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## **Polyphenols Content, Antioxidant and Antimicrobial Activities of *Ampelocissus grantii* (Baker) Planch. (Vitaceae): A Medicinal Plant from Burkina Faso**

<sup>1</sup>C. Zongo, <sup>1</sup>A. Savadogo, <sup>1</sup>L. Ouattara, <sup>1</sup>I.H.N. Bassole, <sup>1</sup>C.A.T. Ouattara,  
<sup>1</sup>A.S. Ouattara, <sup>1</sup>N. Barro, <sup>2</sup>J. Koudou and <sup>1</sup>A.S. Traore

<sup>1</sup>Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles (CRSBAN), UFR/SVT,  
Université de Ouagadougou, 03 BP 7131 Ouagadougou 03, Burkina Faso

<sup>2</sup>Centre de Recherche en Pharmacopée et Médecine Traditionnelle (CERPHAMETRA),  
Université de Bangui, BP 1450 Bangui, République Centrafricaine

**Abstract:** The main goal of this study was to determine the phenol content, the antimicrobial and the antioxidant activities of acetone and water extracts from the rhizomes of *Ampelocissus grantii* (Baker) Planch (Vitaceae). The polyphenol content of extracts was determined colorimetrically using Folin-Ciocalteu method. The antioxidant activity of the same extracts was also screened using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. The antimicrobial activity was performed by disc diffusion and broth microdilution assays against 9 reference bacterial strains including gram-negative and gram-positive bacteria and 1 fungal strain. The acetone extract exhibited the higher level of Total Polyphenol Contents (TPC) which correlated with strong antioxidant activity ( $IC_{50} = 10.87 \mu\text{g mL}^{-1}$  and  $AAI = 5.6$ ) and antimicrobial activity. The polyphenol content of the acetone extract was 124.26 gallic acid equivalent/100 g (GAE/100 g), that of water extract was 74.25 GAE/100 g. The largest diameter of inhibition zone (DIZ) (15 mm) and the lowest Minimal Inhibitory Concentration (MIC) value ( $0.625 \text{ mg mL}^{-1}$ ) were recorded with *Enterococcus faecalis* 10907 CIP and *Bacillus subtilis* ATCC 21332. This study is a scientific base on the use of this plant in traditional health cure system.

**Key words:** *Ampelocissus*, folk medicine, antibacterial activity, DPPH, antioxidant

### **INTRODUCTION**

Plants are important source of therapeutic drugs and play a significant role in the survival of the tribal and ethnic communities. It is estimated that an amount of 20000 species from several families are useful for these purposes (Penso, 1983). Natural products are proposed as a therapeutic alternative to conventional antimicrobial drugs, whose effectiveness is often limited by the resistance that the infectious agents have developed against drugs (Ali and Azhas, 1999; Nimri *et al.*, 1999; Jukemura *et al.*, 2007). Besides, herbal-based therapies, once used only in traditional medical systems are now recommended for treatment of several degenerative disorders and chronic conditions where modern pharmaceutical agents have proved inadequate (Iwu and Wootton, 2002). Natural products with their potential to act as antioxidants, play a major role in the prevention of various pathological conditions such as cancer, cardiovascular and neurodegenerative troubles. Furthermore, in developing countries, particularly in West Africa, effective drugs are not often affordable. Thus, up

to 80% of the population uses medicinal plants to solve their health problems (Kirby, 1996; Hostettmann and Marston, 2002; Karou *et al.*, 2006). This is why, studies on the plants of the folk medicine are necessary with the goal on the one hand to discover new chemical structures and the other hand to develop alternative and complementary drugs cheaper and disposable for populations.

In this study, we evaluated the antibacterial, antifungal and antioxidant activities of *Ampelocissus grantii* (Baker) Planch., an african medicinal plant widely used in folk medicine to treat many diseases including bacterial, parasitic, viral, protozoa and fungal diseases. Ethnobotanical studies revealed that the plant is used alone or mixed with other medicinal plants to treat shigellosis and fever including malaria (Adjanohoun *et al.*, 1980), urinary schistosomiasis (Bah *et al.*, 2006). It is also used to cure old wound (2 to 3 years) (Inngjerdigen *et al.*, 2004), to treat cancer (Muhammad and Amusa, 2005), diarrhoea (Etuk *et al.*, 2009), trypanosomiasis, rheumatism and muscular pain (Bizimana *et al.*, 2006). The trypanocidal activity has been demonstrated by *in vitro* and *in vivo* studies

(Bizimana *et al.*, 2006; Aderbauer *et al.*, 2008). Some bioactive compounds with significant cancer cell growth inhibitory activity against a panel of human cancer cell lines have been isolated from methanol extract of *Ampelocissus* sp. from Philippines (Pettit *et al.*, 2008).

The aim of this study was to establish the scientific bases of the use of the plant in folk medicine to combat bacteria and its uses in the treatment of certain cancers which could be due to its antioxidant properties.

## MATERIALS AND METHODS

**Plant material:** The rhizomes of *Ampelocissus grantii* were collected in the area of Sapone at about 45 km from Ouagadougou (Burkina Faso), in March 2009. The plant was taxonomically authenticated at the Department of Plant Biology and Ecology of the University of Ouagadougou.

The rhizomes were washed, cut to small fragments and carefully dried during 2 weeks at ambient temperature in the laboratory under continuous ventilation, away from sunlight and dust. The samples were then crushed to fine powder with a mechanical crusher and the powder was hermetically sealed in polythene bags and stored away from light and moisture until the time of extraction.

**Preparation of plant extracts:** Different extracts were made from powdered rhizomes using acetone (70%v/v) and water. Aliquots of 25 g were made and each aliquot was soaked with the appropriate solvent. Acetone extract was left under shaking conditions at room temperature for 24 h. Water extract was boiled for 1 h. At the end of the extraction, each extract was filtered using Whatman No. 1 filter paper. Acetone was completely removed at low pressure with a rotary evaporator (BÜCHI, Labortechnik, Switzerland). The acetone extract and the water extract were concentrated, lyophilized and stored at 4°C until analysis.

**Phytochemical screening:** Extracts were screened for phytochemical constituents using standard procedures of analysis (Harborne, 1998; Sofowora, 1993; Trease and Evans, 2002). Dragendorff's test, FeCl<sub>3</sub> test, Frothing test, Libarman-Burchard's test, Killer-killani's test were performed for respectively alkaloids, flavonoids, saponins, terpenes and steroids, cardiac glycosides detection. All chemicals were from Sigma-Aldrich, Germany, obtained with local suppliers.

**Determination of total phenolic contents:** Total Phenolic Contents (TPC) from extracts were quantified using Folin-Ciocalteu's method adapted to a 96 well-plate

(Dicko *et al.*, 2002) with minor modifications. One hundred microliters of Folin-Ciocalteu's reagent (10%, v/v) was added to 20 µL of each lyophilized plant extract dissolved in water and adjusted to 100 µg mL<sup>-1</sup>. After 5 min incubation at room temperature, 80 µL of sodium carbonate (75 g L<sup>-1</sup>) were added to each well containing the previous mixtures. The final volume per well was 200 µL. The 96 well-plate (Greiner Labortechnik, Germany) was slightly shaken and incubated for 30 min at room temperature in the darkness. The absorbance was measured at 735 nm using a multiwell plate reader (µQuant Bio-Tek Instrument, Inc., USA) on line interfaced with a computer. Absorbencies were automatically recorded using KC junior software version 1.31.5 (Bio-Tek instrument, Inc., USA). Gallic acid was used as standard and the calibration equation was  $Y = 0.061X + 0.003$  ( $R^2 = 0.998$ ), where X is the gallic acid concentration in µg mL<sup>-1</sup> and Y is the absorbance read at 735 nm. All the analyses were done in triplicate and results (average of triplicate analysis) were expressed as gallic acid equivalent per gram of lyophilized sample.

**Flavonoid content determination:** Total flavonoid contents (TFC) were determined by the aluminium chloride (AlCl<sub>3</sub>) colorimetric assay method (Quettier-Deleu *et al.*, 2000) adapted to 96 well-plate, using quercetin as a standard. The extracts were dissolved in ethanol 75% and 100 µL of this solution were mixed with 100 µL of 2% AlCl<sub>3</sub>. After 15 min of incubation at ambient temperature, the absorbance was measured at 435 nm using the multiwell plate reader. Three replicates were made for each test sample. For each sample, a blank was prepared by substituting AlCl<sub>3</sub> by the solution used to dissolve extracts. Quercetin was used as a standard for the calibration and a calibration curve (ranging from 0 to 50 µg mL<sup>-1</sup>) was generated, with the regression equation of  $Y = 0.035X + 0.026$  ( $R^2 = 0.999$ ), where X is the concentration of Quercetin in µg mL<sup>-1</sup> and Y is the absorbance reading at 435 nm. All experiments were performed in triplicate. The total flavonoid contents (average of the triplicate analysis) were expressed as Quercetin equivalent in milligrams per gram of lyophilized sample.

**Antioxidant activity index determination:** The Antioxidant Activity Index (AAI) was determinate according to the method described by Scherer and Godoy (2009). This method is based on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical test. Briefly, the scavenging activity of the extracts against DPPH was evaluated spectrophotometrically by a slightly modified method of Mensor *et al.* (2001) with adaptation on the 96-well plate.

Graded concentrations of extracts ranging from 0.781 to 100  $\mu\text{g mL}^{-1}$  obtained by two-fold dilutions were prepared and 100  $\mu\text{L}$  of each dilution were mixed with 100  $\mu\text{L}$  of DPPH (100  $\mu\text{g mL}^{-1}$  in ethanol). Samples were kept for 15 min at room temperature in the dark and then the absorbance was measured at 517 nm. Ascorbic acid (Vitamin C) and Butylated Hydroxyanisole (BHA) were used as references. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{RSA} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

where, RSA is the percentage of free radical scavenging activity, Abs(control) the absorbance of DPPH radical + ethanol and Abs(sample) the absorbance of DPPH radical + sample extract or standard. The  $\text{IC}_{50}$  (concentration providing 50% inhibition) of extracts and standards was determinate using regression curves in the linear range of concentrations. The antioxidant activity index (AAI) was then calculated as follows:

$$\text{AAI} = \frac{[\text{DPPH}] (\mu\text{g mL}^{-1})}{\text{IC}_{50} (\mu\text{g mL}^{-1})}$$

where, [DPPH] is the final concentration of DPPH.

### Antimicrobial assays

**Disc diffusion method:** The *in vitro* antibacterial activity of the extract was studied by the paper disc diffusion method of Bauer *et al.* (1966) as described previously (Zongo *et al.*, 2009). Bacteria grown on Mueller-Hinton Broth (MHB) (Liofilchem, Italy) at 37°C for 18 to 24 h were suspended in saline solution (0.9% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standard corresponding to  $10^8$  colony forming units (cfu)  $\text{mL}^{-1}$  (McFarland, 1907; Sittiwet and Puangpronpitag, 2009). Bacterial suspensions were used to inundate sterile Petri dishes (90 mm diameter) containing 15 mL Mueller-Hinton Agar (MHA) (Liofilchem, Italy). Plant extracts were dissolved in DMSO at 100  $\text{mg mL}^{-1}$  and filtered with a Millipore filter (0.22  $\mu\text{m}$ ) for sterilization. Ten microliters were pipetted to impregnate sterile paper discs (6 mm diameter). The discs were then placed onto the surface of inoculated Petri dishes. Gentamicin (10  $\mu\text{g}$ ) and Ciprofloxacin (5  $\mu\text{g}$ ) (Liofilchem, Italy) were used as the positive control. Paper discs soaked in DMSO without extract were used as negative control and DMSO didn't show inhibition effects to the growth microorganism. Petri dishes were incubated aerobically at 37°C for 18 to 24 h. The results were recorded by measuring the zones of growth inhibition surrounding the disc indicating the presence of

antimicrobial activity. All tests were performed in duplicate and the antibacterial activity was expressed as the mean of Diameters of Inhibition Zone (DIZ) produced.

**Broth microdilution assay:** A broth microdilution method was performed to determine the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) as recommended by NCCLS (2008). The inocula of the bacterial strains were prepared from 18 to 24 h broth cultures and suspensions were adjusted to 0.5 Mc Farland standard turbidity. The extracts were first dissolved in 10% DMSO and then sterilized as described previously. One hundred microliter of this stock extract solution were transferred into the first well of a 96-well sterile plate (Cellstar, Greiner Labortechnik, Germany) previously filed with 100  $\mu\text{L}$  of nutrient broth. Serial twofold dilutions were made in to 11 consecutive wells. Into each well, 95  $\mu\text{L}$  of nutrient broth and 5  $\mu\text{L}$  of the inocula were added to achieve concentrations of extracts ranging from 5 to 0.0049  $\text{mg mL}^{-1}$ . The final volume in each well was 200  $\mu\text{L}$ . On the same plate, some wells with specific medium and microorganism were used as control of the growth and other inoculated wells containing only 200  $\mu\text{L}$  of the media were used for sterility control. Each plate was mixed on a plate shaker at 300 rpm for 20 sec and then incubated at 37°C for 24 h. Bacterial growth was indicated by the presence of turbidity and a pellet on the well bottom. The MIC is the lowest extract concentration demonstrating no visible growth in the broth. The MBC which is the lowest concentration of the extract at which 99.99% or more of the initial inoculum was killed was determinate as described previously (Zongo *et al.*, 2009; Akomo *et al.*, 2009). One hundred microliter from each well demonstrating no visible growth were removed to spread onto Petri dishes filed with sterilized Plate Count Agar (PCA) (Liofilchem, Italy) medium. Petri dishes were incubated at 37°C for a total period of 48 h.

**Antifungal screening:** The antifungal assay was performed by disc diffusion method and microdilution technique (NCCLS, 2008; Singh *et al.*, 2008) as described above with minor modifications. A strain of *Candida albicans* was used for this test. Potato Dextrose Broth (PDB) and Potato Dextrose Agar (PDA) were used. The plates were incubated at 28°C for 48 to 96 h. Standard disc of Nystatin (100 IU) (Liofilchem, Italy) was used as positive control.

## RESULTS

The result of the phytochemical screening is presented in Table 1. This reveals presence of flavonoids

and saponins and absence of alkaloids in both extracts. Lieberman-Burchard's test was negative with water extracts and weakly positive with acetone extract indicating that terpenes and steroids are slightly present in hydro-acetone extract and absent in water extract. Likewise, cardiac glycosides have been detected in water extract but not in acetone extract.

Results of TPC and TFC determination by Folin-Ciocalteu method and aluminium chloride colorimetric assay method are summarised in Table 2. These results show that the TPC and TFC levels were higher in the acetone extract than in the water extract. The TPC and TFC amount in acetone extracts were, respectively 124.26 mgQE/100 g and 12.41 mgQE/100 g of lyophilized extract while in the water extracts, TPC amount was 74.25 mgQE/100 g and TFC 6.32 mgQE/100 g.

The free radical scavenging activity of the extracts by DPPH method shows that acetone extract is more efficient to scavenge DPPH free radicals than the water extract (Fig. 1). However, both extracts showed lower activity than BHA and vitamin C. The AAI of the extracts are shown in Table 3, these results show that acetone extract has strong antioxidant activity (AAI = 4.6) but this activity remain lower than activity of BHA and vitamin C (AAI = 7.30 and 10.66 respectively in this study). Water extract has a weak antioxidant activity (AAI = 0.21).

The results in Table 4 show the antimicrobial activity of the extracts of *A. grantii* against bacteria and fungal strains recorded by disc diffusion method. Our results show that both extracts exhibited weak to moderate activity against all microorganisms used in this study

Table 1: Phytochemical screening of acetone extract (AGae) and water extract (AGwe) of *A. grantii*

Chemical groups	Extracts	
	AGae	AGwe
Alkaloids	-	-
Flavonoids	+++	++
Saponins	++	+
Terpenes and steroids	+	-
Cardiac glycosides	-	+

-: Negative (not detected), +: Positive (detected)

Table 2: Total phenolic compounds (TPC) by Folin Ciocalteu's method and total flavonoids content (TFC) by AlCl<sub>3</sub> method

Extracts	TPC (mgGAE/100 g)	TFC (mgQE/100 g)
Agae	124.26	12.41
AGwe	74.25	6.32

Table 3: Antioxidant activity of *A. grantii* extracts by DPPH free radical scavenging method

Extracts	Equation	R <sup>2</sup>	IC <sub>50</sub> (µg mL <sup>-1</sup> )	AAI
Vit C	y = 10.36x+1.313	R <sup>2</sup> = 0.993	4.69	10.66
BHA	y = 5.615x+11.53	R <sup>2</sup> = 0.966	6.85	7.30
AGae	y = 4.759x+6.476	R <sup>2</sup> = 0.99	10.87	4.60
AGwe	y = 0.237x-1.147	R <sup>2</sup> = 0.996	215.81	0.21

excepted *Pseudomonas aeruginosa* which gave no inhibition with the water extract. The largest DIZ (15 mm) has been recorded with *Enterococcus faecalis* 10907 CIP followed by that obtained with *Bacillus subtilis* ATCC 21332 (13 mm). The MICs and MBCs values of extracts of *A. grantii* obtained with microdilution assay are shown in Table 5. The lowest MIC value (0.625 mg mL<sup>-1</sup>) was recorded with *E. faecalis* and *B. subtilis* confirming their susceptibility in disc diffusion method. MIC values ranged from 0.625 to 5 mg mL<sup>-1</sup>.

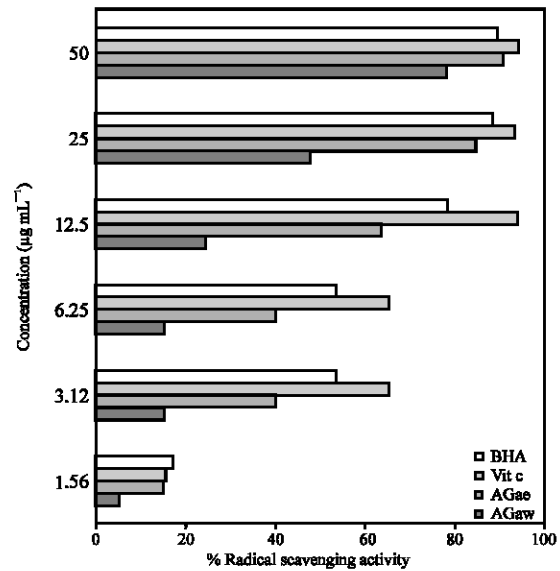


Fig. 1: DPPH radical scavenging activity of acetone extract (AGae) and water extract (AGwe) of *A. Grantii* compared with BHA and Vitamin C. percentage Radical Scavenging Activity = [abs(control)-abs(sample)]/ abs(control)] × 100

Table 4: Diameters (mm) of inhibition zone by disc diffusion assay

Bacteria	Diameters of inhibition zone (mm)				
	Extracts		Standards		
	AGae	AGwe	Cipro	Genta	Nyst
<i>Enterococcus faecalis</i> 10907 CIP	15	12	34	30	Nd
<i>Shigella dysenteriae</i> 5451 CIP	12	9	26	24	Nd
<i>Proteus mirabilis</i> ATCC 35659	10	9	28	Nd	Nd
<i>Salmonella enterica</i> CIP 105 150	12	10	34	28	Nd
<i>Enterobacter aerogenes</i> CIP 104 725	10	10	8	20	Nd
<i>Bacillus subtilis</i> ATCC 21 332	13	9	32	28	Nd
<i>Listeria monocytogenes</i> CRBIB 13 134	12	11	30	26	Nd
<i>Pseudomonas aeruginosa</i> ATCC 19249	10	0	30	20	Nd
<i>Staphylococcus aureus</i> ATCC 9144	12	12	29	29	Nd
Fungi					
<i>Candida albicans</i>	10	0	Nd	Nd	20

Nd: not determined, Cipro: Ciprofloxacin, Genta: Gentamicin, Nyst: Nystatin

Table 5: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or fungicidal concentration (MFC) in mg mL<sup>-1</sup> obtained by microdilution method

Bacteria	Extracts			
	Agae		Agwe	
	MIC	MBC	MIC	MBC
<i>Enterococcus faecalis</i> 10907 CIP	0.625	1.25	2.5	5
<i>Shigella dysenteriae</i> 5451 CIP	2.5	2.5	2.5	5
<i>Proteus mirabilis</i> ATCC 35659	2.5	5	5	>5
<i>Salmonella enterica</i> CIP 105 150	2.5	5	5	nd
<i>Enterobacter aerogenes</i> CIP 104 725	1.25	5	5	>5
<i>Bacillus subtilis</i> ATCC 21 332	0.625	1.25	2.5	5
<i>Listeria monocytogenes</i> CRBIB 13 134	1.25	1.25	2.5	5
<i>Pseudomonas aeruginosa</i> ATCC 19249	>5	>5	>5	>5
<i>Staphylococcus aureus</i> ATCC 9144	2.5	5	5	2.5
Fungi	MIC	MFC	MIC	MFC
<i>Candida albicans</i>	5	>5	>5	>5

## DISCUSSION

The indiscriminate use of commercial antimicrobial drugs has led to multiple drug resistant strains (Motamedi *et al.*, 2010). Beyond the increasing prevalence of drug resistance, some synthetic antibiotics are demonstrating undesirable side effects. This has highlighted the urgent need for new drugs particularly affordable for poorest countries (Raja *et al.*, 2010). In many parts of the world, the extracts of medicinal plants are used for their antibacterial, antifungal and antiviral properties (Hassawi and Kharma, 2006). *A. grantii* is a plant well known in traditional medicine for its properties. It has been reported that the family Vitaceae include 14 genera and 800 species described but only a few number of them contain no significant alkaloids. Like in this study, previous studies did not detect alkaloids with *Ampelocissus* extracts (Raffauf, 1996). Our results showed that polyphenolic compounds amount is higher in acetone extract than water extract. It has been reported that 70% aqueous acetone was more efficient to extract polyphenolic compounds compared to water and other solvent such as alcohol (Lamien-Meda *et al.*, 2008; Wang *et al.*, 2009). Phenolic compounds are generally more soluble in polar organic solvents than in water. However, there are contradictory reports in the literature regarding the best solvent for polyphenols extraction. Some studies have demonstrated that ethanol is the best solvent (Mohsen and Ammar, 2009). The presence of these compounds in water extract is interesting because water is a universal solvent and is generally used in traditional settings to prepare the plant decoctions for health remedies. It was observed that the extracts containing high levels of TPC were also potent DPPH radical scavenger. The acetone extract showed a strong antioxidant activity. An extract is considered to show poor antioxidant activity when AAI<0.5, moderate

antioxidant activity when AAI between 0.5 and 1.0, strong antioxidant activity when AAI between 1.0 and 2.0 and very strong when AAI>2.0 (Scherer and Godoy, 2009). The relationship between phenols content and antioxidant activity has been underlined in literature (Sthishkumar *et al.*, 2009; Hussein *et al.*, 2010). Apart from the antioxidant activity exhibited, acetone extract showed the better antimicrobial effect.

An antimicrobial activity can be considered when the diameter of inhibition zone (DIZ) observed is 9 mm or more around the paper disc (Kitzberger *et al.*, 2006). Generally, DIZ obtained with gram-positive bacteria are larger than those obtained with gram-negative bacteria. This is in phase with several previous studies which demonstrated that gram-positive bacteria are more sensitive to plant extracts and their components than gram-negative bacteria (Kelmanson *et al.*, 2000; Masika and Afolayane, 2000; Sahin *et al.*, 2002; Karaman *et al.*, 2003; Karou *et al.*, 2005; Masoodi *et al.*, 2008; Zongo *et al.*, 2009; Khanahmadi *et al.*, 2010). Indeed, the Gram-negative bacteria which are responsible for a large number of infectious diseases have a unique outer membrane that contains lipopolysaccharides which render them impermeable to certain antibacterial substances including antibiotics (Tortora *et al.*, 2001; Clements *et al.*, 2002). In some cases the MIC was equivalent to the MBC, indicating a bactericidal action of the extracts. Lower MIC and MBC value and higher zones of inhibition for acetone extracts connote higher solubility of phytoconstituents in the acetone extract compared to the water extract. Obtained results are in agreement with what was reported in literature concerning the biological activities of polyphenols. Indeed, there are several data in literature demonstrating the antibacterial, antifungal and antioxidant activity of these compounds (Haslam, 1996; Rice-Evans *et al.*, 1996; Yoshida *et al.*, 2000; Karou *et al.*, 2006; Fattouch *et al.*, 2007; Jimoh *et al.*, 2008; Mohsen and Ammar, 2009). However it is important to notice that over compounds such as alkaloids have strong antimicrobial activities (Karou *et al.*, 2005; Zongo *et al.*, 2009). What is important with plant extract is the fact that antibiotic resistance does not interfere with the antibacterial action of plant extracts. Plant extracts with antimicrobial activities are generally active on multi-drug resistant human pathogens (Oskay *et al.*, 2009).

## CONCLUSION

This study justifies the uses of this plant to treat several infectious diseases and cancers in folk medicine. Investigations may continue in order to know the limit of toxicity of this plant and its *in vivo* potencies. The studies

can lead to new active structures or lead to Enhanced Traditional Medicines (ETM) which are cheaper and disposable for population in developing countries.

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