



Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

**Antibacterial and Antioxidant Activities of Ethanolic Leaves Extracts of
Dissotis multiflora triana (Melastomataceae)**

**Afagnigni Alian Désiré^{1*}, Nyegue Maximilienne Ascension¹, Ndoye Foe Chantal
Florentine², Voundi Olugu Steve¹, Fonkoua Marie Christine³, Etoa Francois-Xavier¹**

¹Laboratory of Microbiology, Department of Microbiology, Faculty of Science, University of Yaounde I, PO Box: 812
Yaounde-Cameroon

²Laboratory of Phytobiochemistry and Medicinal Plants Study, Department of Biochemistry, Faculty of Science,
University of Yaounde I, PO Box: 812 Yaounde-Cameroon

³Laboratory of Bacteriology, Centre Pasteur of Cameroon

ABSTRACT

Dissotis multiflora leaves are commonly used in the Cameroon traditional medicine to heal infectious diseases without solid scientific basis. This study aimed to investigate the phytochemical screening, *in vitro* antibacterial activity, phenol content and antioxidant activity of its ethanolic leaves extracts. Antibacterial test was assessed by agar well diffusion and microdilution methods against seven clinical isolates species namely *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Shigella flexneri*, *Proteus mirabilis*, *Salmonella typhi* and *Bacillus cereus*. Total phenol content was determined by the Folin-Ciocalteu test while antioxidant activity was evaluated by DPPH, FRAP and ABTS methods. The phytochemical screening revealed the presence of phenols, tannins, alkaloids, saponins, anthraquinones, steroids and flavonoids. Extract inhibited the growth of all species tested with inhibition zone diameter ranged from 10.33 ± 1.24 mm (*Klebsiella pneumoniae*) to 28 ± 2.94 mm (*Shigella flexneri*) and MIC varying between $97.65 \mu\text{g/ml}$ (*Salmonella typhi*) and $781 \mu\text{g/ml}$ (*Bacillus cereus* and *Enterobacter cloacae*). Total phenol content was found to be 813.68 ± 1.27 mg ascorbic acid equivalent/g of dry material. The scavenging kinetics of DPPH by *Dissotis multiflora* extracts shows a steady state at 60 minutes while EC_{50} was 0.7×10^3 g/mol in DPPH. FRAP assay shows a reducing power of 7 ± 0.35 mg ascorbic acid equivalent/g of dry material. ABTS assay displayed an inhibition percentage of 93.269% at $4.54 \mu\text{g/ml}$. These results justify the use of *Dissotis multiflora* in traditional medicine to heal diarrhea and can be use as potential biomolecules reservoir.

Keywords: *Dissotis multiflora*, ethanolic extract, antibacterial activity, antioxidant activity.

DOI: 10.25004/IJPSDR.2016.080108

Int. J. Pharm. Sci. Drug Res. 2016; 8(1): 50-56

***Corresponding author: Mr. Afagnigni Alian Désiré,**
of
Microbiology, Faculty of Science, University of
Yaounde I, PO Box: 812 Yaounde-Cameroon; **Tel.:**
+237698325924; E-mail: afagnigni2007@yahoo.fr
Received: 13 January, 2016; **Accepted:** 25 January, 2016

INTRODUCTION

Infectious diseases are the major human health problem because of limited chemotherapy or unavailability of vaccines. [1] They are responsible for 26.3% of death in the world and those of bacterial origin are the most important. Low income and middle-income countries are particularly burdened with the preventable and treatable infection. [2-3] Many drugs as antibiotics are

used to fight bacterial infections but their progress face numerous problems due to inappropriate usage and the poor management of infections. With the advents of ever-increasing resistant bacterial strains, there has been a corresponding rise in the universal demand for natural antimicrobial therapeutics. Moreover, infectious diseases stimulate lipid peroxidation [4] associated with aging, membrane damage, heart diseases and cancer. [5] The common antioxidants used are suspected to possess toxic effects. Therefore it is necessary to find new efficient substances with particular attention given to those which possess both antibacterial and antioxidant activities. One of the solutions is to investigate medicinal plants in the treatment of diseases since they are sources of active molecules and they are accessible and available. Phenolic compounds and flavonoids are widely distributed in plants as natural products with revealed antioxidant and antibacterial activities. [6] That is why herbal medicines are widely used and now form an integral part of the primary health care in many countries. Significant increase and amelioration are been noted in phytotherapy but several plants with medicinal properties remain underexploited. [7] In Cameroon, many plant species are used as traditional medicine in the treatment of several infectious diseases and several interesting openings have originated for further inquiry following *in vitro* antimicrobial activity evaluation. [8]

Aqueous decoctions and powdered leaves of *D. multiflora triana* are used as traditional medicine in west Cameroon to heal diarrhea without strong scientific supports. To the best of our knowledge no previous study has been reported on *D. multiflora*. However, many studies revealed that many plants of the Melastomataceae family are used for the treatment of dysentery. [9] Antidiarrheal and antibacterial activities of ethanolic and methanolic leaves extract from *Dissotis* genera have been revealed as well as toxicological activity. [10-11] Thus it is important to carry out phytochemical screening and biological activities of *D. multiflora triana*. The present study was therefore undertaken to investigate the phytochemical screening, *in vitro* antibacterial and antioxidant activities of ethanolic leaf extracts of *D. multiflora*.

MATERIAL AND METHODS

Collection and identification of plant materials

D. multiflora triana plants were collected at Nkoupa-Matapit in the West region of Cameroon in December 2013. The plant identification was done at the Cameroon National Herbarium by comparison with specimen N° 20950/HNC.

Microorganisms

Seven bacterial species including *E. cloacae*, *K. pneumoniae*, *E. coli*, *S. flexneri*, *P. mirabilis*, *S. typhi* and *B. cereus* commonly associated with diarrheal infection where used. These clinical isolates were obtained from Centre Pasteur of Cameroon and the General Hospital

in Yaoundé. Bacterial strains kept at +4°C were activated before any test.

Preparation of ethanolic extracts

The leaves of *D. multiflora triana* were dried for one week under the shade at room temperature and weighed. The samples were then ground in a mortar and 200 g of dried powder of each sample was soaked for 48 hours in 600 ml of ethanol 95°. The mixing was filtered with Whatman n°1 filter paper and concentrated using a rotar vapor (Buchi) at 70°C.

Phytochemical screening

The different secondary metabolites such as alkaloids, tannins, saponins, flavonoids, steroids and phenols in the extracts were revealed using standard methods. [12]

Antimicrobial assays

Agar-well diffusion method

The assay was conducted by agar well diffusion method. [13] 100 mg of crude extracts were dissolved in 1 ml of distilled water to obtain a final concentration of 100 mg/ml. The solutions were prepared under sterile conditions in fumes cupboard. 25µl of gentamicin (40 mg/ml) was diluted in 975µl of sterile distilled water to a final concentration of 1 mg/ml. The bacterial strains were cultured on nutrient agar at 37°C for 18 hours. A pure colony of each strain was suspended in sterile distilled water and adjusted to a turbidity of 0.5 McFarland standards at 1.5×10^8 CFU/ml. [14] Further dilution was made to obtain 1.5×10^6 CFU/ml. The suspension was used to inoculate the Petri dishes (90 mm in diameter) previously poured with agar medium using a sterile cotton swab. Wells of 6 mm of diameter were punched on the agar medium and each well was filled with 50µl of extract or gentamicin. The Petri dishes were left at room temperature for 15 minutes and then incubated at 37°C for 24 hours. The diameters of inhibition zones around the well were measured using a vernier caliper. The susceptibility of the bacterial strains to the extracts was determined using the inhibition diameter (ID) as: non sensitive (ID ≤ 8 mm); sensitive (9 ≤ ID ≤ 14 mm); more sensitive (15 ≤ ID ≤ 19 mm) and extremely sensitive (20 mm ≤ ID). [15]

Determination of the Minimal Inhibitory Concentration (MIC) by microdilution method

Preparation of inoculums of microorganisms

A colony of bacteria from an overnight pure culture spread on solid Mueller Hinton Agar and incubated at 37°C was suspended in a tube containing 1 ml of sterile distilled water. The concentration of the suspension was then standardized as previously described.

Preparation of working solutions of the plant extracts and reference antibiotic

100 mg/ml of crude extracts of *D. multiflora triana* were prepared as described above while 0.2 mg/ml of gentamicin (standard antibiotic) was prepared by dilution made from solution of 1 mg/ml above.

Preparation of plates

The microdilution was performed in 96-well microtiter plates with U-shaped bottoms. [16] Each well was filled with 100µl of nutrient broth. 100µl of each extract were

added in the first wells and a series of two fold dilution of each crude extract was made until the eleventh well. The standard antibiotic was prepared in the same way. The final concentrations ranged from 25×10^3 to $24 \mu\text{g/ml}$ for each extract and from 0.05 to $0.048 \mu\text{g/ml}$ for gentamicin. The microplates were inoculated with $100 \mu\text{l}$ of a culture containing 1.5×10^6 CFU/ml of each organism, covered and incubated for 16-24 hours at 37°C . After incubation, $40 \mu\text{l}$ of 2, 3, 5-triphenyltetrazolium chloride (TTC) at 0.01% in sterile distilled water was added to each well except for the negative control wells containing only extract and nutrient broth. The microplates were incubated for 30 minutes. The MIC was determined as the lowest concentration of extract inhibiting visible growth of each microorganism on the nutrient broth. Inhibition of bacterial growth in the plates containing extracts was judged by comparison with growth in control microplates and by the turning of uncolored TTC to red. Each experiment was carried out in triplicate. The third column without TTC was left for determination of minimal bactericidal concentration.

Minimal bactericidal concentration (MBC) was determined by transferring $50 \mu\text{l}$ of well without any growth in $100 \mu\text{l}$ of nutrient broth and incubated at 37°C for 48 hours. After incubation, $40 \mu\text{l}$ of TTC was added to each well and plate were incubated at 37°C for 30 minutes. MBC was considered as the concentration of extract at which no growth was observed. The absence of growth indicated bactericidal effects while the presence of microbial growth indicated bacteriostatic effects. [16]

Total Phenol content

The total phenol content was determined using Folin-Ciocalteu (1/10) diluted reagent. [17] $23 \mu\text{l}$ of each extract (1 mg/ml) was introduced in a test tube containing $115 \mu\text{l}$ of Folin-Ciocalteu reagent in $1817 \mu\text{l}$ of distilled water. After 5 minutes, $345 \mu\text{l}$ of Na_2CO_3 (15%) solution was added. Ascorbic acid was used as standard. Absorbance was read at 765 nm with a spectrophotometer (Jenway 6305) after 120 minutes of incubation in the dark. Total phenol content was expressed in mg equivalent of ascorbic acid /g dried extract.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH radical scavenging kinetic was carried out as described by Brand-Williams *et al.* (1994) [18] by measuring the decrease in absorbance each 15 minutes for 2 hours. The scavenging activity was followed after 120 minutes of incubation. $50 \mu\text{l}$ of extracts prepared in ethanol at different concentrations (0.8 mg/ml; 0.4 mg/ml; 0.2 mg/ml; 0.1 mg/ml; 0.05 mg/ml; 0.025 mg/ml) was mixed in $1950 \mu\text{l}$ ethanolic solution of DPPH (0, 04 g/l) in different tubes. Final concentrations in 2 ml of DPPH solution were 0.02 mg/ml; 0.01 mg/ml; 0.005 mg/ml; 0.0025 mg/ml; 0.00125 mg/ml. After 120 minutes of incubation in dark, the absorbance was read at 517 nm with spectrophotometer (Jenway 6305) against control (containing DPPH + ethanol).

Ascorbic acid was used as standard with final concentrations of 0.01 mg/ml; 0.005 mg/ml; 0.0025 mg/ml; 0.00125 mg/ml; 0.000626 mg/ml. Scavenging activity was calculated using the following formula:

$$\% \text{ Scavenging} = (A_{\text{ref}} - A_{\text{mes}} / A_{\text{ref}} - A_{100}) \times 100$$

Where A_{Ref} = Absorbance at $t=120$ minutes of control (DPPH solution without antiradical); A_{mes} = Absorbance at $t=120$ minutes of DPPH solution with antiradical and A_{100} = Absorbance at the end of reaction = 0.

Antioxidant activity of the extracts was expressed as efficient concentration 50 (EC_{50}) (Concentration of sample (g) to scavenge 50% of the DPPH free radicals (mol)) from the linear regression graph.

Ferric Reducing Antioxidant Power Assay (FRAP)

FRAP assay was performed as described by Benzie and Strain (1996). [19] 3.1 mg of 2, 4, 6 tripyridyl-1, 2, 5-triazine (TPTZ) was diluted in 1000 ml of HCl 40 mM. Ferric chloride (FeCl_2) 20 mM and acetate buffer 300 mM, pH = 3.6 were respectively mixed in 1; 1; 10 ratio to obtain FRAP (Ferric Reducing Antioxidant Power) solution. $50 \mu\text{l}$ of extract prepared in ethanol at different concentrations (2 mg/ml; 1 mg/ml; 0.5 mg/ml; 0.25 mg/ml; 0.125 mg/ml; 0.0625 mg/ml) was mixed in $1950 \mu\text{l}$ of ethanolic solution of FRAP in different tubes to a final concentration of 0.5 mg/ml; 0.25 mg/ml; 0.125 mg/ml; 0.0625 mg/ml and 0.03125 mg/ml. After 30 min of incubation in dark, the absorbance was read at 593 nm with spectrophotometer (Jenway 6305) against control. Ascorbic acid was used as standard.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay

The test was carried out as described by Re *et al.* (1999). [20] The working solution was made up of ABTS 7 mM and potassium persulfate 2.4 mM (1:1, v/v) prepared in distilled water. The solution was left in dark for 12 hours at room temperature. The resulting solution was diluted by mixing 1 ml of fresh solution of ABTS to obtain an absorbance of 0.706 ± 0.001 at 734 nm with spectrophotometer (Jenway 6305). The fresh solution was prepared daily. Extract ($20 \mu\text{l}$) at different concentrations (31.25 $\mu\text{g/ml}$; 62.5 $\mu\text{g/ml}$; 125 $\mu\text{g/ml}$; 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$) was mixed with 2 ml of ABTS solution for final concentrations of 2.84 $\mu\text{g/ml}$, 0.56 $\mu\text{g/ml}$, 1.13 $\mu\text{g/ml}$, 2.24 $\mu\text{g/ml}$ and 4.54 $\mu\text{g/ml}$. Absorbance was read at 734 nm after 7 minutes with spectrophotometer (Jenway 6305). Ascorbic acid was used as standard.

$$\text{ABTS radical scavenging activity} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

Where A_{control} = Absorbance at $t=7$ min of control (ABTS solution without antiradical); A_{sample} = Absorbance at $t=7$ min of ABTS solution with antiradical.

Statistical analysis

Data were represented as mean \pm standard deviation (SD) and subjected to one-way analysis of variance (ANOVA) using the Fisher test at threshold of $p < 0.05$ with Stat graphics plus 5.0 for windows. Excel was used to draw figures.

Table 1: Inhibition zone diameters of ethanolic leaves extracts of *D. multiflora* and gentamicin

Extract/ gentamicin	Inhibition zones (mm)						
	ST	KP	BC	SF	EC1	PM	EC2
<i>D. multiflora</i>	21.5 ± 0.40	10.33 ± 1.24	13.5 ± 1.47	28 ± 2.94	18 ± 4.6	13.17 ± 2.49	12 ± 1.87
Gentamicin	25.83 ± 0.62	24.5 ± 2.67	22.33±0.94	29 ± 0.81	21.5 ± 0.40	29.55 ± 0.42	17.66 ± 0.47

ST: *Salmonella typhi*; KP: *Klebsiella pneumoniae*; BC: *Bacillus cereus*; EC1: *Escherichia coli*; PM: *Proteus mirabilis*; EC2: *Enterobacter cloacae*; SF: *Shigella flexneri*

Table 2: Inhibition parameters of ethanolic extracts of *D. multiflora triana* and gentamicin

Extract/ gentamicin (µg/ml)	Parameters	ST	KP	BC	SF	EC1	PM	EC2
<i>D. multiflora</i>	MIC	97.65	195.31	781.25	195.31	390.62	390.62	781.25
	MBC	195.31	1562.5	3215	781.25	390.62	390.62	3215
	MBC/MIC	2	8	4	4	1	1	4
Gentamicin	MIC	0.390	0.390	0.195	0.195	3.125	12.5	0.781
	MBC	0.781	0.781	0.390	0.195	12.5	25	1.562
	MBC/MIC	2	2	2	1	4	2	2

ST: *Salmonella typhi*; KP: *Klebsiella pneumoniae*; BC: *Bacillus cereus*; EC1: *Escherichia coli*; PM: *Proteus mirabilis*; EC2: *Enterobacter cloacae*; SF: *Shigella flexneri*

RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical screening of the crude extracts of *D. multiflora* reveals the presence of phenols, tannins, alkaloids, saponins, anthraquinones, steroids and flavonoids. Among the metabolites present in *D. multiflora* ethanolic extract, tannins, alkaloids and saponins were also found in accordance with results obtain with aqueous ethanol leaf of *Dissotis rotundifolia*, a plant of the same genera. [11] These chemical compounds are responsible for antimicrobial activities. [21-22]

Antibacterial assay

Determination of inhibition diameter zones

The presence of inhibition zones after incubation revealed the activity of the extracts on the bacteria tested. The results are reported in Table 1 below. It shows that the ethanolic extracts of *D. multiflora* inhibited the growth of all bacteria tested (*E. cloacae*, *B. cereus*, *K. pneumoniae*, *S. aureus*, *S. typhi*, *S. flexneri* and *E. coli*). Inhibition zones ranges from 10.33 ± 1.2 against *K. Pneumoniae* to 28 ± 29.4 mm against *S. flexneri*. As can be observed, *K. Pneumoniae*, *B. cereus*, *P. mirabilis* and *E. cloacae* are sensitive; *E. coli* was more sensitive while *S. flexneri* and *S. typhi* were extremely sensitives. [15] No significant difference was observed between inhibition zones of ethanolic extracts of *D. multiflora* and gentamicin against *S. typhi*, *S. flexneri* and *E. coli*. *Shigella flexneri* and *S. typhi* were most sensitive in solid medium while *E. cloacae*, *B. cereus*, *K. pneumoniae* and *P. mirabilis* were less sensitive. These different sensibilities of microorganism to extract may depend on the chemical composition of the extracts, certain specific internal factors of each microorganism and the environment of action. [24] However, *K. Pneumoniae*, *B. cereus*, *P. mirabilis* and *E. cloacae* which appear less sensitive may have some kind of resistance mechanisms like enzymatic inactivation, target sites modification and decrease intracellular drug accumulation. [25] Moreover, the mechanisms of action of the active principles can vary from one strain to another and from one species to another and depend equally on the genetic material of each microorganism.

[26] The antibacterial effect exhibited by ethanolic extracts of *D. multiflora* may be due to the presence of flavonoids, polyphenols and anthraquinones. Previous works revealed that polyphenols inhibit a wide range of microorganisms. Flavonoids can form a complex with extracellular soluble proteins and the bacteria cell wall. [27] Tannins inactivate microbial adhesion, enzymes and proteins of the cellular envelop. Alkaloids insert themselves between DNA and / or cellular membranes. [28] The secondary metabolites identified in the plant material used in this study could be responsible for the antibacterial activity exhibited by this plant. The relatively high activity of *D. multiflora*'s crude extracts may be due to the higher concentration of active compounds.

Inhibitory parameters: Minimal Inhibitory Concentration (MIC) of ethanolic leaves extracts

MIC of extracts and gentamicin are shown in Table 2. It can be noticed that *D. multiflora*, inhibited growth of Gram positive and Gram negative bacterial strains with MIC ranging between 97.65 (*S. typhi*) to 781.25µg/ml (*E. cloacae* and *B. cereus*). The results of the susceptibility test were confirmed by microdilution since the bacteria tested showed different sensibilities in the same manner. Hence, *S. typhi* and *S. flexneri* were the most sensitive strains with 97.65 and 195.31µg/ml respectively. However, ethanolic extracts of *D. multiflora* exhibited a MIC of 195.31µg/ml on *K. pneumoniae* which was less sensitive in the solid medium. This could be explained by the fact that in liquid medium, bacteria is directly in contact with extract which might not completely diffuse on agar medium. This result is important since *Klebsiella sp* is known to be particularly resistant. The MBC/MIC ratio showed that ethanolic extracts of *D. multiflora* exhibited bactericidal effect on all bacterial strains tested except *K. pneumoniae* on which the extracts exhibited only bacteriostatic effect. The secondary metabolites found in ethanolic leaves of *Dissotis multiflora triana* are responsible for the antibacterial activity and may work in synergism. Despite the fact that there is no previous work on *D. multiflora*, these results concord with those obtained with ethanolic leaves of *Dissotis rotundifolia*, a

plant of the same genera which revealed antibacterial activity against *E. coli* and *S. typhi* among others. [11]

Antioxidant tests

Total phenol content

Total phenol content was determined from linear calibration curve of ascorbic acid within the range of 0.00125-0.01 mg/ml ($Y = 20.21x - 0.024$; $R^2 = 0.957$) shown by figure 1. The total phenol was found to be 813.689 ± 1.27 mg ascorbic acid equivalent/g of dry material. This result shows that ethanolic leaves of *D. multiflora* possess important amount of dried material. This further justifies the antioxidant activity of plant extracts since it has been proven that phenols, alkaloids and flavonoids possess antioxidant activity. [29-30]

Ferric Reducing Antioxidant Power Assay (FRAP)

The reducing power was first express in terms of absorbance at various concentrations of *D. multiflora* extracts and ascorbic acid (Figure 2). From the regression curve (ascorbic acid: $Y = 0.036x + 0.090$, $R^2 = 0.996$), the reducing power of ethanolic leaves extracts was expressed in terms of mg equivalent ascorbic acid/g of dry material. The FRAP assay shows an absorbance of 1.701 at 50µg/ml and a reducing power of 7 ± 0.35 mg ascorbic acid equivalent/g of dry material. Absorbance was concentration dependant. The reducing capacity serves as a significant indicator of potential antioxidant activity for a given compound. [31] *D. multiflora triana* extracts can act as electron donors and react with free radicals and convert them to stable products, thus terminating the radical chain reactions. [32]

2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

When DPPH encounters a proton-donating substance (H^+), the radical is scavenged by changing colour from purple to yellow and the absorbance is reduced. In the present test, *D. multiflora triana* reduced the DPPH radical to a yellow-colored compound due to the DPPH radical accepting an electron or hydrogen to become a stable molecule. [33] The antiradical activity of a substance can therefore be expressed as its ability to scavenge the DPPH radical.

Scavenging kinetic of DPPH

The scavenging kinetic of the reactivity of DPPH was evaluated. It aimed to evaluate reactivity of DPPH at various time with different test compound by measuring absorbance every 15 minutes during 120 minutes. There are three types of scavenging kinetic corresponding to the time at which stationnary state is obtained namely fast (<10 minutes), intermediate (20 minutes) and low (>20 minutes). [34] The kinetic of *D. multiflora triana* extracts (figure 3) and ascorbic acid (figure 4) are low since their scavenging kinetic showed steady state after 60 min.

The evaluation of DPPH reactivity revealed that ethanolic extract of *D. multiflora triana* possess low scavenging kinetic corresponding to the steady state obtain after 60 minutes. The same result was obtain with reference antioxidant. However, evaluation of

different kinetics depends of the nature of tested antiradicals. [34] *D. multiflora triana* is a potential source of antiradical molecules such as flavonoid and phenol found in this plant as revealed by phytochemical test.

Antiradical activities

The DPPH radical-scavenging capacity in the studies was reported after 120 minutes of reaction. The radical scavenging activity of extract and reference was express in terms of EC_{50} value which is the concentration of antioxidant required for 50% scavenging of DPPH radicals during reaction. The smaller EC_{50} value, the higher antioxidant activity of the plant extract/reference. The results were expressed in terms of scavenging percentage at various concentrations of extracts and ascorbic acid (Figure 5). It can be noticed that at lower concentration less than 6µg/ml, *D. multiflora* exhibited a scavenging percentage higher than that of Ascorbic acid while the reverse effect is observed as from 6µg/ml. From the above plot (*D. multiflora*: $Y = 7.246x + 1.249$, $R^2 = 0.975$ and Ascorbic acid : $Y = 9.816x - 9.362$, $R^2 = 0.987$), the EC_{50} was found to be 0.70×10^3 (g/mol) for ethanolic extract of *D. multiflora*.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) free radical scavenging activity

The scavenging capacities of extract and Ascorbic acid for the ABTS radical were measured and compared (Figure 6). As in the case of DPPH radical scavenging, the scavenging effect of extract and ascorbic acid increased with increasing concentration. As can be seen, a steady state is observed with *D. multiflora triana* extract at higher concentration from 1.13 to 4.5µg/ml. *D. multiflora triana* extract exhibited the higher ABTS antiradical properties with an inhibition of 93.25% for the ABTS radical at 4.54µg/ml while at the same concentration ascorbic acid exhibited an inhibition of 63.16%. The ABTS test was performed by evaluating the inhibition percentage at different concentrations of extracts. The $ABTS^{*+}$ is a relatively stable radical that is easily reduced by an antioxidant. [35] By reducing the color of the ABTS radical, ethanolic extract of *D. multiflora triana* have been identified as potential antioxidants. This indicates that it have the ability to donate hydrogen atoms to the free radicals, slowing the propagation of lipid peroxidation process. As can be seen, scavenging activity increased with antiradical concentration for both extract and reference but steady state was observed with extract at from 1.13µg/ml, concentration at which any increasing concentration shows no increasing scavenging activity. At the same concentration, *D. multiflora triana* was more active than ascorbic acid, the reference antioxidant.

The phytochemical screening of the crude extracts of *D. multiflora* revealed the presence of active chemical compounds (phenols, tannins, alkaloids, saponins, anthraquinons, steroids and flavonoids). The antibacterial assay shows that ethanolic crude extracts of *D. multiflora* inhibit the growth of bacterial strains tested.

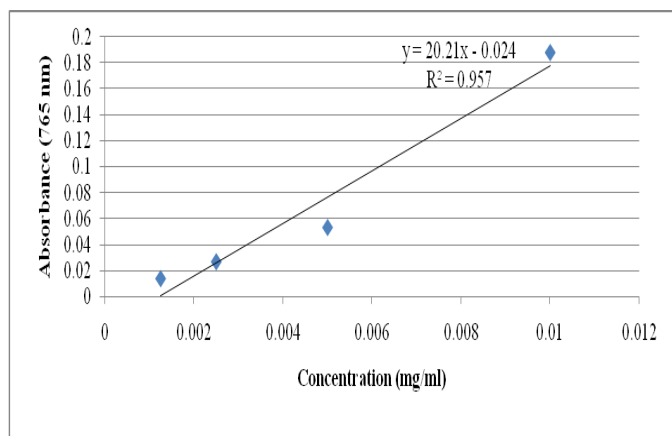


Fig. 1: Calibration curve of standard ascorbic acid for determination of total phenol content

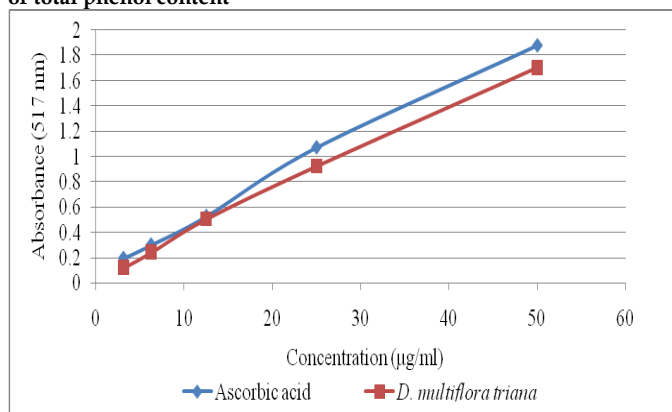


Fig. 2: Total ferric reducing power (FRAP) at different concentrations (3.12–50 µg/ml) of ethanolic extract of *D. multiflora* and reference antioxidant.

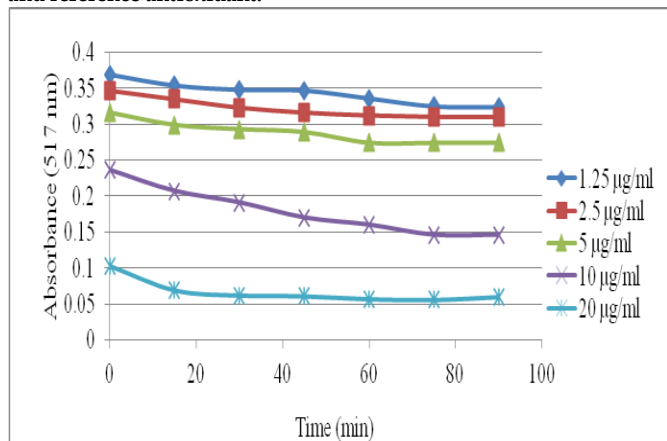


Fig. 3: Scavenging kinetic of *D. multiflora* in terms of absorbance at different times

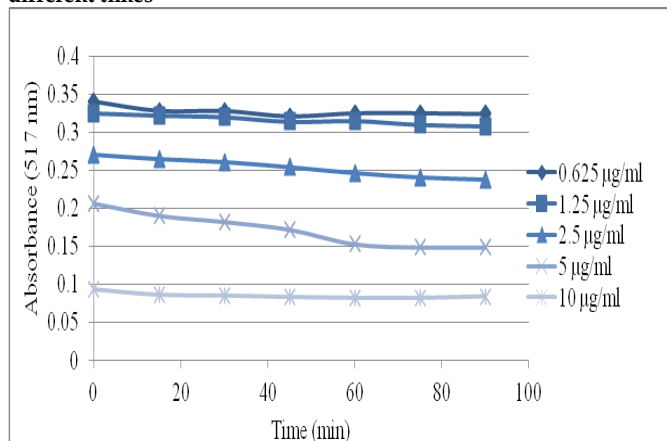


Fig. 4: Scavenging kinetic of Ascorbic acid in terms of absorbance at different times

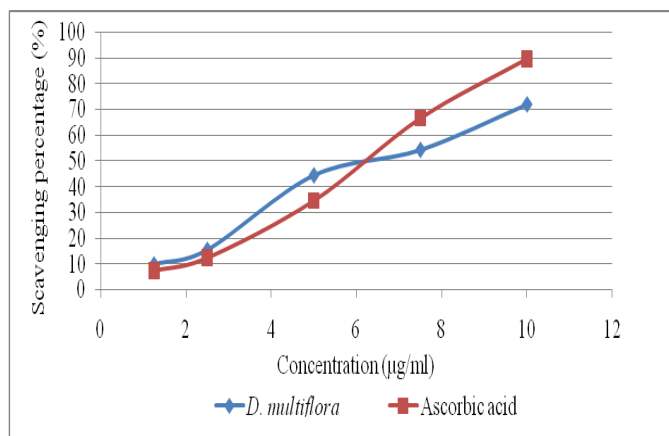


Fig. 5: DPPH free radical scavenging activity at different concentrations (1.25–10 µg/ml) of ethanolic extract of *D. multiflora* and reference antioxidant.

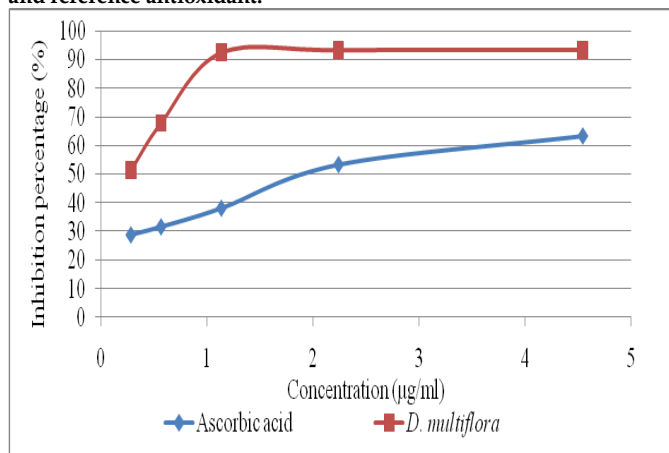


Fig. 6: ABTS radical scavenging activity at different concentrations (0.284–4.54 µg/ml) of ethanolic extract of *D. multiflora* and reference antioxidant

Bactericidal activity was observed on *S. typhi*, *E. cloacae*, *P. mirabilis*, *S. flexneri* and *E. coli*. An *in vitro* antioxidant study provides scientific evidence and additional information to prove the traditional claims to the Melastomataceae member, *D. multiflora*. On the basis of the results obtained in the present study, it was concluded that the ethanolic leaf extract of this species possess significant antioxidant activity. Presence of adequate amount of phenols and other compounds may account for the observed activities. So the findings of this study suggest that this plant is a potential source of natural antioxidant. These results support the use of leaves of *D. multiflora* in Cameroon traditional medicine to treat diarrhea and also reveals that it constitutes an important source of biomolecules to be exploited. Further studies are required for antidiarrheal activity, isolation and characterization of antibacterial and antioxidant compounds, and *in vivo* studies are needed to understand their mechanism of action as antibacterial and antioxidant compounds as well as their toxicity.

ACKNOWLEDGEMENTS

The authors acknowledge contributions from the National Herbarium of Cameroon; Centre Pasteur of Cameroon, Yaoundé General Hospital and the

Laboratory of Microbiology of the Department of Microbiology, University of Yaoundé I, Cameroon.

REFERENCES

- Arya V, Yadav S, Kumar S, Yadav JP. Antimicrobial activity of *Cassia occidentalis* L (leaf) against various human pathogenic microbes. *Life Sci Med Res.* 2010; 9:1-11.
- OMS. Stratégie de l'OMS pour la médecine traditionnelle pour 2002-2005. *WHO/EDM/TRM/2002*, Genève, 2002, pp. 65.
- Jules CA, Henri LK, Dickson SN, Anna N, Peter FN, Emmanuel AA, Abdel JN, Bertrand S, Véronique BP. Antimicrobial and toxicological activities of five medicinal plant species from Cameroon Traditional Medicine. *BMC Complementary and Alternative Medicine* 2011; 11:70.
- Gorowara S, Sapru S, Ganguly NK. Role of intracellular second messengers and ROS in the pathophysiology of *V. cholerae* 0139 treated rabbit ileum. *Biochim Biophys Acta.* 1998; 1407:21-30.
- Inoue K, Garner C, Ackermann BL, Oe T, Blair IA. Liquid chromatography/tandem mass spectrometry characterization of oxidized amyloid beta peptides as potential biomarkers of Alzheimer's disease. *Rapid Commun Mass Spectrom* 2006; 20:911-918.
- Mukund S, Sivasubramanian V, Senthil kumar NS. *In vitro* antioxidant activity of the Methanolic Extract of *Oscillatoria terebriformis* C.A. Agardh ex Gomont. *J Algal Biomass Utiln.* 2013; 4(1):17-25
- Lalitha V, Raveesha KA, Kiran B. Antimicrobial activity of *Solanum torvum Swart* against important seed borne pathogens of paddy. *Iranica J Energy Environ.* 2010; 1(2):160-164.
- Kuete V. Potential of Cameroonian plants and Derived products against microbial infections: A review. *Planta med.* 2010; 76:1479-1491.
- Noumi E, Yomi A. Medicinal plants used in intestinal diseases in Mbalmayo region. *Fitoterapia* 2001; 246-254.
- Ateufack G, Tadjoua TH, Yousseu NW, Sama FL, Kuate JR, Kamanyi A. Antidiarrheal and antibacterial activity of aqueous and methanolic leaves extracts of *Dissotis thollonii Cogn. (Melastomataceae)*. *Asian Pac J Trop Biomed.* 2014; 4 (Suppl 2): 837-843
- Abere TA, Okoto PE, Agoreyo FO. Antidiarrhea and toxicological evaluation of the leaf extract of *Dissotis rotundifolia triana (Melastomataceae)*. *BMC Complementary and Alternative Medicine* 2010; 10:71.
- Harbone JB. *Phytochemical methods. A guide to modern techniques of plants analysis.* Chapman and Hall, London. 1998, pp. 150.
- Perez C, Paul M, Bazerque P. An Antibiotic assay by the agar well diffusion method. *Acta Bio Med Exp.* 1990; 15:113-115.
- Tereshuck ML, Riera MVQ, Castro GR, Abdala LR. Antimicrobial activity of flavonoid from leaves of *targets minuta*. *J Ethnopharmacol* 1997; 56(3):227-232
- Moreira MR, Ponce AG, Del VC, Roura SI. Inhibitory parameters of essential oils to reduce a foodborne pathogen. *Lebensmittel-Wissenschaft und-Technologie* 2005; 38: 56.
- Eloff JN. A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extracts for bacteria. *Plant Med.* 1998; 64: 711-713.
- Vinson JA, Yong H, Xuehui S, Ligia Z. Phenol antioxidant quantity and quality in foods: vegetable. *J Agric Food Chem.* 1998; 46: 3630-3634.
- Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und -Technologie* 1995; 28:25-30.
- Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal Biochem.* 1996; 239:7076.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol Med.* 1999; 26:1231-1237.
- Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Tian Y. Evaluation of antioxidant capacity and total phenolic content of different fraction of selected microalgae. *Food Chem.* 2007; 102:771-776.
- Gordana SC, Jasna MC, Sonja MD. Antioxidant potential, lipid peroxidation inhibition and antimicrobial activities of *Satureja Montana L. Sub sp. kitaibelii* extracts. *Int J Mol Sci.* 2007; 8:1013-1027.
- Obasi NB, Igboechi AC, Anuforo DC. Studies of the antidiarrheal potential of some composite plants. Medicinal plants in the Developing Economy. Proceedings of a Workshop organized by the Nigeria society of Nigeria 1990; 108-116.
- Meyer A, Deiana J. *Cours de microbiologie générale.* Do in éditeurs, Paris. 1988, pp. 201-224, 234.
- Yala D, Merad A S, Mohamedi D, Ouar Korick MN. Classification et mode d'action des antibiotiques. *Médecine du Maghreb* 2001; 91:5-12.
- Takeo O, Masato K, Keiko S, Rika O, Junko M, Hiroshi I, Hiroyuki K, Toshi A, Tosshifumi A, Shigeo M. *In vitro* and *in vivo* antimicrobial activities of tricyclic ketolide Te-802 and its analogs. *Journal of Antibiotics* 2004; 57:518-527.
- Cowan MM. Plants products as antimicrobial agents. *Clin Microbiol Rev.* 1999; 14:564-584.
- Ghestem A, Seguin E, Paris M, Orecchioni AM. Le préparateur en pharmacie. Dossier 2, Botanique-Pharmacognosie-Phytotherapie-homeopathie. *Tec et Doc Ed.* 2001, pp. 272.
- Sokol-Letowska A, Oszmianski J, Wojdylo A. Antioxydant activity of the phenolic compounds of Hawthorn, pine and skullcap. *Food chem.* 2007; 103:853-859.
- Centenaro GS, Mellado MS, Prentice-Hernández C. Antioxidant Activity of Protein Hydrolysates of Fish and Chicken Bones. *Adv J Food Sci Technol.* 2011; 3(4):280-288.
- Zhu QY, Hackman RM, Ensuna JL, Holt RR, Keen CL. Antioxidative activities of oolong tea. *J Agric Food Chem.* 2002; 50:6929-34.
- Sowndhararajan K, Jince MJ, Dharmar R, Sellamuthu M. *In vitro* antioxidant characteristics of different parts of *Melothria maderaspatana (L.) Cogn.* *Int J Pharm Sci.* 2010; 2(3):111-23.
- Liu Q, Kong B, Xiong YL, Xia X. Antioxidant activity and functional properties of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis. *Food Chem.* 2010; 118: 403-410.
- Nyegue MA. Propriétés chimiques et biologiques des huiles essentielles de quelques plantes aromatiques et/ou médicinales du Cameroun : évaluation de leurs activités antiradicalaires, anti-inflammatoires et antimicrobiennes. Thèse de doctorat des universités de Montpellier II et Yaoundé I, 2006.
- Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci.* 1993; 84: 407-412.

Source of Support: Nil, Conflict of Interest: None declared.