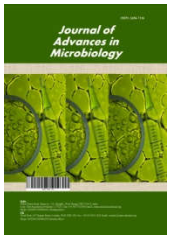

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Biological Properties and Phytochemical Analysis of Fractions and Total Extracts of *Cylicodiscus gabunensis* Harms and *Morinda lucida* Benth

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Authors' contributions

This work was carried out in collaboration among all authors. Author CSO did the conceptualization methodology, software writing. Author RLNMM did the visualization investigation. Author EON'N did the formal analysis of mass spectra in phytochemistry. Author JPO managed the writing - editing and revision. Author GRNA managed the software validation. Author LCOE did the preparation of the original project. Author ENE did the supervision and responsibility for planning. All authors read and approved the final manuscript.

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ABSTRACT

Two plants used in traditional medicine from Gabon were studied. The aim of this study was to study the biological properties and phytochemical analysis of *Cylicodiscus gabunensis* Harms and *Morinda lucida* Benth. These extracts were subjected to antimicrobial (diffusion, dilution), antioxidant (DPPH, ABTS) and inhibition of protein denaturation. The water-ethanol, water-acetone extracts and the partitioned extracts (F1 and F2) of *Cylicodiscus gabunensis* showed the greatest antibacterial activity on the majority of microbial strains and a good inhibition of denaturation with IC_{50} of 58.75 ± 2.10; 19.16 ± 2.00; 69.50 ± 2.00 and 17.49 ± 1.20 µg / mL respectively, compared to standards. Similarly, these extracts of *Cylicodiscus gabunensis* have anti-free radical capacities

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close to standards including vitamin C and butylated hydroxyanisole. The phytochemicals present in the extracts of *Cylicodiscus gabunensis* and *Morinda lucida* may be responsible for the biological activities studied.

Keywords: Antibacterial; denaturation inhibition; anti-free radical; DPPH; ABTS.

1. INTRODUCTION

In Gabon, as in other developing countries, infectious diseases are a public health problem because of their frequency and severity [1]. The situation is more worrisome because of the emergence of multi-resistant microorganism strains and the emergence of uncommon infections that compromise treatment with existing drugs [2,3].

Besides microbial infections, free radicals are involved in a large number of pathologies considered to be a public health problem. Rheumatism, cancer, inflammatory lesions and metabolic disorders are noted, among others. During oxidative stress, free radicals that are not trapped induce tissue damage [4].

Faced with these numerous obstacles, it is essential to look for new natural substances that are effective and broad-spectrum. One of the strategies for this research is to explore the plants used in traditional medicine.

Indeed, WHO [5] estimates that traditional medicine covers a wide variety of therapies and practices which vary fom country to country and region to region. Majority of therapies involve the exploitation of the active ingredients of medicinal plants. These plant species of great importance for the health of populations deserve to be studied scientifically for their best use.

Cylicodiscus gabunensis Harms (Leguminosae) is a large cylindrical tree, regular and very straight. Its trunk is brown with brown thorns in young trees [6]. The decoction of its bark is used in enemas against stomach pains, or in lotions against rheumatism. The root decoction of *Cylicodiscus gabunensis* is given to treat pulmonary and gastrointestinal diseases. Finally in mouthwash, it is used against gingivitis [7,8].

Morinda lucida Benth (Rubiaceae) is a tree of generally small dimensions, always green. Its gray bark is not thick. Its foliage is simple and bright green. Bark of *Morinda lucida* Benth is used to treat stomachaches, constipation, hernia, sterility of women and to promote childbirth [8,9].

This study aims to study the biological properties and phytochemical analysis of *Cylicodiscus gabunensis* Harms and *Morinda lucida* Benth

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant material of the study consists of the stem bark of *Cylicodiscus gabunensis* Harms and *Morinda lucida* Benth; these plants of the traditional medicine were collected in Oyem (North of Gabon), in June 2017 and identified with National herbarium (IPHAMETRA). The voucher specimens (SOC25: *Cylicodiscus gabunensis* and SOC50: *Morinda Lucida*) were deposited at the National Herbarium. The bark of these harvested plants was dried and milled under laboratory conditions and used for extractions.

2.2 Preparation of Total Extracts

The water-ethanol (30/70), water-acetone (30/70) and water (100%) extracts were prepared from the dry powder of *Cylicodiscus gabunensis* and *Morinda lucida*. Each sample (25 g) was mixed with 250 mL of suitable solvents. The water extracts were boiled for 60 min. All extracts were filtered, concentrated and lyophilized. The extracts obtained were stored at 4 °C. until they were used for the various tests.

2.3 Preparation of Partitioned Extracts (Fractions)

The different partitioned extracts (fractions) were obtained by flash chromatography. The dichloromethane / methanol solvent system (1: 0 - 0: 1) was chosen as the elution mode, varying in each case the polarity of the solvent. The fractions of the same nature were grouped by the thin layer chromatography method.

2.4 Bacterial Germs Tested

The bacterial carrier used in our study consisted of six reference bacterial strains (*Escherichia coli* 105182 CIP, *Listeria innocua* LMG 135668 BHI,

Staphylococcus aureus ATCC 25293 BHI, *Enterococcus faecalis* 103907 CIP, *Bacillus cereus* LMG 13569 BHI, *Staphylococcus camorum* LMG 13567 BHI, *Shigella dysenteria* 5451 CIP) and eleven clinical strains isolated from pathological products are *Pseudomonas aeruginosa*, *Salmonella enterica*, *Salmonella typhi*, *Neisseria gonorrhea*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Salmonella sp.* and *Neisseria meningitidis*.

2.5 *In vitro* Antibacterial Activity

2.5.1 Sensitivity test

The inhibition of bacterial growth has been studied by the diffusion method [10,11]. Bacterial colonies were used to prepare the inoculum in order to have a density equivalent to that of 0.5 McFarland. The flood inoculated agar was left for 10 min. Wattman 1 paper discs, sterilized and impregnated with a concentration of extract (100 µg / mL), were placed on the agar plate. Petri dishes were incubated for 18-24 hours at 37°C. Antibacterial activity was determined by measuring the diameter of the zone of inhibition.

2.5.2 Minimum Inhibitory Concentration (MIC)

MICs were determined by the microdilution method [10]. A series of seven dilutions of each extract (double dilutions ranging from 0.0049 to 5 mg/ml) were made in Muller Hinton Nutrient Broth (Liofilchem, Italy). Each well of the series is inoculated with an inoculum having a turbidity equivalent to that of 0.5 McFarland. After 18 hours of incubation at 37°C., the MIC of the extract tested is determined.

2.5.3 Minimum Bactericidal Concentration (MBC)

Nutrient agar poured into Petri dishes is inoculated into 100 µL of the contents of the wells with a concentration greater than or equal to MIC in the previous dilution series. MBC is determined after a 24 hour incubation at 37°C.

2.5.4 Time-kill test

In this study, the measure of mortality was determined by the actual reduction in viable counts at 6 hours for each isolate [12,13]. The plant extracts exhibiting the lowest minimum bactericidal concentrations were chosen to carry out a bacterial destruction test as a function of time. Thus, the bacterial suspensions were diluted in a nutrient broth (0.5 Mac Farland standard), the concentrations of total extracts or of fractions corresponding to the best MBC, were respectively added to the corresponding culture. The cultures were incubated at 37°C. At 0, 1, 2, 3, 4, 5 and 6 hours, a 100 µL aliquot was taken and diluted with 5 ml of sterile broth. The resulting suspension was used to inoculate Petri dishes. After 24 hours of incubation at 37 ° C, the viability of the bacteria was assessed by the presence of colonies on the plates.

2.6 Protein Denaturation Inhibition Method

Denaturation inhibition of the crude extracts and fractions from *Cylicodiscus gabunensis* and *Morinda lucida* was carried out according to the method of inhibition of the denaturation of proteins [14]. Briefly, a mixture of fresh chicken egg albumin (0.1 mL), pH 6.4 saline phosphate buffered saline (0.9 mL) and varying concentrations of the crude extracts and fractions (1.9 mL) was run so that endings become 31.25, 62.5, 125, 250 and 500 µg / mL. Distilled water served as a negative control. Then, the mixtures were incubated at 37°C in an incubator (Ecocell, LSIS-B2V / EC55, Germany) for 20 min and then heated at 70 °C for 5 min. After cooling, the absorbances were measured at 660 nm by spectrophotometer (60S evolution, United States). Diclofenac sodium and Paracetamol were used as reference drugs and treated under the same conditions as crude extracts.

> % Inhibition = [(Abs sample - Abs control) / Abs control] x 100, Abs = Absorbance.

The concentration of the extract for 50% inhibition (IC_{50}) was determined by the dose response curve.

2.7 Assessment of Anti-free Radical Activity

2.7.1 Measurement of the trapping power of the DPPH radical

The measurement of the anti-radical activity was tested according to the method of Blois [15] as described by Sima et al. [16] with some modifications. The principle of this method is based on the measurement of the free radical scavenging of DPPH (diphenyl picryl hydrazyl)

dissolved in methanol. The addition of an anti-free radical in a solution of DPPH leads to a discoloration of the latter, which is directly proportional to the anti-free radical capacity of the added product. This discoloration can be followed spectrophotometrically by measuring the decrease in absorbance at 517 nm. It provides a convenient way to measure the anti-free radical activity of crude extracts and fractions of *Cylicodiscus gabunensis* and *Morinda lucida*. DPPH solutions are incubated for 30 min in the absence (control) or in the presence of increasing concentrations of plant crude extracts and fractions (0.91, 1.81, 3.62, 7.25, 14.5, 25, 50 and 100 µg / mL).

At the end of the incubation period, the absorbance at 517 nm is read and the anti-free radical activity is calculated according to the following formula:

$$\% \text{ RSA} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100.$$

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**.**

2.7.2 ABTS scavenging activity

The ABTS test is based on the ability of an antioxidant to stabilize the $ABTS^{\bullet+}$ radical by transforming it into $ABTS^{+}$ [17]. A mixture of ABTS solution (7 mM) and potassium persulfate (2.4 mM) was incubated for 12 h in the dark at room temperature until formation of the ABTS radical complex ($ABTS^{+}$). To 60 µL of extract, 2.94 mL of $ABTS^{\bullet+}$ solution were added. The mixture was incubated at 37 °C for 20 min in the dark. Vit C and BHA were used as references. After incubation, the absorbance was measured in a spectrophotometer at 734 nm. The percent inhibition (PI) was calculated by the following method:

$$\text{Percentage inhibition} = [(A_0 - A) / A_0] \times 100$$

Where, A_0 is the absorbance of ethanol, A is the absorbance of sample extract or standard.

2.8 Preliminary Phytochemical Screening

Phytochemical screening was carried out on each total extract and each fraction in order to highlight the different important chemical groups (Total phenolic, tannin gallic, tannin catechol, total flavonoids, alkaloids, proanthocyanidins) that could explain the biological activities observed. These tests are based on the appearance of characteristic stains after addition of specific reagents [16].

2.9 Quantitative Study of Phenolic Compounds

2.9.1 Determination of total phenols

The total phenol content of the crude extracts or fractions was determined by the method of Folin-Ciocalteu [18]. The absorption is read spectrophotometer at the wavelength of 735 nm.

2.9.2 Determination of total flavonoids

The total flavonoid content of the crude extracts and fractions was determined by the aluminum trichloride method [19]. Absorbances were measured at 435 nm spectrophotometer.

2.9.3 Determination of the total tannins

Tannin content was determined by using Standard method for determining the tannins in sorghum [20]. Absorbances were measured after at 525 nm with a spectrophotometer.

2.9.4 Determination of the total proantho-cyanidins

The proanthocyanidin assay is demonstrated by HCl-butanol method [12]. Optical densities were read at a wavelength of 550 nm at the spectrophotometer.

2.10 Statistical Analysis

The results of the various tests carried out *in vitro* are expressed as mean ± SD. The data were analyzed by the univariate ANOVA test followed by Dunnet / Tukey test for multiple comparisons and determination of significance rates. Values of $p \leq 0.05$ are considered statistically significant.

3. RESULTS

3.1 Antibacterial Activity

In this study, seventeen bacterial strains were used. The results of the inhibition diameters are presented in Table 1. The fractions and crude

extracts of *Cylicodiscus gabunensis* and *Morinda lucida* have antimcrobial activity on the majority of bacteria tested. The diameters of inhibition vary from 7±1.0 to 22±1.0 mm. Thus, the bacterial strain *Neisseria gonorrhea* was significantly more sensitive to total extracts and fractions of *Cylicodiscus gabunensis* compared to other bacteria tested. All extracts and fractions of *Cylicodiscus gabunensis* inhibit the growth of the bacterial strains used, with the exception of the F3 fraction, which only inhibits the bacterial growth of *Bacillus cereus* LMG 13569 BHI, *Shigella dysenteriae* 5451 CIP, *Neisseria gonorrhea* and *Klebsiella pneumonia*. The F2 fraction of *Morinda lucida* showed inhibitory activity on all bacterial strains. The other extracts and fractions of *Morinda lucida* showed weak inhibitions on most bacterial strains.

The diameters of inhibitions of two plants studied remain lower than those of certain reference antibiotics, in particular gentamicin, tetracycline, doxycycline and amoxicillin (Table 2).

The results in Table 2 summarize the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of *Cylicodiscus gabunensis* and *Morinda lucida*. MIC and CMB values range from 0.6 to 5 mg / mL from bacteria to bacteria. The F1 and F2 fractions of *Cylicodiscus gabunensis* have the best minimum bactericidal concentration (1.2 mg / mL) on gonococcus. The water-ethanol and water-acetone extracts of *Cylicodiscus gabunensis* also reveal the best minimum bactericidal concentration (1.2 mg / mL) on certain bacterial strains (*Enterococcus faecalis* 103907 CIP, *Shigella dysenteriae* 5451 CIP, *Salmonella enterica*). However, the crude extracts and fractions of *Morinda lucida* demonstrate the relatively high bactericidal concentration on certain bacteria.

Regarding the variability of the bacterial strains exposed to plant extracts with the lowest minimum bactericidal concentrations (Table 3), the results show that, after 5 hours, the bacteria exposed to fractions F1, F2 and the water acetone extracts (*Cylicodiscus gabunensis*) are destroyed while the water-ethanol extract of *Cylicodiscus* kills bacteria such as Enterococcus from CIP 103907 and Shigella dysenteriae 5451 CIP after 4 hours of exposure.

3.2 Protein Denaturation Inhibition Method

The anti-denaturation potential of crude extracts and fractions of two medicinal plants (*Cylicodiscus gabunensis* and *Morinda lucida*) is evaluated by the method of inhibition of denaturation of proteins (Table 4). Fractions F_1, F_2; and the water-ethanol and water-acetone extracts of *Cylicodiscus gabunensis* show good denaturation inhibition ($P< 0,05$) with respectively IC_{50} of 58.75 ± 2.10, 19.16 ± 2.00, 69.50 ± 2.00 and 17.49 ± 1.20 µg / mL compared to standards (Diclofenac sodium and Paracetamol). Except for water extracts, all crude extracts and fractions of *Morinda lucida* have very low denaturation inhibition compared to standards.

3.3 Anti-free Radical Activity

The antioxidant activity of extracts (total extracts and fractions) of *Cylicodiscus gabunensis* and *Morinda lucida* is presented in Table 5. The results of DPPH method show that water-ethanol extract (IC_{50} = 7.39 ± 0.58 µg / mL), water-acetone extract (IC_{50} = 7.77 ± 0.98 µg / mL) and fractions F_1 (IC_{50} = 6.25 ± 0.50 µg / mL) and F_2 (IC_{50} = 7.22 ± 0.00 µg / mL) of *Cylicodiscus gabunensis* have anti-free radical capacity close to standards, in particular Vitamin C (IC_{50} = 7.12 ± 0.60 µg / mL) and Butylated Hydroxyanisole (IC_{50} = 6.59 ± 0.30 µg / mL). The scanning of the crude extracts and fractions by ABTS method confirms the good anti-free radical activity of the water-ethanol extract, water-acetone extract and F_1 and F_2 fractions. Also, the fraction F_4 and the raw water extract of *Cylicodiscus gabunensis* reveal a good anti-free radical potential; the other extracts or fractions of *Morinda lucida* have a more or less low activity in the whole.

3.4 Phytochemical Analysis

Phytochemical studies show that *Cylicodiscus gabunensis* and *Morinda lucida* are rich in phenolic compounds (Table 6). The assay of the phenolic compounds reveals that the fractions F1 and F2; the water-ethanol and water-acetone extracts of stem bark of *Cylicodiscus gabunensis* significantly contain the highest levels of phenolic compounds (Table 7).

Table 1. Inhibition zone diameters (mm) produced by total extracts and fractions from *Cylicodiscus gabunensis* and *Morinda lucida*

Bacteria	Inhibition zone diameters (mm)											
	Total extracts and fractions											
	Cg WE	Cg WEE	Cg WAE	Cg F_1	Cg F_2	Cg F_3	Cg F_4	MI WE	MI WEE	MI WAE	MI F_1	MI F_2
E. coli 105182 CIP	^{a}7 ± 0.0	^{ab}10 ± 1.0	^{b}13 ± 0.6	^{b}15 ± 1.0	^{bc}16 ± 0.0	0 ± 0.0	^{a}8 ± 0.0	0 ± 0.0	^{a}7 ± 1.0	0 ± 0.0	0 ± 0.0	^{a}8 ± 1.0
L. innocua LMG 135668 BHI	^{a}7 ± 0.5	^{ab}11 ± 1.0	^{ab}12 ± 0.0	^{b}15 ± 2.0	^{ab}11 ± 0.0	0 ± 0.0	^{ab}10 ± 1.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	^{a}7 ± 0.0
S. aureus ATCC 25293 BHI	^{a}7 ± 0.0	^{a}8 ± 1.0	^{a}9 ± 0.0	^{ab}12 ± 1.0	^{ab}11 ± 0.0	0 ± 0.0	^{b}13 ± 0.0	^{a}7 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	^{a}9 ± 0.0
E. faecalis 103907 CIP	^{a}7 ± 0.0	^{ab}10 ± 0.5	^{ab}11 ± 2.0	^{ab}11 ± 0.0	^{ab}10 ± 1.0	0 ± 0.0	^{ab}10 ± 1.0	0 ± 0.0	^{a}8 ± 1.0	^{a}8 ± 0.0	0 ± 0.0	^{a}8 ± 1.0
B. cereus LMG 13569 BHI	^{a}9 ± 0.0	^{a}9 ± 1.0	^{ab}10 ± 0.5	^{b}13 ± 1.0	^{b}13 ± 0.0	^{ab}10 ± 1.0	^{b}13 ± 0.5	0 ± 0.0	^{ab}11 ± 1.0	^{ab}10 ± 1.0	0 ± 0.0	^{ab}11 ± 1.0
S. dysenteria 5451 CIP	^{a}7 ± 0.0	^{ab}11 ± 0.0	^{ab}11 ± 0.0	^{b}13 ± 0.0	^{ab}12 ± 1.0	^{ab}10 ± 0.0	^{ab}12 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	^{a}7 ± 0.0
P. aerugenosa	^{a}7 ± 1.0	^{ab}11 ± 0.0	^{ab}11 ± 1.0	^{a}9 ± 1.0	^{ab}10 ± 0.0	0 ± 0.0	^{ab}12 ± 1.0	0 ± 0.0	^{ab}10 ± 1.0	^{a}8 ± 1.0	0 ± 0.0	^{ab}10 ± 1.0
S. enterica	^{a}7 ± 1.0	^{b}12 ± 0.5	^{b}12 ± 0.0	^{a}9 ± 0.0	^{ab}11 ± 1.0	0 ± 0.0	^{ab}12 ± 0.0	0 ± 0.0	^{a}7 ± 1.0	0 ± 0.0	0 ± 0.0	^{a}9 ± 1.0
S. thyphimurium	^{a}8 ± 1.0	^{ab}10 ± 0.0	^{b}13 ± 0.0	^{ab}12 ± 1.0	^{ab}10 ± 1.0	0 ± 0.0	^{ab}10 ± 0.0	0 ± 0.0	^{a}7 ± 1.0	^{a}8 ± 0.0	0 ± 0.0	^{a}9 ± 1.0
N.gonorrhea	^{b}14 ± 1.0	^{bc}18 ± 1.0	^{bc}18 ± 1.0	^{c}22 ± 1.0	^{c}19 ± 1.0	^{ab}10 ± 1.0	^{b}15 ± 0.0	0 ± 0.0	^{a}7 ± 1.0	^{a}7 ± 0.0	0 ± 0.0	^{a}8 ± 1.0
E. coli	^{a}9 ± 1.0	^{a}9 ± 1.0	^{a}9 ± 1.0	^{ab}12 ± 1.0	^{ab}10 ± 1.0	0 ± 0.0	^{ab}10 ± 0.0	0 ± 0.0	0 ± 0.0	^{a}7 ± 1.0	0 ± 0.0	^{a}9 ± 0.0
S. aureus	^{a}8 ± 0.0	^{a}9 ± 1.0	^{a}9 ± 1.0	^{ab}11 ± 1.0	^{ab}12 ± 0.0	0 ± 0.0	^{a}9 ± 0.0	^{a}7 ± 0.0	0 ± 0.0	^{a}8 ± 1.0	^{a}7 ± 0.0	^{a}9 ± 0.0
K. pneumonia	^{a}8 ± 1.0	^{a}7 ± 0.0	^{a}8 ± 1.0	^{ab}12 ± 2.0	^{ab}10 ± 0.0	^{a}7 ± 0.0	^{a}8 ± 1.0	0 ± 0.0	^{a}7 ± 1.0	^{a}7 ± 0.0	0 ± 0.0	^{a}9 ± 1.0
A. baumannii	^{a}8 ± 1.0	^{ab}11 ± 1.0	^{b}13 ± 1.0	^{b}12 ± 0.0	^{ab}10 ± 0.0	0 ± 0.0	^{ab}10 ± 0.0	^{a}8 ± 1.0	^{a}7 ± 1.0	^{a}7 ± 0.0	^{a}7 ± 0.0	^{a}8 ± 1.0
E. aerogenes	^{a}7 ± 0.0	^{ab}10 ± 1.0	^{b}14 ± 2.0	^{ab}11 ± 0.0	^{ab}10 ± 1.0	0 ± 0.0	^{ab}10 ± 0.0	^{a}7 ± 1.0	^{a}7 ± 0.0	^{a}8 ± 1.0	^{a}7 ± 0.0	^{a}9 ± 0.0
Salmonella Sp	^{a}8 ± 1.0	^{ab}11 ± 1.0	^{ab}11 ± 1.0	^{b}13 ± 1.0	^{ab}12 ± 1.0	0 ± 0.0	^{ab}11 ± 0.5	^{a}7 ± 0.0	^{a}7 ± 1.0	^{a}7 ± 1.0	^{a}7 ± 1.0	^{a}9 ± 1.0
N. meningitidis	^{a}7 ± 0.0	^{ab}10 ± 1.0	^{ab}12 ± 0.0	^{ab}12 ± 0.0	^{ab}12 ± 0.0	0 ± 0.0	10 ± 0.0	^{a}7 ± 1.0	^{a}8 ± 1.0	^{a}7 ± 1.0	^{a}7 ± 0.0	^{a}9 ± 1.0

WAE = Water-acetone extract; WEE=Water-ethanol extract; F = Fraction; WE= Water extract; MI = Morinda lucida; Cg = Cylicodiscus gabunensis. In each column, the assigned values of different alphabetic letters (a, b, c) indicate significantly different yields (P <0.05).

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plants extracts from *Cylicodiscus gabunensis* and *Morinda lucida*

Bacteria	MIC and MBC (mg/mL)																							
	Cg WE		Cg WEE		Cg WAE		Cg F_1		Cg F_2		Cg F_3		Cg F_4		Ml WE		Ml WEE		Ml WAE		Ml F_1		Ml F_2	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli 105182 CIP	-	-	1.2	2.5	1.2	2.5	2.5	2.5	1.2	2.5	-	-	5.0	>5	-	-	>5	>5	-	-	-	-	5.0	>5
L. innocua LMG 135668 BHI	2.5	5.0	2.5	2.5	2.5	5.0	1.2	2.5	2.5	5.0	-	-	2.5	5.0	-	-	-	-	-	-	-	-	-	-
S. aureus ATCC 25293 BHI	5.0	>5	2.5	5.0	2.5	5.0	2.5	5.0	2.5	5.0	-	-	2.5	5.0	>5	>5	-	-	-	-	-	-	5.0	>5
E. faecalis 103907 CIP	2.5	2.5	1.2	1.2	1.2	2.5	2.5	5.0	5.0	5.0	-	-	2.5	5.0	-	-	5	>5	5	>5	-	-	5.0	5.0
B. cereus LMG 13569 BHI	2.5	2.5	1.2	2.5	1.2	2.5	1.2	2.5	1.2	2.5	5.0	>5	2.5	2.5	-	-	2.5	5.0	2.5	5.0	-	-	2.5	5.0
S. dysenteria 5451 CIP	2.5	5.0	0.6	1.2	1.2	1.2	1.2	2.5	2.5	5.0	5.0	>5	2.5	2.5	-	-	-	-	-	-	-	-	-	-
P. aerugenosa	2.5	2.5	0.6	2.5	0.6	2.5	5.0	>5	5.0	>5	-	-	2.5	2.5	-	-	2.5	5.0	5.0	>5	-	-	2.5	5.0
S. enterica	2.5	5.0	0.6	1.2	1.2	2.5	5.0	>5	5.0	>5	-	-	2.5	5.0	-	-	>5	>5	-	-	-	-	2.5	5.0
S. thyphimurium	2.5	5.0	1.2	2.5	1.2	2.5	2.5	5.0	5.0	5.0	-	-	5.0	5.0	-	-	5.0	>5	5.0	>5	-	-	5.0	>5
N. gonorrhea	2.5	5.0	2.5	2.5	2.5	2.5	0.6	1.2	1.2	1.2	5	>5	2.5	2.5	5.0	>5	5.0	>5	5.0	>5	-	-	5.0	>5
E. coli	2.5	5.0	5.0	5.0	2.5	5.0	2.5	5.0	5.0	5.0	-	-	2.5	5.0	>5	>5	5.0	>5	5.0	>5	-	-	5.0	>5
S. aureus	5.0	5.0	2.5	5.0	2.5	5.0	2.5	5.0	2.5	5.0	-	-	5.0	>5	5.0	>5	5.0	>5	5.0	>5	>5	>5	5.0	>5
K. pneumonia	5.0	>5	2.5	5.0	2.5	5.0	2.5	5.0	5.0	5.0	-	-	5.0	>5	5.0	>5	>5	>5	5.0	>5	-	-	5.0	>5
A. baumannii	5.0	>5	1.2	2.5	2.5	5.0	5.0	5.0	5.0	5.0	-	-	5.0	5.0	5.0	>5	5.0	>5	5.0	>5	-	-	>5	>5
E. aerogenes	5.0	>5	5.0	5.0	2.5	2.5	2.5	5.0	2.5	2.5	-	-	2.5	5.0	>5	>5	5.0	>5	5.0	>5	>5	>5	>5	>5
Salmonella Sp	2.5	5.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	5.0	-	-	2.5	5.0	5.0	>5	5	>5	5.0	>5	5.0	>5	>5	>5
N. meningitidis	5.0	>5	1.2	2.5	1.2	2.5	5.0	5.0	2.5	5.0	-	-	5.0	>5	5.0	>5	5	>5	5.0	>5	5.0	>5	>5	>5

WAE=Water-acetone extract; WEE=Water-ethanol extract; WE=Water extract; F=Fraction; Ml=Morinda lucida; Cg=Cylicodiscus gabunensis.

Table 3. Viability of bacteria after 6 hours exposure of plant extracts with the lowest bactericidal minimum concentrations

Plants extracts (MBC = 1.2 mg/mL)	Bacteria	Time-kill (h)						
		0 h	1 h	2 h	3 h	4 h	5 h	6 h
Cg Water-ethanol	*E. faecalis* 103907 CIP	+ (UC)	+ (UC)	15 ± 02	06 ± 01	-	-	-
	S. dysenteria 5451 CIP	+ (UC)	+ (UC)	25 ± 01	08 ± 02	-	-	-
	S. enterica	+ (UC)	+ (UC)	36 ± 02	13 ± 01	10 ± 02	-	-
Cg Water-acetone	*S. dysenteria* 5451 CIP	+ (UC)	+ (UC)	40 ± 01	32 ± 06	15 ± 02	-	-
Cg Fraction 1	*N. gonorrhea*	+ (UC)	+ (UC)	30 ± 03	20 ± 00	06 ± 00	-	-
Cg Fraction 2	*N. gonorrhea*	+ (UC)	+ (UC)	28 ± 02	20 ± 02	12 ± 01	-	-

The results are the means of number of the colonies ± standard deviations ; +: For the presence of the colonies ; -: for absence of colonies ; UC: uncountable. Cg = Cylicodiscus gabunensis.

Table 4. Effect of *Cylicodiscus gabunensis* and *Morinda lucida* against protein denaturation

Plants species	Extracts	IC_{50} (µg/mL)
Cylicodiscus gabunensis	Water-acetone extract	^{a}17.49 ± 1.20
	Water-ethanol extract	^{b}69.50 ± 2.00
	Water extract	^{b}72.10 ± 1.00
	Fraction 1	^{ab}58.75 ± 2.10
	Fraction 2	^{a}19.16 ± 2.00
	Fraction 3	^{c}175.87 ± 5.80
	Fraction 4	^{c}149.81 ± 6.55
Morinda lucida	Water-acetone extract	^{d}637.10 ± 9.50
	Water-ethanol extract	^{e}726.13 ± 10.20
	Water extract	^{c}116.01 ± 8.50
	Fraction 1	^{f}425.52 ± 10.50
	Fraction 2	^{g}836.23 ± 6.25
Standards	Diclofenac sodium	^{b}70.26 ± 5.20
	Paracetamol	^{h}294.97 ± 7.25

In each column, the assigned values of different alphabetic letters (a, b, c, d, e, f, g, h) indicate significantly different yields (P <0.05).

Table 5. Anti-free radical activity of *Cylicodiscus gabunensis* and *Morinda lucida*

Plants species	Extracts	DPPH	ABTS
		IC_{50} (µg/mL)	IC_{50} (µg/mL)
Cylicodiscus gabunensis	Water-acetone extract	^{a}7.39 ± 0.58	^{a}3.25 ± 0.10
	Water-ethanol extract	^{a}7.77 ± 0.98	^{a}3.63 ± 0.15
	Water extract	^{b}32.97 ± 1.20	^{b}20.35 ± 0.50
	Fraction 1	^{a}6.25 ± 0.50	^{a}3.59 ± 0.00
	Fraction 2	^{a}7.22 ± 0.00	^{a}4.25 ± 0.50
	Fraction 3	^{ab}45.52 ± 2.10	^{b}25.58 ± 0.50
	Fraction 4	^{ab}25.00 ± 2.00	^{ab}15.66 ± 1.00
Morinda lucida	Water-acetone extract	^{c}158.93 ± 2.00	135.65 ± 1.1
	Water-ethanol extract	^{d}362.7 ± 0.75	^{b}24.73 ± 0.56
	Water extract	^{b}58.41 ± 0.89	^{ab}15.44 ± 0.64
	Fraction 1	^{c}125.50 ± 2.00	^{c}95.56 ± 2.5
	Fraction 2	^{bc}99.56 ± 1.50	^{bc}56.25 ± 0.50
Standards	Vitamin C	^{a}7.12 ± 0.60	^{a}5.01 ± 0.55
	Butylated Hydroxyanisole	^{a}6.59 ± 0.30	^{a}4.26 ± 0.25

In each column, the assigned values of different alphabetic letters (a, b, c, d) indicate significantly different yields (P <0.05)

Table 6. Phytochemical screening of *Cylicodiscus gabunensis* and *Morinda lucida* stem bark

Extracts	Total phenolic	Tannin gallic	Tannin catechic	Total flavonoids	Alkaloids	Proanthocyanidins
Cg WAE	+++	+++	++	+++	-	++
Cg WEE	+++	+++	++	++	-	++
Cg WE	+++	+++	+++	++	-	++
Cg F_1	+++	+++	+++	+	-	+
Cg F_2	+++	++	+++	++	-	+
Cg F_3	++	++	+	+	-	+
Cg F_4	+++	++	++	++	-	++
Ml WAE	++	-	+	++	++	+
Ml WEE	++	-	-	++	-	+
Ml WE	+++	++	+++	+++	++	++
Ml F_1	++	-	+	++	-	+
Ml F_2	++	++	++	+	-	+

+++ = Very abundant; ++= Abundant; + = not abundant, — = Not Detected. Cg: Cylicodiscus gabunensis; Ml: Morinda lucida; WAE: Water-acetone extract; WEE: Water-ethanol extract; WE: Water extract ; F : Fraction

Table 7. Total phenolic content, total flavonoid content, total tannins content and total proanthocyanidins content from *Cylicodiscus gabunensis* and *Morinda lucida* extracts

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)
Cg WAE	^{ab}5 352 ± 7.92	^{ab}1 146.63 ± 3.96	^{b}1 301.63 ± 3	^{a}786.22 ± 2.59
Cg WEE	^{a}6 688.67 ± 6.67	^{b}808.71 ± 4.31	^{b}1 489.78 ± 1	^{b}186.22 ± 1.67
Cg WE	^{b}1 766.44 ± 8.08	^{bc}563.71 ± 0.49	^{c}338.67 ± 3.7	^{c}95.11 ± 7.41
Cg F_1	^{a}7 453 ± 9.82	^{a}2 126.50 ± 2.56	^{a}2 350 ± 7.5	^{a}896.25 ± 8.52
Cg F_2	^{a}6 850 ± 10.30	^{b}989.70 ± 5.30	^{ab}1 895 ± 8.5	^{b}326.12 ± 6.20
Cg F_3	^{bc}850.80 ± 6.35	250 ± 3.25	^{c}373.40 ± 5.25	^{b}250.40 ± 10.50
Cg F_4	^{b}1 852.50 ± 9.20	^{bc}625.51 ± 8.25	^{c}289.60 ± 9.56	^{bc}125.25 ± 8.95
Ml WAE	^{bc}760.89 ± 1.25	^{b}746.63 ± 2.71	^{c}473.48 ± 1.67	^{bc}158.44 ± 8.33
Ml WEE	^{bc}440.89 ± 9.17	^{c}240.79 ± 1.60	-	^{bc}143.67 ± 10.00
Ml WE	^{bc}783.11 ± 4.58	^{c}302.04 ± 0.90	^{d}39.41 ± 2.47	^{a}744 ± 1.11
Ml F_1	^{c}268.52 ± 10.52	^{c}103.25 ± 12.36	-	^{c}59.95 ± 9.5
Ml F_2	^{c}356.50 ± 9.25	^{c}205.10 ± 6.25	^{d}25.56 ± 3.50	^{c}26.06 ± 1.25

TPC: Total phenolic content; TFC: Total flavonoid content; TTC: Total tannins content; TPRC: Total proanthocyanidins content; Cg: Cylicodiscus gabunensis; Ml: Morinda lucida; WAE: Water-acetone extract; WEE: Water-ethanol extract; WE: Water extract ; F: Fraction; TAE: Tannic acid equivalent; APE: Apple procyanidins equivalent. In each column, the assigned values of different alphabetic letters (a, b, c, d) indicate significantly different yields (P <0.05).

4. DISCUSSION

This study shows that all the extracts total and fractions tested exhibited antibacterial activity on at least one bacterial strain. The activity of the total extracts based on organic solvents is markedly greater than that of the aqueous extracts for each plant. However, the inhibition diameters of the water-ethanol and water-acetone extracts of *Cylicodiscus gabunensis* are higher than all other total extracts of the two medicinal plants studied. Partitioned extracts (fractions) of *Cylicodiscus gabunensis* gave different responses to the bacterial strains tested compared to the total extracts. These responses are justified by the principle of serial extraction, which consists of using several solvents of different polarities in a precise order in order to extract all the compounds extractable in solvent after solvent [21]. The activities of the F1 and F2 fractions of *Cylicodiscus gabunensis* were greater than those of the total extracts. These details could be explained by the purification and concentration of bioactive compounds. For the partitioned extracts (fractions) which did not show a bactericidal action on the strains tested, several reasons can be mentioned. These fractions do not contain the active ingredient (s) necessary for a bactericidal action. The activity of total and partitioned extracts of *Cylicodiscus gabunensis* corroborates the results of Kouitcheu et al. [22]. Indeed, these authors have shown

that extracts of ethyl acetate from the stem bark of *Cylicodiscus gabunensis* were bactericidal against *Staphylococcus aureus* and *Bacillus cereus*. These results justify the use in traditional medicine of this plant in the treatment of various pathologies caused by these strains [8]. However, the total extracts and fractions of *Morinda lucida* showed poor antibacterial activities; the work of Apenteng et al. [23] also demonstrate the low activity of *Morinda lucida* with minimal inhibitory concentrations ranging from 50 to 80 mg / mL.

The effectiveness of an antibacterial agent is measured by its ability to inhibit and kill bacteria [24]. Killing 90% of bacteria at 6 a.m. is one of the most reliable methods [13]. In general, the effects of total extracts and fractions on bacteria depended on the concentration of the extract and the killing time of the bacteria. The result also confirms that extracts *of Cylicodiscus gabunensis* and *Morinda lucida* may be useful in the treatment of microbial diseases.

Many studies have shown the inflammatory effect of bacterial infections. In fact, certain proteins make it possible to detect different types of pathogens, thus triggering inflammation [25]. Several studies have shown the influence of certain microorganisms on the parameters of oxidative stress [26]. The plant extracts of *Cylicodiscus gabunensis* and *Morinda lucida* have an anti-free radical potential which would allow them to play a beneficial role in terms of preventive actions for human health. In addition to the study of the antibacterial, anti-denaturing and anti-radical activities, a qualitative and quantitative study of secondary metabolites was carried out to identify large families of molecules and to measure those which are abundant in the total extracts and the fraction of *Cylicodiscus. gabunensis* and *Morinda lucida*. The antimicrobial and anti-denaturing activities of most of these compounds, including flavonoids, tannins and proanthocyanidins, have already been demonstrated by several authors [12].

5. CONCLUSION

These works have made it possible to show that the crude extracts and the fractions of *Cylicodiscus gabunensis* and *Morinda lucida* have good antioxidant, antimicrobial and anti-inflammatory properties. They demonstrate that these plants can be used to treat infectious diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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