Asian Pacific Journal of Tropical Biomedicine

Original Article

doi: www.apjtb.org

Phytochemical analyses, antimicrobial and antioxidant activities of stem bark extracts of *Distemonanthus benthamianus* H. Baill. and fruit extracts of *Solanum torvum* Sw. from Gabon

Cédric Sima Obiang[1,2]✉, Rick Léonid Ngoua Meye Misso[1,2], Guy Roger Ndong Atome[1,2], Joseph Privat Ondo[1,2], Louis Clément Obame Engonga[1,2], Edouard Nsi Emvo[1]

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

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

ARTICLE INFO

Article history:
Received 18 February 2019
Revision 13 March 2019
Accepted 5 May 2019
Available online 24 May 2019

Keywords:
Antioxidants
Phytochemical
Antimicrobial
Distemonanthus benthamianus
Solanum torvum

ABSTRACT

Objective: To evaluate the phytochemical constituents, antimicrobial and antioxidant activities of the extracts of *Distemonanthus benthamianus* (*D. benthamianus*) stem bark and *Solanum torvum* (*S. torvum*) fruit which have been used as traditional medicinal herbs in Gabon.

Methods: Plant extracts were subjected to a qualitative study (phytochemical screening) and a quantitative (dosing) study of secondary metabolites. Antioxidant activity was tested by 1,1-diphenyl-2-picrylhydrazyl and 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid assay. Bacteria and fungi susceptibility tests were performed on Mueller Hinton medium and solid Sabouraud, respectively, using the diffusion method, while minimum inhibitory concentration, minimum fungicidal concentration and minimum bactericidal concentration were evaluated by microdilution method.

Results: The total phenol and tannin contents were significantly higher in the water-ethanol extract compared to the other extracts of *D. benthamianus* and *S. torvum*. The water-ethanol and water-acetone extracts showed significantly higher antioxidant activity than the aqueous extracts of the two medicinal plants. However, the extracts presented weak antioxidant activities compared to standards (Vitamin C, BHA). The water-acetone and water-ethanol extracts of *S. torvum* showed the highest antimicrobial activity against *Bacillus cereus* LMG 13569 BHI, *Shigella dysenteriae* 5451 CIP, *Shigella dysenteriae* and *Neisseria gonorrhoeae*.

Conclusions: Our results show that *D. benthamianus* and *S. torvum* can be promising sources of natural products with potential antimicrobial and antioxidant activities.

1. Introduction

Medicinal plants have always been used to relieve and cure human diseases[1]. Currently, the development of microbial resistance to antibiotics and the toxicity of synthetic antioxidants have led researchers to exploit the plant world in order to search for effective natural molecules that are free of any adverse effects[2].

Distemonanthus benthamianus (*D. benthamianus*) H. Baill (Leguminosae) is a tree distributed in tropical Africa, its bark powder associated with that of red wood (padouk) is used traditionally against skin conditions. It is also administered in

✉First and corresponding author: Cédric Sima Obiang, Laboratory of Research in Biochemistry, University of Sciences and Technology of Masuku, P. O. Box 943 Franceville, Gabon.
Tel: (+241) 07883962
Fax: (+241) 671660
E-mail: cedricsima@gmail.com, cedricsima@univ-masuku.ga
Funding: This work is supported by USTM Biochemistry Research Laboratory (SG / CIS / SDM / SA / sa grant n° 77).

How to cite this article: Sima Obiang C, Ngoua Meye Misso RL, Ndong Atome GR, Ondo JP, Obame Engonga LC, Nsi Emvo E. Phytochemical analyses, antimicrobial and antioxidant activities of stem bark extracts of *Distemonanthus benthamianus* H. Baill. and fruit extracts of *Solanum torvum* Sw. from Gabon. Asian Pac J Trop Biomed 2019; 9(5): 209-216.

enemas for diarrheal diseases[3]. This species is rich in phenolic compounds such as oxyayanine, cyanine and alkaloids[4]. Certain compounds derived from *D. benthamianus* have anti-antiadrenergic, antioxidant, antitumor and contact dermatitis effects[5]. *Solanum torvum* Sw (*S. torvum*) (Solanaceae) is a slender shrub, its fruits and leaves can fight series of microbial diseases. The heated leaves of *S. torvum* are applied to cutaneous infections[3]. *S. torvum* is rich in phytochemicals such as steroidal saponins, steroidal alkaloids and phenols[6]. The antimicrobial, antiaggregant, analgesic, anti-inflammatory and cytotoxic activities of this plant have been described by Yousaf *et al*.[6].

Microbial infections are diseases caused by the development of bacteria or yeasts, some of which are pathogenic[7]. In addition to microbial infections, free radicals are implicated in the etiology of a large number of pathologies that are now considered to be one of the major public health problems[8].

However, plants have an anti-radical and antimicrobial potential that would allow them to play a beneficial role in terms of preventive action, which is very important for human and animal health[9]. The purpose of this work is to determine the medicinal properties of stem bark extracts of *D. benthamianus* H. Baill. and fruit extracts of *S. torvum* Sw in Gabon by evaluating the phytochemical constituents as well as the antimicrobial and antioxidant activities of the extracts of these plants.

2. Materials and methods

2.1. Plant material

The choice of stem bark of *D. benthamianus* H. Baill. and fruits of *S. torvum* Sw. was made according to traditional medicinal use. Plant samples were collected in Oyem in 2018. Identification of the species was carried out at the National Herbarium of the Institute of Pharmacopoeia and Traditional Medicine. The identification numbers of *D. benthamianus* H. Baill. and *S. torvum* Sw. were Bernard SRFG 320 and Bourobou 255, respectively.

2.2. Treatment of plant material

The plant samples were freeze-dried, powdered, kept at ambient temperature, and protected from light. Each sample (20 g) was 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 ℃.

2.3. Chemical products

Butylated hydroxyanisole (BHA), 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethanol, sulfuric acid, hydrochloric acid, sodium chloride, Folin-Ciocalteu, gallic acid and ascorbic acid (vit C) were from Sigma-Aldrich (St Louis, MO, USA).

2.4. Preliminary phytochemical study

Each extract was tested for the presence of flavonoids, coumarins, tannins, total phenolics, saponosides, triterpenoids, alkaloids and anthracenoids as described by Aiyegoro *et al*[10].

2.5. Quantitative phytochemical analysis

2.5.1. Total phenol content

To determine the total phenol content, the Folin-Ciocalteu method was used[11]. Absorbance was measured at 735 nm. All experiments were performed in triplicate and the phenolic compounds were expressed in gallic acid equivalents (GAE).

2.5.2. Total flavonoid content

The aluminum trichloride method was used to determine the flavonoid content and the absorbance was measured at 435 nm. The flavonoid content was expressed in quercetin equivalent (QE)[12].

2.5.3. Tannin content

The reference method by Sima-Obiang *et al* was used to determine the tannin content[13]. Absorbance was measured at 525 nm and tannic acid was used as a standard. The tannin contents were expressed in mg of tannic acid equivalent (TAE)/100 g of extract.

2.5.4. Proanthocyanidin content

The determination of proanthocyanidins was carried out by the HCl-Butanol method[14]. Absorbance was read at 550 nm and apple procyanidin was applied as standard. Proanthocyanidin levels were expressed in apple procyanidins equivalent (APE).

2.6. Antioxidant activity assay

2.6.1. DPPH assay

The measurement of the anti-radical activity was conducted according to the method of Blois[15] as described by Brand-Williams *et al*[16] with some modifications. The principle of this method is based on the measurement of the free radical scavenging of diphenyl picryl hydrazyl (DPPH) dissolved in methanol. The addition of an antioxidant in a solution of DPPH leads to a discoloration of the latter which is directly proportional to the antioxidant 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 antioxidant activity of *D. benthamianus* and *S. torvum* extracts. DPPH solutions were incubated for 30 min in the absence (control) or in the presence of increasing concentrations of plant extracts. Vit C and BHA were used as references.

At the end of the incubation period, the absorbance at 517 nm was read and the antioxidant activity was calculated according to the

following formula:

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

2.6.2. ABTS method

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 ℃ 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:

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.

2.7. Microorganism test

Microorganisms used in this study included *Escherichia coli* (*E. coli*) 0157 ATCC, *E. coli* 105182 CIP, *Listeria innocua* (*L. innocua*) LMG 135668 BHI, *Staphylococcus aureus* (*S. aureus*) ATCC 25293 BHI, *Enterococcus faecalis* (*E. faecalis*) 103907 CIP, *Bacillus cereus* (*B. cereus*) LMG 13569 BHI, *Shigella dysenteriae* (*S. dysenteriae*) 5451 CIP, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella enterica* (*S. enterica*), *Salmonella typhimurium* (*S. typhimurium*), *Shigella flexneri* (*S. flexneri*), *S. dysenteriae*, *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *E. coli*, *E. faecalis*, *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* (*C. albicans*) ATCC 10231, *C. albicans* ATCC 90028 and *C. albicans*.

Gentamicin, ampicillin and tetracycline were used as positive controls for the bacterial strains tested.

2.7.1. Antibacterial sensitivity test

The diffusion method was used to study the susceptibility of microorganisms to plant extracts[18]. Bacteria and fungi were respectively grown in Muller Hinton and Sabouraud broths. Each culture was then suspended in a solution of sodium chloride (NaCl, 0.9%) to a turbidity equivalent to that of the standard Mac Farland 0.5[19]. The extracts were diluted in dimethylsulfoxide at 100 mg/mL. Each extract (10 μL) was loaded onto each filter paper disc. The agar was suspended in distilled water, heated to complete dissolution and autoclaved at 121 ℃ and poured into Petri dishes. Disks were plated on cultures and antimicrobial activity was estimated after incubation at 37 ℃ for 24 h by measuring the inhibition diameter.

The relative percentage inhibition (RPI) of the plant extracts relative to the positive control (Gentamicin) was calculated using the following formula[18].

RPI = 100 × (X-Y) / (Z-Y)

Where X is the total zone of inhibition of the plant extract, Y is the total zone of inhibition of the solvent and Z is the total zone of inhibition of the standard drug (Gentamicin).

Table 1. Phytochemical screening of *D. benthamianus* and *S. torvum*.

Chemical groups	*D. benthamianus*			*S. torvum*		
	WE	WEE	WAE	WE	WEE	WAE
Saponosides	++	-	-	++	-	-
Tannin gallic	+	+	+	+++	++	++
Tannin catechin	+++	++	+	++	-	+
Total phenolics	+++	+++	+++	+++	++	++
Total flavonoids	++	++	+++	++	++	+
Reducing sugars	++	++	++	+++	++	-
Alkaloids	++	-	-	-	+	+
Proanthocyanidins	++	++	++	++	++	-
Anthracenosides	++	++	++	++	++	+
Coumarins	++	++	+++	+++	+++	+++
Triterpenoids	+++	+	+	+++	+++	++

+++ = very abundant; ++= abundant; + = not abundant, - = not detected. WAE=water-acetone extract; WEE=water-ethanol extract; WE= water extract.

Table 2. Phenolic compounds of *D. benthamianus* and *S. torvum* extracts.

Phenolic compounds	Yield of extraction (%)	Total phenolic content (mg GAE/100 g of extracts)	Total flavonoid content (mg QE/100 g of extracts)	Proanthocyanidin content (mg APE/100 g of extracts)	Total tannin content (mg TAE/100 g of extracts)
D. benthamianus					
Water	2.10	$1\,180.8 \pm 5.0^{b}$	110.2 ± 2.0^{a}	470.6 ± 10.4^{a}	556.6 ± 5.7^{a}
Water-ethanol	3.11	$2\,760.7 \pm 5.2^{c}$	110.9 ± 5.3^{a}	$1\,180.7 \pm 5.0^{b}$	$1\,350.8 \pm 9.0^{b}$
Water-acetone	3.71	$1\,710.2 \pm 6.8^{b}$	250.7 ± 5.2^{a}	$1\,020.6 \pm 9.5^{b}$	536.3 ± 10.2^{a}
S. torvum					
Water	5.14	660.2 ± 4.3^{a}	110.7 ± 2.2^{a}	400.9 ± 6.6^{a}	101.3 ± 3.5^{a}
Water-ethanol	5.37	$1\,710.4 \pm 9.2^{b}$	91.0 ± 2.2^{a}	417.3 ± 6.3^{a}	$1\,050.3 \pm 9.5^{b}$
Water-acetone	10.83	$1\,190.6 \pm 8.5^{b}$	100.7 ± 5.0^{a}	440.4 ± 7.2^{b}	650.2 ± 9.5^{a}

GAE=gallic acid equivalent; QE= quercetin equivalent; TAE= tannic acid equivalent; APE =apple procyanidins equivalent. For both plants, on the same line, data with different letters (a,b,c) indicate the signifcant difference ($P < 0.05$) for three replicates.

2.7.2. Minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and minimum fungicidal concentrations (MFCs)

MICs, MBCs and MFCs were determined by the microdilution technique[13,19]. Briefly, the nutrient broth was dispensed into the wells of a microplate. One hundred microliters of extracts were added to the first well of one row and double dilution was performed in other wells. Ninety microliters of nutrient broth and 10 μL of inoculum were added to the wells. A concentration range of the extract of 0.004 9 to 5 mg/mL was obtained. The plates were gently shaken and incubated at 37 ℃ for 24 h; the inhibition was evaluated by the absence of turbidity in the wells.

To determine MBCs and MFCs, 100 μL of each well showing no visible growth were collected and seeded in agar plates containing agar. The plates were incubated at 37 ℃ for 24-48 h and the number of colonies was counted[13].

The action of an antimicrobial on a microorganism can be characterized with several parameters such as MIC and MBC or MFC. According to the MBC/MIC or MFC/MIC report, antimicrobials with MBC/MIC ratios of 1 are considered to be microbicides; while those with the MBC/MIC ratio as 2 or greater are considered to be bacteriostatic or fungistatic[17,20].

2.8. Statistical analyses

The experimental results were expressed as mean ± standard deviation. All measurements were replicated three times. The data were analyzed by the univariate ANOVA test followed by the Dunnet/Tukey test for multiple comparisons and determination of significance rates. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Phytochemical screening

Phytochemical screening of extracts was performed to detect major chemical groups. Table 1 shows that total phenols, total flavonoids, proanthocyanidins, anthracenosides and coumarins were abundant in the crude extracts of *D. benthamianus* and *S. torvum*.

The total phenolic, total flavonoids, total tannins and total proanthocyanidins contents of *D. benthamianus* and *S. torvum* extracts are shown in Table 2. The total phenolic content ranged from (660.2 ± 4.3) to (2 760.7 ± 5.2) mg GAE/100 g of extracts. The water-ethanol extract of *D. benthamianus* had the highest phenolic content and the water extract of *S. torvum* was the lowest in phenolic compounds. The results of the total flavonoids did not show a significant difference between *D. benthamianus* and *S. torvum* extracts. The amount of tannin was highest in the water-ethanol extract of *D. benthamianus* [(1 350.8 ± 9.0) mg TAE/100 g extracts].

3.2. Antioxidant activities

The antioxidant activities of the extracts of *D. benthamianus* and *S. torvum* by the DPPH and ABTS methods are shown in Table 3. The extracts reduced the free radicals of DPPH and ABTS. In the case of the DPPH method, the water-ethanol and water-acetone extracts of *D. benthamianus* and *S. torvum* showed significantly higher antioxidant activity compared to the aqueous extracts of the two plants studied. ABTS method confirmed the antioxidant activity of the water-ethanol and water-acetone extracts of the two medicinal plants with the IC_{50} values which varied from (23.5 ± 1.2) μg/mL to (47.5 ± 1.5) μg/mL. By comparing plant extracts and references (Vit C and BHA), the antioxidant activities of the extracts were significantly lower than those of Vit C and BHA.

3.3. Sensitivity test of extracts

Screening of antimicrobial properties of six samples showed that all extracts of *D. benthamianus* and *S. torvum* had antimicrobial activities (Table 4). The antimicrobial activity of the two plants studied varied from one extract to another. In fact, *B. cereus* LMG 13569 BHI and *S. dysenteriae* were most sensitive among all microbial strains studied. Extracts of *S. torvum* had the higher inhibition diameters compared to extracts of *D. benthamianus*. Several microbial strains such as *B. cereus* LMG 13569 BHI, *S. dysenteriae* 5451 CIP, *S. dysenteriae*, *N. gonorrhoeae* and *E. faecalis* were more sensitive on the majority of crude extracts compared to standard (gentamicin, tetracycline, ampicillin).

3.4. Percentage of inhibition of crude extracts

Gentamicin was used to determine the relative percentage inhibition (RPI) of antimicrobial activity of *D. benthamianus* and *S. torvum* extracts in various solvents (Table 5). The water-ethanol extracts of *S. torvum* exhibited the maximum RPI (100.00%, 184.62%, 121.05% and 123.08%) against *S. aureus* ATCC 25293 BHI, *B. cereus* LMG 13569 BHI, *S. dysenteriae* and *E. faecalis,* respectively. The water-acetone and water extracts of *S. torvum* also showed a relative percentage of maximal inhibition against *B. cereus* LMG 13569 BHI, *S. dysenteriae* and *E. faecalis*, while all stem bark extracts of *D. benthamianus* showed the maximum RPI againt *B. cereus* LMG 13569 BHI compared with other test strains.

Table 3. Antioxidant activities of *D. benthamianus* and *S. torvum* extracts.

Extracts	IC_{50} (μg/mL)	
	DPPH	ABTS
D. benthamianus		
Water	97.6 ± 2.1[a]	84.5 ± 1.5[a]
Water-ethanol	32.0 ± 1.0[b]	23.5 ± 1.2[b]
Water-acetone	51.3 ± 0.6[b]	47.5 ± 1.5[b]
S. torvum		
Water	105.5 ± 6.3[a]	95.2 ± 7.0[a]
Water-ethanol	48.5 ± 2.6[b]	40.3 ± 3.6[b]
Water-acetone	42.6 ± 8.4[b]	43.3 ± 6.3[b]
Standards		
Vitamin C	9.8 ± 0.5[c]	7.5 ± 0.5[c]
BHA	7.5 ± 0.5[c]	6.3 ± 0.2[c]

For both plants, on the same line, data with different letters (a,b,c) indicate the signifcant difference ($P < 0.05$) for three replicates.

Table 4. Inhibition zone diameters produced by the crude extracts from *D. benthamianus* and *S. torvum* in disc diffusion (mm).

Strains	Gram	Extracts						Standards		
		Db water	Db water ethanol	Db water acetone	St water	St water ethanol	St water acetone	Gen	Am	Te
Bacteria reference strains										
E. coli 0157 ATCC	-	10.0 ± 2.0^{a}	13.0 ± 1.0^{b}	9.0 ± 1.0^{a}	11.0 ± 2.0^{b}	12.0 ± 2.0^{b}	13.0 ± 0.0^{b}	17.0 ± 1.0^{c}	Nd	Nd
E. coli 105182 CIP	-	11.0 ± 1.0^{b}	14.0 ± 0.0^{b}	10.0 ± 1.0^{a}	10.0 ± 0.0^{a}	12.0 ± 0.0^{b}	11.0 ± 1.0^{b}	15.0 ± 0.0^{b}	7.0 ± 1.0^{a}	7.0 ± 0.0^{a}
L. innocua LMG 135668 BHI	+	9.0 ± 1.0^{a}	11.0 ± 1.0^{b}	10.0 ± 0.0^{a}	8.0 ± 1.5^{a}	11.0 ± 0.0^{b}	11.0 ± 2.0^{b}	13.0 ± 0.0^{b}	7.0 ± 0.0^{a}	14.0 ± 0.0^{b}
S. aureus ATCC 25293 BHI	+	8.0 ± 2.0^{a}	10.0 ± 1.0^{a}	12.0 ± 0.0^{b}	9.0 ± 0.0^{a}	15.0 ± 1.0^{b}	13.0 ± 0.0^{b}	15.0 ± 0.3^{b}	Nd	17.0 ± 0.6^{c}
*E. faecalis*103907 CIP	+	12.0 ± 0.0^{b}	13.0 ± 2.0^{b}	11.0 ± 1.0^{b}	10.0 ± 0.0^{a}	13.0 ± 0.5^{b}	13.0 ± 2.0^{b}	30.0 ± 0.0^{c}	7.0 ± 1.0^{a}	19.0 ± 0.0^{c}
B. cereus LMG 13569 BHI	+	13.0 ± 0.0^{b}	14.0 ± 1.0^{b}	15.0 ± 0.0^{b}	19.0 ± 1.0^{c}	24.0 ± 1.0^{c}	24.0 ± 0.0^{c}	13.0 ± 0.5^{b}	Nd	18.0 ± 0.6^{c}
S. dysenteriae 5451 CIP	-	11.0 ± 1.0^{b}	15.0 ± 1.0^{b}	16.0 ± 1.0^{c}	14.0 ± 2.0^{b}	18.0 ± 1.0^{c}	17.0 ± 0.0^{c}	24.0 ± 0.5^{c}	Nd	16.0 ± 0.0^{c}
Bacteria clinical isolates										
P. aeruginosa	-	7.0 ± 1.0^{a}	9.0 ± 1.0^{a}	7.0 ± 0.0^{a}	7.0 ± 1.0^{a}	10.0 ± 0.0^{a}	9.0 ± 1.0^{a}	20.0 ± 0.0^{c}	7.0 ± 1.0^{a}	21.0 ± 1.0^{c}
S. enterica	-	7.0 ± 1.0^{a}	11.0 ± 0.5^{b}	12.0 ± 0.0^{b}	10.0 ± 1.0^{b}	13.0 ± 0.0^{b}	12.0 ± 2.0^{b}	28.0 ± 1.0^{c}	7.0 ± 1.0^{a}	16.0 ± 0.3^{c}
S. typhimurium	-	10.0 ± 1.0^{a}	15.0 ± 0.0^{b}	12.0 ± 0.0^{b}	11.0 ± 2.0^{b}	15.0 ± 0.0^{b}	13.0 ± 0.0^{b}	20.0 ± 0.5^{c}	7.0 ± 0.0^{a}	15.0 ± 0.5^{b}
S. flexneri	-	13.0 ± 1.0^{b}	12.0 ± 0.0^{b}	10.0 ± 2.0^{a}	12.0 ± 1.0^{b}	11.0 ± 2.0^{b}	11.0 ± 2.0^{b}	18.0 ± 0.0^{b}	Nd	13.0 ± 0.0^{b}
S. dysenteriae	-	Nd	8.0 ± 0.0^{a}	7.0 ± 1.0^{a}	20.0 ± 2.0^{c}	23.0 ± 1.0^{c}	24.0 ± 0.0^{c}	19.0 ± 0.0^{c}	7.0 ± 0.0^{a}	12.0 ± 1.0^{b}
N. gonorrhoeae	-	13.0 ± 1.0^{b}	16.0 ± 0.0^{c}	12.0 ± 2.0^{b}	14.0 ± 1.0^{b}	17.0 ± 1.0^{c}	18.0 ± 0.0^{c}	22.0 ± 1.2^{c}	7.0 ± 1.0^{a}	10.0 ± 1.0^{a}
E. coli	-	9.0 ± 0.0^{a}	10.0 ± 1.0^{a}	11.0 ± 1.0^{b}	9.0 ± 1.0^{a}	10.0 ± 2.0^{a}	13.0 ± 1.0^{b}	16.0 ± 1.0^{c}	7.0 ± 0.0^{a}	9.0 ± 1.0^{a}
E. faecalis	+	7.0 ± 0.0^{a}	10.0 ± 1.0^{a}	9.0 ± 1.0^{a}	16.0 ± 2.0^{c}	16.0 ± 1.0^{c}	15.0 ± 1.0^{b}	13.0 ± 1.0^{b}	Nd	10.0 ± 0.0^{a}
S. aureus	+	7.0 ± 1.0^{a}	8.0 ± 0.0^{a}	8.0 ± 1.0^{a}	11.0 ± 0.0^{b}	11.0 ± 1.0^{b}	12.0 ± 1.0^{b}	16.0 ± 1.0^{c}	7.0 ± 0.0^{a}	8.0 ± 1.0^{a}
K. pneumoniae	-	Nd	9.0 ± 0.0^{a}	7.0 ± 1.0^{a}	Nd	10.0 ± 0.0^{a}	9.0 ± 0.0^{a}	18.0 ± 1.0^{c}	7.0 ± 0.0^{a}	Nd
A. baumannii	-	8.0 ± 1.0^{a}	10.0 ± 1.0^{a}	12.0 ± 2.0^{b}	10.0 ± 1.0^{a}	13.0 ± 2.0^{b}	13.0 ± 1.0^{b}	16.0 ± 0.5^{c}	Nd	10.0 ± 2.0^{a}
E. aerogenes	-	7.0 ± 0.0^{a}	10.0 ± 0.0^{a}	13.0 ± 1.0^{b}	9.0 ± 0.0^{a}	10.0 ± 2.0^{a}	12.0 ± 2.0^{b}	16.0 ± 0.0^{c}	7.0 ± 1.0^{a}	10.0 ± 0.0^{a}
Salmonella spp	-	9.0 ± 0.0^{a}	12.0 ± 1.0^{b}	12.0 ± 0.0^{b}	10.0 ± 1.0^{a}	14.0 ± 2.0^{b}	13.0 ± 0.0^{b}	25.0 ± 0.0^{c}	7.0 ± 1.0^{a}	14.0 ± 1.5^{b}
N. meningitidis	-	7.0 ± 0.0^{a}	11.0 ± 0.0^{b}	11.0 ± 1.0^{b}	10.0 ± 0.0^{a}	12.0 ± 1.0^{b}	12.0 ± 2.0^{b}	16.0 ± 0.0^{c}	7.0 ± 1.0^{a}	Nd
Fungi										
C. albicans ATCC 10231		8.0 ± 0.0^{a}	8.0 ± 0.0^{a}	9.0 ± 1.0^{a}	7.0 ± 1.0^{a}	10.0 ± 1.0^{a}	9.0 ± 1.0^{a}	Nd	Nd	Nd
C. albicans ATCC 90028		12.0 ± 1.0^{b}	13.0 ± 2.0^{b}	10.0 ± 1.0^{a}	9.0 ± 0.0^{a}	14.0 ± 0.0^{b}	12.0 ± 1.0^{b}	Nd	7.0 ± 1.0^{a}	Nd
C. albicans		10.0 ± 0.0^{a}	14.0 ± 2.0^{b}	7.0 ± 0.0^{a}	13.0 ± 1.0^{b}	12.0 ± 2.0^{b}	13.0 ± 0.0^{b}	Nd	Nd	Nd

Nd = not determinated; Gen = Gentamicin; Te = Tetracycline; Am = Ampicillin; Db = *D. benthamianus*; St = *S. torvum*. For both plants, on the same line, data with different letters (a,b,c) indicate the signifcant difference ($P < 0.05$) for three replicates.

Table 5. Determination of relative percentage inhibition of water, water ethanol and water acetone crude extracts from *D. benthamianus* and *S. torvum* to standard antibiotic (Gentamicin).

Bacteria	Extracts					
	Db water	Db water ethanol	Db water acetone	St water	St water ethanol	St water acetone
E. coli 0157 ATCC	58.82	76.47	52.94	64.71	70.59	76.47
E. coli 105182 CIP	73.33	93.33	66.67	66.67	80.00	73.33
L. innocua LMG 135668 BHI	69.23	84.62	76.92	61.54	84.62	84.62
S. aureus ATCC 25293 BHI	53.33	66.67	80.00	60.00	100.00	86.67
E. faecalis 103907 CIP	40.00	43.33	36.67	33.33	43.33	43.33
B. cereus LMG 13569 BHI	100.00	107.69	115.38	146.15	184.62	184.62
S. dysenteriae 5451 CIP	45.83	62.50	66.67	58.33	75.00	70.83
P. aerugenosa	35.00	45.00	35.00	35.00	50.00	45.00
S. enterica	25.00	39.29	42.86	35.71	46.43	42.86
S. typhimurium	50.00	75.00	60.00	55.00	75.00	65.00
S. flexneri	72.22	66.67	55.56	66.67	61.11	61.11
S. dysenteriae	0.00	42.11	36.84	105.26	121.05	126.32
N. gonorrhoeae	59.09	72.73	54.55	63.64	77.27	81.82
E. coli	56.25	62.50	68.75	56.25	62.50	81.25
E. faecalis	53.85	76.92	69.23	123.08	123.08	115.38
S. aureus	43.75	50.00	50.00	68.75	68.75	75.00
K. pneumoniae	0.00	50.00	38.89	0.00	55.56	50.00
A. baumannii	50.00	62.50	75.00	62.50	81.25	81.25
E. aerogenes	43.75	62.50	81.25	56.25	62.50	75.00
Salmonella Spp	36.00	48.00	48.00	40.00	56.00	52.00
N. meningitidis	43.75	68.75	68.75	62.50	75.00	75.00

Db = *D. benthamianus*; St = *S. torvum*.

3.5. MIC and MBC or MFC of crude extracts of D. benthamianus and S. torvum

The results in Table 6 summarize MICs, MBCs, and MFCs of the crude extracts of *D. benthamianus* and *S. torvum*. MIC and MBC values ranged from 0.62 to 5.00 mg/mL from one microorganism to another. The water-ethanol extract of *S. torvum* had the best minimum microbicide concentrations (1.25 mg/mL) on *E. coli* 0157 ATCC, *B. cereus* LMG 13569 BHI, *S. dysenteriae* 5451 CIP and *C. albicans*. The aqueous and water-acetone extracts of *S. torvum* also revealed

Table 6. MIC and MBC or MFC of crude extracts of *D. benthamianus* and *S. torvum* obtained by microdilution method (mg/mL).

Strains	Gram	Db water		Db water ethanol		Db water acetone		St water		St water ethanol		St water acetone	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacteria reference strains													
E. coli 0157 ATCC	-	1.25	>5.00	1.25	>5.00	2.50	>5.00	1.25	2.50	1.25	1.25	1.25	2.50
E. coli 105182 CIP	-	2.50	5.00	2.50	2.50	2.50	>5.00	2.50	5.00	2.50	2.50	2.50	2.50
L. innocua LMG 135668 BHI	+	5.00	>5.00	2.50	5.00	2.50	5.00	>5.00	>5.00	2.50	5.00	5.00	5.00
S. aureus ATCC 25293 BHI	+	>5.00	>5.00	5.00	>5.00	2.50	5.00	5.00	>5.00	2.50	5.00	2.50	2.50
*E. faecalis*103907 CIP	+	2.50	5.00	2.50	2.50	2.50	5.00	2.50	5.00	1.25	2.50	1.25	2.50
B. cereus LMG 13569 BHI	+	2.50	5.00	2.50	2.50	2.50	2.50	1.25	2.50	0.62	1.25	1.25	1.25
S. dysenteriae 5451 CIP	-	5.00	5.00	1.25	2.50	1.25	2.50	2.50	5.00	1.25	1.25	1.25	2.50
Bacteria clinical isolates													
P. aeruginosa	-	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00	2.50	>5.00	1.25	>5.00	1.25	2.50
S. enterica	-	>5.00	>5.00	5.00	>5.00	2.50	5.00	5.00	5.00	1.25	2.50	1.25	2.50
S. typhimurium	-	5.00	5.00	2.50	2.50	2.50	5.00	5.00	5.00	2.50	5.00	2.50	2.50
S. flexneri	-	2.50	>5.00	2.50	>5.00	2.50	>5.00	0.62	2.50	1.25	2.50	2.50	>5.00
S. dysenteriae	-	Nd	Nd	Nd	Nd	Nd	Nd	1.25	2.50	1.25	2.50	1.25	2.50
N. gonorrhoeae	-	2.50	5.00	1.25	2.50	2.50	5.00	2.50	5.00	1.25	2.50	1.25	2.50
E. coli	-	>5.00	>5.00	5.00	>5.00	2.50	5.00	>5.00	>5.00	2.50	>5.00	2.50	5.00
E. faecalis	+	1.25	>2.50	1.25	>2.50	2.50	>2.50	0.62	2.50	0.62	2.50	0.62	2.50
S. aureus	+	2.50	>5.00	2.50	>5.00	2.50	>5.00	2.50	>5.00	1.25	2.50	2.50	>5.00
K. pneumoniae	-	Nd	Nd	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00	5.00	>5.00	>5.00	>5.00
A. baumannii	-	>5.00	>5.00	5.00	5.00	2.50	5.00	5.00	5.00	1.25	2.50	2.50	5.00
E. aerogenes	-	>5.00	>5.00	2.50	5.00	2.50	5.00	>5.00	>5.00	5.00	5.00	2.50	2.50
Salmonella spp	-	5.00	>5.00	2.50	2.50	2.50	5.00	5.00	5.00	2.50	5.00	2.50	2.50
N. meningitidis	-	>5.00	>5.00	2.50	5.00	5.00	5.00	>5.00	>5.00	2.50	5.00	1.25	2.50
Fungi		MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C. albicans ATCC 10231		>5.00	>5.00	5.00	>5.00	>5.00	>5.00	>5.00	>5.00	2.50	5.00	2.50	5.00
C. albicans ATCC 90028		2.50	5.00	2.50	2.50	2.50	5.00	5.00	>5.00	2.50	2.50	2.50	2.50
C. albicans		1.25	>5.00	1.25	>5.00	1.25	>5.00	1.25	1.25	0.62	1.25	1.25	1.25

Nd = Not determinated; Db = *D. benthamianus*; St = *S. torvum*.

Table 7. MBC/MIC of plant extracts from *D. benthamianus* and *S. torvum*.

Strains	Db water	Db water ethanol	Db water acetone	St water	St water ethanol	St water acetone
Bacteria reference strains						
E. coli 0157 ATCC	-	-	-	2#	1*	2#
E. coli 105182 CIP	2#	1*	-	2#	1*	1*
L. innocua LMG 135668 BHI	-	2#	2#	-	2#	1*
S. aureus ATCC 25293 BHI	-	-	2#	-	2#	1*
*E. faecalis*103907 CIP	2#	1*	2#	2#	2#	2#
B. cereus LMG 13569 BHI	2#	1*	1*	2#	2#	1*
S. dysenteriae 5451 CIP	1*	2#	2#	2#	1*	2#
Bacteria clinical isolates						
P. aeruginosa	-	-	-	-	-	2#
S. enterica	-	-	2#	1*	2#	2#
S. typhimurium	1*	1*	2#	1*	2#	1*
S. flexneri	-	-	-	2#	2#	-
S. dysenteriae	-	-	-	2#	2#	2#
N. gonorrhoeae	2#	2#	2#	2#	2#	2#
E. coli	-	-	2#	-	-	2#
E. faecalis	-	-	-	2#	2#	2#
S. aureus	-	-	-	-	2#	-
K. pneumoniae	-	-	-	-	-	-
A. baumannii	-	1*	2#	1*	2#	2#
E. aerogenes	-	2#	2#	-	1*	1*
Salmonella spp	-	1*	2#	1*	2#	1*
N. meningitidis	-	2#	1*	-	2#	2#
Fungi						
C. albicans ATCC 10231	-	-	-	-	2@	2@
C. albicans ATCC 90028	2@	1&	2@	-	1&	1&
C. albicans	-	-	-	1&	2@	1&

*bactericidal; #bacteriostatic; &fungicidal; @fungistatic.

the best minimum fungicidal concentrations (1.25 mg/mL) on *C. albicans*. However, the crude extracts of *D. benthamianus* and *S. torvum* demonstrated relatively high minimum microbicidal concentrations on certain bacteria and fungi.

3.6. Antimicrobial effects of crude extracts of D. benthamianus and S. torvum

The results in Table 7 show the antimicrobial effects of the crude extracts of *D. benthamianus* and *S. torvum*. Water-acetone extracts of *S. torvum* showed bactericidal actions on *E. coli* 105182 CIP, *L. innocua* LMG 135668 BHI, *S. aureus* ATCC 25293 BHI, *B. cereus* LMG 13569 BHI, *S. typhimurium*, *E. aerogenes* and *Salmonella* spp, while water ethanol extract of *S. torvum* showed bactericidal actions on *E. coli* 0157 ATCC, *E. coli* 105182 CIP, *S. dysenteriae* 5451 CIP and *E. aerogenes*. Moreover, water extracts of *S. torvum* presented bactericidal activity on *S. enterica*, *S. typhimurium*, *A. baumannii* and *Salmonella* spp. Bacteriostatic actions were also highlighted by extracts of *S. torvum* and bactericidal actions were highlighted by extracts of *S. torvum* on the majority of bacterial strains.

Extracts of *D. benthamianus* also indicated bactericidal actions on certain bacterial strains. In addition, the water-ethanol extracts of *D. benthamianus* and water-acetone extracts of *S. torvum* showed the fungicidal effects on *C. albicans* ATCC 90028, while water and acetone extracts of *S. torvum* showed the fungicidal actions on *C. albicans*.

4. Discussion

Traditional healers make use of medicinal plants to treat microbial diseases without any scientific basis[21]. This experimental study was used to evaluate the antioxidant and antimicrobial potential of plant extracts rich in phenolic compounds (water-acetone, water-ethanol and water extracts of *D. benthamianus* and *S. torvum*). Phytochemical screening in this study revealed the presence of a few secondary metabolites in the stem bark of *D. benthamianus* and the fruits of *S. torvum*. The work of Mounguengui *et al*.[5] also showed the presence of tannins and flavonoids in the extracts of *D. benthamianus*. The qualitative study of *D. benthamianus* and *S. torvum* highlights secondary metabolites in the six extracts studied. Phenolic compounds are active substances that may have biological or pharmacological activities[13,22].

Angiolella *et al*.[23] also reported that phenolic compounds have antibacterial, antioxidant and anticancer effects. Therefore, the use of *D. benthamianus* bark and *S. torvum* fruit in traditional medicine could be attributed to the high content of phenolic compounds[5,24]. This content contributes to the antioxidant power of the plant. These antioxidants can act according to two major mechanisms, either by transfer of hydrogen atom or by electron transfer[25]. In the present study, two methods were used to demonstrate the antioxidant activity of the crude extracts of *D. benthamianus* and *S. torvum*. Thus, the capacity of the water-ethanol and water-acetone extracts to reduce the free radicals DPPH and ABTS is greater than that of the aqueous extract. The results of our study on the antioxidant activity of *D. benthamianus* extracts are compatible with the work of Mounguengui *et al*[5]. However, Kumar *et al*[26] demonstrated that water extracts of *S. torvum* had a high antioxidant capacity compared to methanol extracts. Antioxidant activity can be directly related to the amount of phenolic compounds present in various extracts[14]. The antimicrobial activity of the crude extracts of *D. benthamianus* and *S. torvum* was evaluated by two methods (diffusion and microdilution). The results obtained in this study show that the water-ethanol and water-acetone extracts of both plants have a great inhibitory effect on the growth of all bacterial and fungal strains tested. These observed activities are also explained by the results of the chemical analysis of plants which reveal the presence of phenolic compounds whose antimicrobial properties have already been demonstrated[9]. The antimicrobial activity of the stem bark of *D. benthamianus* and the fruits of *S. torvum* varies from one extract to another and from one microorganism to another[27].

These results support Evina *et al*.[28] which showed the antimicrobial activity of *D. benthamianus* extracts against several Gram-positive and Gram-negative bacteria. This variability of inhibition may be due to the resistance capacity linked to the bacterial groups or to the nature of the compounds present in the plant extracts. The work of Lalitha *et al*[29] on antimicrobial activity of *S. torvum* also corroborates with the results of our study. These results support the traditional use of *D. benthamianus* and *S. torvum* in the treatment of microbial infections[30].

Ultimately, the study of crude extracts of *D. benthamianus* and *S. torvum* showed effective antioxidant and antimicrobial activities. These activities could be due to secondary metabolites.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are very grateful to Shell Gabon for the financial support of the equipment at the USTM Biochemistry Research Laboratory (SG / CIS / SDM / SA / sa grant n °77).

Funding

This work is supported by USTM Biochemistry Research Laboratory (SG / CIS / SDM / SA / sa grant n °77).

References

[1] Szopa A, Ekiert R, Ekiert H. Current knowledge of *Schisandra chinensis* (Turcz.) Baill.(Chinese magnolia vine) as a medicinal plant species: A review on the bioactive components, pharmacological properties, analytical and biotechnological studies. *Phytochem Rev* 2017; **16**(2): 195-218.

[2] Silva LN, Zimmer KR, Macedo AJ, Trentin DS. Plant natural products targeting bacterial virulence factors. *Chem Rev* 2016; **116**(16): 9162-9236.

[3] Raponda-Walker A, Sillans R. Les plantes utiles du Gabon. *Quarterly J Crude Drug Res* 1961; **1**(1): 27

[4] Aiyegoro O, Akinpelu D, Afolayan A, Okoh A. Antibacterial activities of crude stem bark extracts of *Distemonanthus benthamianus* Baill. *J Biol Sci* 2008; **8**(2): 356-361.

[5] Mounguengui S, Tchinda JBS, Ndikontar MK, Dumarçay S, Attéké C, Perrin D, et al. Total phenolic and lignin contents, phytochemical screening, antioxidant and fungal inhibition properties of the heartwood extractives of ten Congo Basin tree species. *An Forest Sci* 2016; **73**(2): 287-296.

[6] Yousaf Z, Wang Y, Baydoun E. Phytochemistry and pharmacological studies on *Solanum torvum* Swartz. *J Appl Pharm Sci* 2013; **3**(4): 152-160.

[7] Chang HH, Cohen T, Grad YH, Hanage WP, O'Brien TF, Lipsitch M. Origin and proliferation of multiple-drug resistance in bacterial pathogens. *Microbiol Mol Biol Rev* 2015; **79**(1): 101-116.

[8] Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, et al. Oxidative stress, prooxidants, and antioxidants: The interplay. *BioMed Res Int* 2014; **2014**: 761264.

[9] Koech K, Wachira FN, Ngure R, Wanyoko J, Bii C, Karori S, et al. Antimicrobial, synergistic and antioxidant activities of tea polyphenols. *Afr Crop Sci J* 2014; **22**(4): 837-846.

[10]Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complement Alter Med* 2010; **10**(1): 21.

[11]Hossain MA, AL-Raqmi KAS, AL-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. *Asian Pac J Trop Biomed* 2013; **3**(9): 705.

[12]Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J Food drug Anal* 2014; **22**(3): 296-302.

[13]Sima-Obiang C, Ngoua-Meye-Misso R, Ndong-Atome G, Privat Ondo J, Obame-Engonga L, Nsi-Emvo E. Phytochemical screening, phenolic compounds content, antioxidant, anti-inflammatory, and antimicrobial properties of *Pachylobus balsamifera* Guillaum from Gabon. *Phytother* 2018. Doi: 10.3166/phyto-2018-0059.

[14]Ngoua-Meye-Misso RL, Sima-Obiang C, Ndong JDLC, Ondo JP, Abessolo FO, Obame-Engonga LC. Phytochemical screening, antioxidant, anti-inflammatory and antiangiogenic activities of *Lophira procera* a. Chev.(Ochnaceae) medicinal plant from Gabon. *Egypt J Basic Applied Sci* 2018; **5**(1): 80-86.

[15]Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature* 1958; **181**(4617): 1199.

[16]Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT–Food Sci Tech* 1995; **28**(1): 25-30.

[17]Obiang CS, Ngoua-Meye-Misso RL, Ndong-Atome GR, Ondo JP, Obame-Engonga LC, Nsi-Emvo E. Chemical composition, antioxidant and antimicrobial activities of stem barks of *Englerina gabonensis* Engler and *Sterculia tragacantha* Lindl from Gabon. *Inter J Phytomed* 2017; **9**(3): 501-510.

[18]Obiang CS, Ondo JP, Atome GRN, Engonga LCO, Siawaya JFD, Emvo EN. Phytochemical screening, antioxidant and antimicrobial potential of stem barks of *Coula edulis* Baill. *Pseudospondias longifolia* Engl. and *Carapa klaineana* Pierre from Gabon. *Asian Pac J Trop Dis* 2016; **6**(7): 557-563.

[19]Obame-Engonga LC, Abdoul M, Ondo JP, Obiang CS, Ngoua-Meye-Misso RL, Traoré A, et al. Phytochemical screening, antioxidant and antibacterial activities of *Guibourtia ehie* and *Syzygium rowlandii* medicinal plants from Gabon. *Inter J Cur Res* 2017; **9**(8): 56354-56360.

[20]Konaté K, Hilou A, Mavoungou JF, Lepengué AN, Souza A, Barro N, et al. Antimicrobial activity of polyphenol-rich fractions from *Sida alba* L.(Malvaceae) against co-trimoxazol-resistant bacteria strains. *An Clin Microbiol Antimicrob* 2012; **11**(1): 1-5.

[21]Rigat M, Vallès J, Iglésias J, Garnatje T. Traditional and alternative natural therapeutic products used in the treatment of respiratory tract infectious diseases in the eastern Catalan Pyrenees (Iberian Peninsula). *J Ethnopharmacol* 2013; **148**(2): 411-422.

[22]Carradori S, Gidaro MC, Petzer Al, Costa G, Guglielmi P, Chimenti P, et al. Inhibition of human monoamine oxidase: Biological and molecular modeling studies on selected natural flavonoids. *J Agric Food Chem* 2016; **64**(47): 9004-9011.

[23]Angiolella L, Carradori S, Maccallini C, Giusiano G, T Supuran C. Targeting *Malassezia* species for novel synthetic and natural antidandruff agents. *Cur Med Chem* 2017; **24**(22): 2392-2412.

[24]Acheampong A, Badu M, Agyemang AY. Comparative total phenolics and antioxidant activities of *Xanthosoma colocasia*, *Solanum torvum* and *Allium ascalonicum* L. *Inter J Chem Biomol Sci* 2016; **2**(4): 73-79.

[25]Tachakittirungrod S, Okonogi S, Chowwanapoonpohn S. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. *Food Chem* 2007; **103**: 381-388.

[26]Kumar S, Karl P, Samuel J, Selvakumar M, Shalini K. Anti-oxidant, anti-diabetic, antimicrobial and hemolytic activity of *Tridax procumbens*. *J Chem Pharm Res* 2016; **8**(2): 808-812.

[27]Valerino-Díaz AB, Gamiotea-Turro D, Zanatta AC, Vilegas W, Gomes Martins CH, de Souza Silva T, et al. New polyhydroxylated steroidal saponins from *Solanum paniculatum* L. leaf alcohol tincture with antibacterial activity against oral pathogens. *J Agri Food Chem* 2018; **66**(33): 8703-8713.

[28]Evina JN, Bikobo DSN, Zintchem AAA, Nyemeck I, Mbabi N, Ndedi EDFM, et al. *In vitro* antitubercular activity of extract and constituents from the stem bark of *Disthemonanthus benthamianus*. *Rev Brasi Farmaco* 2017; **27**(6): 739-743.

[29]Lalitha V, Raveesha K, Kiran B. Antimicrobial activity of *Solanum torvum* Swart. against important seed borne pathogens of paddy. *Iran J Energy Env* 2010; **1**(2): 160-164.

[30]Korem T, Zeevi D, Zmora N, Weissbrod O, Bar N, Lotan-Pompan M, et al. Bread affects clinical parameters and induces gut microbiome-associated personal glycemic responses. *Cell Metab* 2017; **25**(6): 1243-1253.