALS AND METHODS

### Plant materials

The bark of *Guibourtia ehie* and *Syzygium rowlandii* were collected in June 2016 from Mebane Endama village, in Oyem (Northern of Gabon). Identification of the species was carried out at National Herbarium of IPHAMETRA, Libreville (Gabon). Voucher specimens have been deposited in Herbarium of IPHAMETRA and in Laboratory of Natural Substances and Organometallic Synthesis (LASNSOM) at Department of Chemistry, Faculty of Sciences of USTM in Franceville-Gabon.

### Compoud extraction and qualitative analysis

The harvest samples were dried in the laboratory at room temperature, afterwards they were pulverized with a mechanical crusher. The powder was used for several extractions. For aqueous extraction, 50 g of powder were percolated for 24 h in 300 mL water. After cooling at room temperature, the extract was filtred and lyophilised. For methanolic extraction, 50 g of powder were percolate in 500 mL of methanol for 24 h. For chloroform extract, 50 g of powder were percolated with 500 mL of chloroform for 24 h. Methanol and chloroform were evaporated with a rotary evaporator.The extract was then washed with hexane in order to eliminate the chlorophyll and other pigments. The solvent were evaporated and the extract was finally lyophilised. For the test, aqueous extract was dissolved in distilled water. Chloroform and methanol extracts were dissolved in dimethyl sulfoxide (DMSO) and a serial dilution was made to have a final concentration of 0.5% DMSO to obtain a twofold serial dilutions ranging from 400 to 2000 µg/mL. Qualitative tests were performed on each extract for chemical groups such as saponosids, tannins, reducing compounds, phenolic compounds, flavonoids, proanthocyanidins, sterols, triterpens, carotenoids, polyphenols and anthracenoside detection as described by Ciulei (1982).

### Quantitative analysis of phytochemicals

**Phenolic content:** The Folin-Ciocalteu method was used to measure total amount of phenolic content (Singleton et *al*., 1999). The original assay was adapted to a microtiter 96-wells plate system (Dicko et *al*., 2005a). Gallic acid (3, 4, 5-trihydroxybenzoic acid) was used as standard. To 20 µL of beverage, 80 µL of Folin-Ciocalteu reagent were added. After 5 min incubation at room temperature (20°C), 80 µL of 20% (w/v) sodium carbonate solution was added and incubated. After 30 min of incubation, the absorbances were read at 760 nm. All tests were carried out in triplicate and results were expressed as gallic acid equivalent (GAE).

**Tanins:** The reference method of European communauty was used to measure total amount of tannins (1994).

**Proanthocyanidins:** The method was quantified with an adaptation to a 96-well plate assay (Dicko et *al*., 2005a). It involved the hydrolysis of proanthocyanidins in a hot acid-alcohol medium into anthocyanidins. This method allows taking into account all the units of flavan-3-ol constituting the polymers (Prigent, 2005). The heating step destroys the anthocyanidins pigments generated by flavan-4-ol and eliminates part of the chlorophyll pigments. The routine assay is performed by mixing 50 µL of the extract with 700 µL of 30% HCl-butanol solution (v/v). The mixture was put in tightly closed 1.5 mL Eppendorf tube and vortexed for 1 min. Subsequently, the tube was heated at 100°C for 2 h and after cooling, 200 µL aliquots were put in triplicate into a 96 multiwell plate and the absorbances were read at 550 nm. Apple procyanidins (DP ≈ 7.4) treated as aforementioned were used as a standard. Results were expressed as apple procyanidins equivalent (APE).

**Flavonoid**: The Dowd method was used to measure total amount of flavonoids (Arvouet-Grant, 1994). The original assay was adapted to a microtiter 96-wells plate system (Dicko et *al*., 2005a). Quercetin was used as standard. 100 µL of $AlCl_3$ 2% in methanol were added to 100 µL of extract. After 10 min incubation at room temperature (20°C), the absorbances were read at 415 nm. All tests were carried out in triplicate and results were expressed as qercetin equivalent (QE).

### Microorganisms, antibiotics and media

Commercially available antibiotics discs, Penicillin (10 IU/IE/UI) and Tetracyclin (30 µg) were purchased from Beckton Dickinson. The reference strains were *Escherichia coli* CIP 105182, *Enterococcus faecalis* CIP 103907, *Bacillus cereus* LMG 13569 BHI, *Listeria innocua* LMG 135668 BHI, *Staphylococcus aureus* ATCC 25293 BHI, *Staphylococcus camorum* LMG 13567 BHI, *Proteus mirabolis* CIP 104588, *Shigella dysenteria* CIP 5451 and *Staphylococcus aureus* ATCC 9144. Clinical isolates were *Staphylococcus aureus* (n = 5), *Enterococcus faecalis* (n = 5), *Pseudomonas aerugenosa* (n

= 10), *Salmonella enterica* (n = 5) and *Streptococcus pyogenes* (n = 10). All these strains were isolated from clinical samples at Laboratory of Research in Biochemistry of USTM-Franceville.

**Determination of antioxidant activity**

**Determination of Antioxidant Activity Index (AAI):** The Antioxidant Activity Index was assessed according to the method described by Scherer and Godoy (2009). This method is based on DPPH radical test. Briefly, the working reagent was prepared by dissolving 10 mg of DPPH in 100 mL ethanol. Graded concentrations of extracts ranging from 0.781 to 100 µg/mL obtained by two-fold dilutions were prepared and 100 µL of each dilution were mixed with 100 µL of the working solution of DPPH in a 96-well plate. Absorbencies were measured at 517 nm after 15 min incubation at room temperature in the dark. Ascorbic acid (Vitamin C) and butylated hydroxyl toluene (BHT) were used as references. The ability to scavenge DPPH radical was calculated by the following equation:

%RSA = [(A control – A sample) / A control] x 100.

A = Absorbance at 517 nm.

The $IC_{50}$ (concentration providing 50% inhibition) of extracts and standards was determinate using regression curves in the linear range of concentrations. The AAI was then calculated as follows:

AAI = [DPPH] ($\mu g.mL^{-}1$) / $IC_{50}$ ($\mu g.mL^{-1}$)

[DPPH] is the final concentration of DPPH.

We considered criteria of Scherer and Godoy (2009) according to which plant extracts show poor antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI between 0.5 and 1.0, strong antioxidant activity when AAI between 1.0 and 2.0, and very strong when AAI > 2.0.

**Determination of Relative Antioxidant Activity (RAA%)**

The antioxidant capacity of the extracts was determined according to method described by Dapkevicus et *al*. (1998). 0.5 mg ofβ-carotene was dissolved in 1 mL of Chloroform (HPLC grade); 25 µL of linoleic acid and 200 mg of tween 40 were added as emulsifier because β-carotene is not water soluble. Chloroform was completely evapored using a vacuum evaporator. Then, 100 mL of distilled water saturated with oxygen was added with vigorous shaking at a rate of 100 mL/min for 30 min; 2500 µL of this reaction mixture was dispersed to test tubes, and 350 µL portions of extracts, prepared in 2 g/L concentrations, were added. The emulsion system was incubated for up to 48 h at laboratory temperature. The same procedure was repeated with a positive control BHT and a blank. After this incubation time, the absorbance of the mixture was measured at 490 nm. Antioxidant capacities of extracts were compared with those at BHT and blank. Tests were carried out in triplicate. The relative antioxidant activity (RAA %) of the extracts was calculated from the equation:
RAA (%) = ($A_{Sample}$ / $A_{BHT}$) x 100. Where $A_{BHT}$ is the absorbance of the positive control BHT and $A_{Sample}$ is the absorbance of the extract.

**Antibacterial assays**

**Agar-well diffusion:** The assay was conducted as described by Perez *et al*. (1990). Briefly, microorganisms from growth on nutrient agar incubated at 37°C for 18 h were suspended in saline solution 0.9% NaCl and adjusted to a turbidity of 0.5 Mac Farland standards ($10^8$ cfu/mL). The suspension was used to inoculate 90 mm diameter Petri plates with a sterile non toxic cotton swab on a wooden applicator. Six millimeters diameter wells were punched in the agar and filled with 50 µL of 2000 µg/mL extract. The dissolution of the extract was aided by 0.5% (v/v) DMSO which did not affect microorganism growth, according to our control experiments. Commercial antibiotics were used as positive reference standard to determine the sensitivity of the strains. Discs were directly placed onto the bacterial culture. Plates were incubated in air at 37°C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters (IZD). The experiments were conducted twice (Obame et *al*., 2014; Sima et *al*., 2017).

**Broth microdilution assay:** Broth microdilution method was used to determine minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of extract against the test microorganisms as recommended by National Committee for Clinical Laboratory Standards (NCCLS, 2000). The tests were performed in 96 well-plates. Extract dissolved in 0.5% DMSO was transferred in plates to obtain a twofold serial dilutions ranging from 0.0625 to 400 µg/mL. Then plates were inoculated with microbial suspensions diluted from the same 0.5 Mac Farland standards to have $10^8$ cfu/mL in each well (Lennette et *al*., 1987). The final volumes in wells were 200 µL. After 24 h incubation in air at 37°C, MIC was recorded as a lowest extract concentration demonstrating no visible growth in broth. MBC was recorded as a lowest extract concentration killing 99.9% of bacterial inocula. MBC values were determined by removing 100 µL of bacterial suspension from subculture demonstrating no visible growth and inoculating nutrient agar plates. Plates were incubated at 37°C for a total period of 48 h (Obame et *al*., 2012; 2014; 2016).

**Statistical analysis:** Data were expressed as mean ± SEM. A one way variance was used to analyse data. P< 0.01 represented significant difference between means (Duncan's multiple range test).

## RESULTS AND DISCUSSION

**Phytochemical screening**

The phytochemical screening of the extracts was first performed to detect the major chemical groups occurring in the extracts. The results of screening are shown in table 1. It appears that two plants studied *Guibourtia ehie* and *Syzygium rowlandii* contain phenolic compounds and triterpenoids. The methanol extracts of *Guibourtia ehie* contained the most detected chemical groups and are rich in tannins, phenolic compounds, flavonoids, proanthocyanidins and sterol. The saponins and anthocyanin are abundant in water extracts. Chloroform extract are rich in reducing compounds and anthracene derivatives. The screening of chemical extracts of *Syzygium rowlandii* shows that they are rich in phenolic compounds.

**Table 1. Results of the preliminary phytochemical screening**

| Chemical Groups | *Guibourtia ehie* | | | *Syzygium rowlandii* | | |
|---|---|---|---|---|---|---|
| | Chloroform | Methanol | Water | Chloroform | Methanol | Water |
| Flavonoids | - | +++ | +++ | - | ++ | ++ |
| Coumarins | + | + | ++ | ++ | ++ | + |
| Tannin | - | +++ | +++ | ++ | ++ | ++ |
| Total phenol | - | +++ | +++ | - | ++ | +++ |
| Anthracenosids | +++ | + | +++ | +++ | ++ | - |
| Saponosids | - | - | +++ | + | - | +++ |
| Sterol and Triterpenoids | - | +++ | +++ | +++ | +++ | - |
| Carotenoids | - | ++ | ++ | - | +++ | + |
| Proanthocyanidin | - | +++ | + | + | +++ | ++ |
| Alkaloids | - | ++ | ++ | ++ | +++ | + |
| Reducing compounds | ++ | +++ | +++ | +++ | +++ | - |

+++ = Very abundant; ++= Abundant; + = Rare, — = Not Detected.

**Table 2. Total phenolic content (TPC), total flavonoid content (TFC), total tannins content (TTC) and total proanthocyanidins content (TPC) of extracts from *Guibourtia ehie* and *Syzygium rowlandii***

| Extracts | TPC (mg GAE/ 100 g of extract) | TFC (mg QE/ 100 g of extract) | TTC (mg TAE/ 100 g of extract) | TPC (mg APE/100 g of extract) |
|---|---|---|---|---|
| Ge WE | 193±5.74 | 560±6.78 | 857.26±27.77 | 1974.89±11.67 |
| Ge CE | Nd | 1002.24±7.34 | 260.77±11.66 | 991.83±13 |
| Ge ME | 180.33±2.50 | 740.94±3.26 | 3201.34±18.06 | 8720.88±26.17 |
| Sr WE | 904.67±3.25 | 380.52±4.65 | 247.26±9.70 | 197.12±11.31 |
| Sr CE | Nd | Nd | 186.33±6.71 | 679.33±15.81 |
| Sr ME | 583.42±2.08 | 615.62±3.19 | 985.41±8.14 | 339.34±13.11 |

Ge = *Guibourtia ehie*; Sr = *Syzygium rowlandii*; WE = Water extract; EE=Ethanol extract; CE= Chloroform extract, Nd = Non determined.

**Table 3. Antioxidant activities of *Guibourtia ehie* and *Syzygium rowlandii* extracts**

| Plants | Antioxidant activity | | Water | Chloroform | Methanol |
|---|---|---|---|---|---|
| *Guibourtia ehie* | DPPH | $IC_{50}$ (µg/mL) | 34.0±0.15 | Nd | 49.0±0.19 |
| | | AAI | 2.31 | Nd | 1.60 |
| | | Activity | Very strong | Nd | Strong |
| | β-carotene | RAA (%) | 68.4±0.05 | Nd | 55.6±0.05 |
| *Syzygium rowlandii* | DPPH | $IC_{50}$ (µg/mL) | 48.8±0.50 | Nd | 9.22±0.02 |
| | | AAI | 1.61 | Nd | 8.54 |
| | | Activity | Strong | Nd | Very strong |
| | β-carotene | RAA (%) | 91.5±0.15 | Nd | 94.4±0.05 |

**Table 4. Inhibition zone diameters IZD (mm) recorded in agar-well diffusion assay**

| | Diameters of inhibition zone (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Guibourtia ehie* | | | *Syzygium rowlandii* | | | Te | P |
| | Gram | WE | CE | ME | WE | CE | ME | | |
| *E. coli* CIP 105182 | - | 13 | 27 | 26 | Nd | 13 | 10 | 11 | Nd |
| *Enterococcus faecalis* CIP 103907 | + | 20 | 26 | 13 | 27 | 0 | 13 | 12 | 19 |
| *Bacillus cereus* LMG 13569 BHI | + | 15 | 25 | 14 | 21 | 9 | 14 | 11 | 18 |
| *Listeria innocua* LMG 135668 BHI | + | 13 | 33 | Nd | 27 | 0 | 10 | 12 | 14 |
| *Staphylococcus aureus* ATCC 25293 BHI | + | 11 | 32 | Nd | 19 | 10 | 10 | 12 | 26 |
| *Proteus mirabolis* CIP 104588 | - | Nd | 20 | Nd | 7 | 15 | 19 | 13 | 15 |
| *Shigella dysenteria* CIP 5451 | + | 22 | 30 | Nd | Nd | 7 | 21 | 23 | 16 |
| *Staphylococcus aureus* ATCC 9144 | - | 16 | 31 | Nd | 19 | 6 | 10 | 5 | 26 |
| *Staphylococcus camorum* LMG 13567BHI | + | 15 | 32 | Nd | 33 | 15.5 | 15 | 15 | 21 |
| *Staphylococcus aureus* (n=5) | + | 17±3 | 32±2 | Nd | 0±0 | 21±3 | 14±3 | 0±0 | 39 |
| *Salmonella enterica* (n=5) | + | 15±2 | 23±3 | Nd | Nd | 10±2 | 13±1 | 12±3 | 16 |
| *Streptococcus pyogenes* (n=10) | + | 14±2 | 26±3 | Nd | 29 | 12±2 | 15±3 | 13±2 | 21 |
| *Enterococcus faecalis* (n=5) | - | 18±4 | 24±2 | Nd | 24 | 4.5±1 | 13±2 | 13±2 | 17 |
| *Pseudomonas aerugenosa* (n=10) | - | 20±5 | 23±3 | Nd | 9 | 12±1 | 15±2 | 13±2 | 21 |

WE= Water extract, CE = Chloroform extract, ME= Methanol extract, P = Penicillin (10 IU/IE/UI), Te = Tetracycline (30 µg), Nd = Non determined.

**Table 5. Means of MIC and MBC recorded in microdilution assay using *Guibourtia ehie* and *Syzygium rowlandii* extracts (mg/mL)**

| Extracts | *Guibourtia ehie* | | | | | | *Syzygium rowlandii* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Water | | Chloroform | | Methanol | | Water | | Chloroform | | Methanol | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| *E. coli* 105.182 CIP | 25 | 25 | >100 | Nd | 50 | 50 | 0.50 | 0.50 | >100 | Nd | 0.25 | 50 |
| *Enterococcus faecalis* 103.907 CIP | 12,5 | 12,5 | Nd | Nd | 50 | 50 | >100 | Nd | 0.50 | 0.50 | 0.50 | 0.5 |
| *Bacillus cereus* LMG 13569 BHI | 25 | 25 | >100 | Nd | 50 | 50 | >100 | Nd | 0.25 | 0.25 | 0.25 | 0.25 |
| *Listeria innocua* LMG 135668 BHI | 12.5 | 12.5 | >100 | Nd | 50 | 100 | >100 | Nd | >100 | Nd | 0.0625 | 0.25 |
| *Staphylococcus aureus* ATCC 25293 BHI | 25 | 25 | Nd | Nd | 25 | 25 | >100 | Nd | >100 | Nd | 0.0625 | 0.0625 |
| *Proteus mirabolis* 104588 CIP | 25 | 25 | Nd | Nd | 50 | 50 | 0.50 | 0.50 | 0.125 | 0.125 | 0.25 | 0.25 |
| *Staphylococcus camorum* LMG 13567 BHI | 50 | 50 | Nd | Nd | 25 | 100 | 0.50 | 0.50 | 0.50 | 50 | 0.25 | 0.25 |
| *Shigella dysenteria* 5451 CIP | 25 | 25 | Nd | Nd | 50 | 100 | >100 | Nd | 0.125 | 0.125 | 0.125 | 0.125 |
| *Staphylococcus aureus* 53154ATCC 9144 | 100 | 100 | Nd | Nd | 25 | 25 | >100 | Nd | 0.50 | 100 | >100 | Nd |
| Staphylococcus aureus | >100 | Nd | Nd | Nd | 25 | 25 | >100 | Nd | 0.25 | 25 | 0.50 | 0.50 |
| *Streptococcus pyogenes* | 25 | 25 | Nd | Nd | 50 | >100 | >100 | Nd | 0.25 | 0.25 | 0.25 | 0.50 |
| *Enterococcus faecalis* | 100 | 100 | >100 | Nd | 50 | >100 | >100 | Nd | 0.50 | 0.50 | 0.25 | 0.50 |
| *Pseudomonas aerugenosa* | 50 | 50 | Nd | Nd | 50 | >100 | >100 | Nd | 0.25 | 0.25 | 0.25 | 0.50 |
| *Salmonella enterica* | 25 | 25 | Nd | Nd | 50 | 100 | >100 | Nd | 0.50 | 100 | 0.50 | 100 |

The tannins are abundant in all extracts. Reducing compounds, anthracene derivatives, alkaloids, sterols and triterpenes are very abundant in methanol and chloroform extracts. The saponins, phenolic compounds, flavonoids and proanthocyanidins are abundant in aqueous extract. The emodols and coumarins are abundant in chloroform extract while methanol extract is rich in carotenoids.

**Phenolic content and DPPH scavenging activity**

The results show that the bark of *Guibourtia ehie* and *Syzygium rowlandii* contains extractable polar compounds superior to non-polar compounds (Table 2). Levels of phenolic content were expressed in terms of gallic acid equivalent (GAE). The equation of the right and side of the proportioning of total phenolic content by Folin-Ciocalteu method gave Y = 0.0012 X – 0.0004 with $R^2$ = 0.9902 (Abdoul-latif et *al*., 2012). The HCl/butanol assay used here for the determination of proanthocyanidins is more specific than many other tests such as vanillin assay (Makkar, 2000; Santos-Buelga and Scalbert, 2000). Levels of proanthocyanidins were expressed in terms of apple procyanidins equivalent (APE). The equation of the right-hand side of the proportioning of proanthocyanidins by HCl-Butanol method gave Y = 0.0006 X + 0.0024 with $R^2$ = 0.9869 (Abdoul-latif et *al*., 2012, Andzi-Barhé et *al*., 2015). In *Guibourtia ehie*, the concentration of proanthocyanidines and tannins is considerably greater in methanol extract than in water and chloroform extracts, while those of total phenols and flavonoid are greater in water and chloroform extracts. The extracts reduce the concentration of DPPH free radical, the $IC_{50}$ values are 34.0 ± 0.15 µg/mL and 49.0± 0.19 µg/mL for water and methanol extracts. Oxidation of β-carotene is inhibited by methanolic and aqueous extracts (55.64 ± 0.05% and 68.4 ± 0.05%). The capacity of aqueous extract to reduce β-carotene is greater than that in methanol extract and exceeds 50% in both cases. The antioxidant effect evaluated by decoloration of β-carotene appears to be greater than that observed with DPPH method. This activity is due to the chemical composition of the extracts rich in highly hydroxylated polyphenols. These two extracts of *Guibourtia ehie* constitute potential antioxidants.

The yield of water, chloroform and methanol of *Syzygium rowlandii* were 4.43% (v/v), 1.49% (v/v) and 2.76% (v/v), respectively. Proanthocyanidin contents were 197.12 µg APE/mL, 679.33 µg APE/mL and 339.34 µg APE/mL of aqueous, chloroformic and methanolic extracts of *S. rowlandii*. The average content in proanthocyanidins was 45 µg APE/mL. Levels of flavonoids were expressed as qercetin equivalent (QE). The equation of the right-hand side of the proportioning of qercetin by Dowd method, gave Y = 0.0032 X + 0.0077 with $R^2$ = 1 (Obame et *al*., 2013). Flavonoid contents were 380.52 µg QE/mL and 615.62 µg QE /mL of aqueous and methanolic extracts. Levels of tannins were expressed in terms of tannic acid equivalent (TAE). The equation of the right-hand side of the proportioning of total tannins by reference method of European communauty (1994) gave Y = 0.0009 X + 0.2088 with $R^2$ = 1. Among *S. rowlandii* extracts, tannins contents were 247.26 µg TAE/mL, 186.33 µg TAE/mL and 985.41 µg TAE/mL. The concentration of total phenols, proanthocyanidins and tannins is greater in methanol extract while flavonoid content is greater in chloroform extract. For DPPH test, the extracts reduce the concentration of free radical. The $IC_{50}$ of *Syzygium rowlandii* are 91.5 ± 0.15 µg/mL and 94.45 ± 0.05 µg/mL for water and methanol extracts, respectively. The relative antioxidant activity of methanol extracts was 95.4 ± 0.05%. The capacity of methanol extract to reduce β-carotene is higher than that of aqueous extract and exceeds 90%. The antioxidant effect by trapping DPPH radical is higher than that observed by method of discoloration of β-carotene. This activity is due to the chemical composition of methanol extract rich in polyphenols, more recently, several scientific studies of their biological activity were performed.

As it can be seen, the AAI of extracts ranged from 1.60 to 8.54 (Table 3). The crude extract of *Guibourtia ehie* and *Syzygium rowlandii* showed a strong activity. However, water extract of *Guibourtia ehie* and methanol extract of *Syzygium rowlandii* had strong effects, AAI are 2.31 and 8.54, and can be compared to AAI of Vitamin C and BHT (AAI values of 11.32 and 7.85, respectively) (Andzi et *al*., 2013; Obame et *al*., 2016; Sima et *al*., 2016; Ngoua et *al*., 2017). These water and methanol extracts of *Guibourtia ehie* and *Syzygium rowlandii* have a potential antioxidant which would enable them to play a beneficial role in terms of very significant preventive actions for human health.

**Antimicrobial activity**

The results of susceptibility testing with extracts show that they exhibit significant antibacterial activity of the strains tested. Extracts of *Guibourtia ehie* and *Syzygium rowlandii* recorded inhibition diameters contained in table 4. The antimicrobial properties of the extracts of *Guibourtia ehie* show that all have a specific activity. The extracts are active on Gram positive and negative bacteria. The aqueous and methanolic extracts gave an important antimicrobial activity with inhibition diameters varying between 11 and 33 mm. The strains most sensitive to aqueous extract were *Staphylococcus camorum* LMG 13567 BHI (22 mm), *Salmonella enterica*, *Enterococcus faecalis* 103.907 CIP (20 mm), *Pseudomonas aeruginosa* (18 mm), *Staphylococcus aureus* (17 mm), *Shigella dysenteria* 5451 CIP (16 mm), *Streptococcus pyogenes*, *Bacillus cereus* LMG 13569 BHI and *Staphylococcus aureus* ATCC 9144 (15 mm). The aqueous extract of *Guibourtia ehie* gave intermediate inhibition zone diameter on *Enterococcus faecalis* (14 mm) and *Listeria innocua* LMG 135668 BHI (13 mm). The methanol extract of *Guibourtia ehie* gave a great antibacterial activity on all the strains tested with inhibition diameters varying from 20 to 33 mm. The extract was very active on *Listeria innocua* LMG 135668 BHI (33 mm), *Staphylococcus aureus* ATCC 25293 BHI, *Staphylococcus aureus* (32 mm), *Shigella dysenteria* 5451 CIP (31 mm) and *Staphylococcus camorum* LMG 13567 BHI (30 mm). The other strains are sensitive with inhibition diameters ranging from 20 to 27 mm, the case of *E. coli* 105182 CIP (27 mm), *Enterococcus faecalis* 103907 CIP, *Enterococcus faecalis* (26 mm), *Pseudomonas aeruginosa* (24 mm), *Streptococcus pyogenes* and *Salmonella enterica* (23 mm). *Proteus mirabolis* 104588 CIP, *E. coli* 105182 CIP, *Staphylococcus camorum* LMG 13567 BHI and *Listeria innocua* LMG 135668 BHI show resistance to aqueous extract with weak areas of inhibition whereas no bacteria tested, is resistant to methanol extract. Aqueous and methanolic extracts of *Guibourtia ehie* have a higher antibacterial activity than Penicillin for Gram-negative bacteria (*Proteus mirabolis*, *Streptococcus pyogenes* and *Salmonella enterica*). *Syzygium rowlandii* exhibited antimicrobial activity greater than some antibiotics such as Penicillin and Tetracycline. The aqueous extract of *Syzygium rowlandii* is more active against *Staphylococcus camorum* LMG 13567 BHI (15.5 mm),

*Proteus mirabolis* CIP 104588 (15 mm) and *E. coli* CIP 105182 (13 mm). The lowest antibacterial activity was observed with *Enterococcus faecalis* (4.5 mm). The aqueous extract shows no inhibition of *Staphylococcus aureus, Listeria innocua* LMG 135668 BHI and *Enterococcus faecalis* CIP 103907. The chloroform extract of *Syzygium rowlandii* presented the largest inhibition of *Staphylococcus aureus*, *Shigella dysenteria* CIP 5451 (21 mm), *Proteus mirabolis* CIP 104588 (19 mm), *Staphylococcus camorum* LMG 13567 BHI, *Streptococcus pyogenes, Pseudomonas aerugenosa* (15 mm) and *Bacillus cereus* LMG 13569 BHI (14 mm). The lowest antibacterial activity of the extract was observed on *E. coli* CIP 105182, *Listeria innocua* LMG 135668 BHI, *Staphylococcus aureus* ATCC 255293 BHI and *Staphylococcus aureus ATCC* 9144 (10 mm). The methanol extract of *Syzygium rowlandii* has the highest antibacterial activities of *Shigella dysenteria* CIP 5451 (23 mm), *Staphylococcus camorum* LMG 13567 BHI (15 mm) and *Staphylococcus aureus* (14 mm). *Staphylococcus aureus* ATCC 9144 is resistant with a zone of inhibition of 5 mm.

**MIC and MBC recorded in microdilution assay**

The results of MIC and MBC of *Guibourtia ehie* and *Syzygium rowlandii* gave values of table 5. The aqueous extract of *Guibourtia ehie* has a bactericidal effect on *E. coli* 105182 CIP, *Enterococcus faecalis* 103907 CIP, *Bacillus cereus* LMG 13569 BHI, *Listeria innocua* LMG 135668 BHI, *Staphylococcus aureus* ATCC 25293 BHI, *Proteus mirabolis* 104588 CIP, *Staphylococcus camorum* LMG 13567 BHI, *Staphylococcus aureus* ATCC 9144, *Streptococcus pyogenes, Pseudomonas aerugenosa* and *Salmonella enterica*. Chloroform extract of *G. ehie* has a low activity and is neither bactericidal nor bacteriostatic on all strains tested. Methanol extract of *G. ehie* has a bactericidal effect on *E. coli* 105182 CIP, *Enterococcus faecalis* 103907 CIP, *Bacillus cereus* LMG 13569 BHI, *Staphylococcus aureus* ATCC 25293 BHI, *Proteus mirabolis* 104588 CIP, *Staphylococcus aureus* ATCC 9144 and *Staphylococcus aureus*. It has a bacteriostatic effect on *Listeria innocua* LMG 135668 BHI, *Staphylococcus camorum* LMG 13567 BHI, *Shigella dysenteria* CIP 5451 and *Salmonella enterica*. This set of results still militates in favor of the use of *Guibourtia ehie* in traditional medicine in the treatment of diseases bacterial infections (Raponda-Walker and Sillans, 1961; Hawthorne, 1995b; White and Abernethy, 1996).

Chloroform and methanol extracts have a bactericidal action on *Bacillus cereus* LMG 13569 BHI, *Enterococcus faecalis* CIP 103907, *Shigella dysenteria* CIP 5451, *Staphylococcus aureus* 252253, *Staphylococcus aureus* ATCC 25293, *Staphylococcus aureus* ATCC 9144, *Proteus mirabolis* CIP 104588, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aerugenosa* and *Enterococcus faecalis*. The aqueous extract was bactericidal against *E. coli* CIP 105182, *Proteus mirabolis* CIP 104588 and *Staphylococcus camorum* LMG 13567 BHI. The chloroform extract is bacteriostatic against *Listeria innocua* LMG 135668 BHI, *Salmonella enterica, Staphylococcus camorum* LMG 13567 BHI. The methanol extract has a bacteriostatic effect on *E. coli* CIP 105182, *Listeria innocua* LMG 135668 BHI, *Salmonella enterica*, *Enterococcus faecalis*, *Streptococcus pyogenes* and *Pseudomonas aerugenosa*. Chloroform and methanol extracts have a bacteriostatic effect for these different bacterial strains. The extracts are biologically active and have antimicrobial activity. This set of results argues for the use of *Syzygium rowlandii* in traditional treatment of diarrhea and other bacterial infections (Raponda-Walker and Sillans, 1961; Kerharo, 1974; Hoffmann, 1988; Michl, 1995; Obame *et al*., 2014; Sima *et al*., 2017). The result showed that different extracts of *Guibourtia ehie* and *Syzygium rowlandii* inhibited the growth of nearly all microorganisms used in the essay, indicating the presence of antimicrobial compounds in these plants. It can be inferred that the presence and the quantity of antimicrobial compounds in *Guibourtia ehie* and *Syzygium rowlandii* could justify the observed results. *Guibourtia ehie* and *Syzygium rowlandii* are used in folk medicine for the treatment of many diseases including bacterial diseases, gonorrhea, syphilis, parasitic diseases and diarrhea.

**Conclusion**

Medicinal plants are the local heritage with global importance and world is endowed with a rich wealth. *Guibourtia ehie* and *Syzygium rowlandii* are riches in phenolic compounds and antimicrobial activity against several microorganisms. The extracts reduce the concentration of free radical. The results of the present study support the traditional medicinal use of *Guibourtia ehie* and *Syzygium rowlandii* and suggest that a great attention should be paid to these plants which are found to have many pharmacological properties. The study confirms the multiple uses of *Guibourtia ehie* and *Syzygium rowlandii* for the treatment of many infectious diseases including bacterial diseases, gonorrhea, syphilis, parasitic diseases and diarrhea. The result place them as candidate for further investigations for traditional drug utilizable as complementary and alternative medicines development and new active compounds.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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