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Full Length Research Paper

Evaluation of antioxidant and antibacterial properties of six Sapindaceae members

Margaret O. Sofidiya[1]*, Florence O. Jimoh[2], Adamu A. Aliero[3], Anthony J. Afolayan[2], Olukemi A. Odukoya[1] and Oluwole B. Familoni[4]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Lagos State, Nigeria.
[2]Department of Botany, University of Fort Hare, Alice 5700, South Africa.
[3]Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria.
[4]Department of Chemistry, Faculty of Science, University of Lagos, Lagos State, Nigeria.

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Free radical scavenging and antibacterial activities were determined for six Sapindaceae members namely: *Allophylus africanus*, *Cardiospermum grandiflorum, Blighia sapida*, *Blighia unijugata, Deinbollia pinnata* and *Zanha golungensis* used for the treatment of wounds, inflammation and infectious diseases in South Western Nigeria. The antioxidant activities were determined by 2,2′-azinobis-3- ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and FRAP assays. Five of the plant species produced above 70% inhibition of ABTS radicals at 0.02 mg/ml. A concentration dependent inhibition of DPPH radicals ranging between 33.8 and 99.2% at 0.1 mg/ml was observed. The ferric reducing capacity was comparable with that of butylated hydroxytoluene (BHT) and ascorbic acid. Positive correlation (R^2 = 0.9359) was found between total phenolics content and DPPH antioxidant activity of the plant extracts. In the antibacterial study, the plant extracts were more active against Gram-positive than Gram-negative bacteria. The most susceptible bacterium was *B. cereus.* Extracts from *B. sapida* and *B. unijugata* did not show any activity on the tested organisms even at 2.0 mg/ml. None of the extracts exhibited activity against *Serratia marcescens, Pseudomonas aeruginosa* and *Klebsiella pneumonae.* The strongest antibacterial activity was found in *A. africanus* and *C. grandiflorum* extracts. Among the six Sapindaceae species, *A. africanus* had the best antioxidant and antibacterial activities.

Key words: Antioxidant, antibacterial, Sapindaceae, polyphenolic contents.

INTRODUCTION

The world health organization (1985) estimates that, 80% of the people living in developing countries almost exclusively use traditional medicine for their primary health care needs. However, the effectiveness of the majority of the herbal remedies that are used today is yet to be validated. Limited knowledge, as well as lack of scientific studies on the practices of local herbalists has led to the neglect of potentially valuable drug containing plants (Sofowora, 1980). In recent time, the screening of plant extracts and plant products has demonstrated that higher plants represent a potential source of novel drug prototypes (Maurer-Grimes et al., 1996; Rabe and van Staden, 1997; Afolayan, 2003).

The family Sapindaceae has a wide spread distribution with 136 genera and 2000 species (Urdampilleta et al., 2005). Ethnomedical information revealed that extracts from members of this family are commonly used for the treatment of boils, ulcers, pain dematological troubles, wounding healing, diarrhea and dysentery (Burkill, 2000; Iwu, 1993; Sofidiya et al., 2007). Many species in this family have been reported to possess a number of biological and pharmacological activities (Henman, 1982; Belliardo et al., 1985; Bempong and Houghton, 1992; Gill, 1992; Espinola et al., 1997; Basile et al., 2005). For

*Corresponding author. E-mail: toyin_sofidiya@yahoo.co.uk. Tel: +234 803 3356 197.

Abbreviations: DPPH, 1,1-Diphenyl-2-picrylhydrazyl; **BHT,** butylated hydroxytoluene; **ABTS,** 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid.

example, extracts from the leaves, stems and roots of *Paullinia pinnata* showed significant antibacterial and antioxidant activities (Kofi and Houghton, 2004; Jimoh et al., 2007), while the roots and seeds of *Lecaniodiscus cupanoides* were reported to be cytotoxic but possess analgesic and CNS depressant activity (Adeyemi and Yemitan, 2004; Adeyemi et al., 2005; Yemitan and Adeyemi, 2005).

Despite the use of extracts of Sapindaceae *species* in Nigerian folk medicine, information on the comparative antioxidant and antibacterial properties of these species are unavailable. This type of information is necessary in the current search for pharmacologically active natural product of plant sources. In this paper, we reported the antioxidant and antibacterial activity of crude extracts from six species of the Sapindaceae family. The choice of these species was based on ethnobotanical information from literature on their uses for the treatment of various ailments such as yaws and ulcers, pain, dermatological troubles and wound healings, disease conditions, in which free radicals have been implicated.

MATERIALS AND METHODS

Collection and extraction of plant materials

The leaves of *Allophylus africanus* Beauv, *Blighia sapida* Koenig, *Blighia unijugata* Baker, *Deinbollia pinnata* Schum and Thonn, and *Zanha golungensis* Hiern and whole plant of *Cardiospermum grandiflorum* Swartz were collected from their natural populations in Ibadan (7° 23' 16'' N; 3° 53' 47'' E) and were identified by Mr. B.O. Daramola of the herbarium unit of Botany Department, University of Lagos, Nigeria. The identities of the plants were confirmed by comparing the voucher specimens of each plant with the herbarium specimens. Voucher numbers and other valuable information on the selected plants are found on Table 1. Portion of air dried leaves (50 g) from each species were extracted in methanol for 24 h and the extracts were filtered with Whatman no. 1 filter paper and concentrated to dryness at 40°C using a rotary evaporator. The extracts were re-dissolved in the same solvent to the required concentrations for the antioxidant and antibacterial studies.

Chemicals used for experimentation

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid (ABTS), potassium ferricyanide; catechin, butylated hydroxytoluene (BHT), ascorbic acid, catechin, tannic acid, quercetin and $FeCl_3$ were purchased from Sigma chemical Co. (St. Louis, MO, USA); vanillin from BDH; Folin-Ciocalteus's phenol reagent and sodium carbonate were from Merck Chemical Supplies (Damstadt, Germany). All the other chemicals used including the solvents, were of analytical grade.

Determination of total phenolics

Total phenol contents in the extracts were determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using the Hewlett Packard UV-VS spectrophotometer. Samples of extract were evaluated at a final concentration of 0.1 mg/ml. Total phenolic content were expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $R^2 = 0.9365$, where x was the absorbance and y was the tannic acid equivalent (mg/g).

Determination of total flavonoids

Total flavonoids were estimated using the method of Ordonez et al. (2006). To 0.5 ml of sample, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. After 60 min at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid contents were calculated as quercetin (mg/g) using the following equation based on the calibration curve: $y = 0.0255x$, $R^2 = 0.9812$, where x was the absorbance and y was the quercetin equivalent (mg/g).

Determination of total proanthocyanidins

Determination of proanthocyanidin was based on the procedure reported by Sun et al. (1998). A volume of 0.5 ml of 0.1 mg/ml of extract solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid; the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm. Extracted samples were evaluated at a final concentration of 0.1 mg/ml. Total proanthocyanidin contents were expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve: $y = 0.5825x$, $R^2 = 0.9277$, where x was the absorbance and y was the catechin equivalent (mg/g).

Determination of antioxidant activity

ABTS radical scavenging assay

For ABTS assay, the method of Re et al. (1999) was adopted. The stock solutions included 7 mM $ABTS^{\cdot +}$ solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml $ABTS^{\cdot +}$ solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh $ABTS^{\cdot +}$ solution was prepared for each assay. Plant extracts (1 ml) were allowed to react with 1 ml of the $ABTS^{\cdot +}$ solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The $ABTS^{\cdot +}$ scavenging capacity of the extract was compared with that of BHT and percentage inhibition calculated as:

ABTS radical scavenging activity (%) = $[(Abs_{control} - Abssample)]/(Abs_{control})] \times 100$

Where, $Abs_{control}$ was the absorbance of ABTS radical + methanol, and Abs_{sample} was the absorbance of ABTS radical + sample extract /standard.

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

The effect of extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02 to 0.1

Table 1. Ethnobotanical information of the selected plant species.

Botanical name	Local name	Part used	Ethnobotanical information[b]	Voucher no.
Allophylus africanus P. Beauv	Akanro, Akaraesu	Bark, root and leaves	Arthritis, analgesic, epilepsy, sedative, induction of labour, hernia, cardiac pains	FHI 89322
Cardiospermum grandiflorum Swart	Ako-ejirin	Whole plant	Dermatological troubles, sores and swellings, scabies	FHI 88315
Blighia unijugata Baker	Akoko-isin	Roots, pods and leaves	Analgesic, sedative, Antihelmintic, amenorrhoea, fever, giddiness	FHI 31544
Blighia sapida Konig	Isin	Stem barks and leaves	Oedema and intercostals pains, yaws and ulcers	LUH 356
Deinbollia pinnata Schum and Thonn	Oju agbigbo	Leaves, roots	Analgesic, jaundice	LUH 2599
Zanha golungensis Hiern	Nago gorii raya	Leaves	Analgesic, broken limbs, wounds and sores, chest complaints, malaria, headache, catarrh	FHI 93419

[b]Burkill (2000), Iwu (1993) and Sofidiya et al. (2007).

mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid and BHT were used as references. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$, where $Abs_{control}$ was the absorbance of DPPH radical + methanol; Abs_{sample} was the absorbance of DPPH radical + sample extract /standard.

Total antioxidant activity (FRAP assay)

A modified method of Benzie and Strain (1996) was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37°C before use. Plant extracts (150 µl) were allowed to react with 2.85 ml of the FRAP solution for 30 min in the dark. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results were expressed in µM Fe (II)/g dry mass and compared with that of BHT, ascorbic acid and catechin.

Antibacterial assay

Laboratory isolates of 10 bacteria species which included five Gram positive and five Gram negative strains were obtained from the Department of Microbiology, Rhodes University. They were *Bacillus cereus*, *Staphylococcus epidermidus*, *Staphylococcus aureus*, *Micrococcus kristinae*, *Streptococcus pyrogens*, *Escherichia coli*, *Salmonella pooni*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Klebsiella pneumonae.* Each organism was maintained on nutrient agar slants and was recovered by sub-culturing in nutrient broth (Biolab No. 2) for 24 h. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth.

The bacteria were streaked in radial patterns on the agar plates and incubated at 37°C and examined after 24 and 48 h. Complete suppression of growth by a specific concentration of an extract was required to be declared active. Each extract was tested at 2.0, 1.0, 0.5, and 0.1 mg/ml. Blank plates containing only nutrient agar and another set containing nutrient and 2% methanol served as control (Meyer and Afolayan, 1995).

Statistical analyses

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Duncan's Multiple Range test using the statistical analysis system (SAS, 1999) program. *P* values < 0.05 were regarded as significant and *P* values < 0.01 as very significant. Correlation between polyphenol contents and antioxidant activity was established by regression analysis.

RESULTS AND DISCUSSION

Total phenolics, flavonoids and proanthocyanidin contents

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators (Agrawal, 1989). The total content of phenolic compounds (mg/g tannic acid equivalent) varied between 6.3 and 21.0 in the plant species investigated (Table 2). The highest amount of phenolic was found in the extract

Table 2. Polyphenol contents and antioxidant activity of six members of the Sapindaceae family.

Plant	Total polyphenol (mg tannic acid/g)	Total flavonoids (mg quercetin/g)	Total proanthocyanidins (mg catechin/g)	FRAP (µmol Fe II/g)
A. africanus	21.046 ± 1.37[a]	6.670 ± 0.35[a]	7.679 ± 0.16[a]	102.899 ± 0.56[a]
B. unijugata	11.360 ± 0.39[b]	7.062 ± 0.09[a]	5.966 ± 0.08[b]	40.366 ± 5.54[b]
B. sapida	10.473 ± 2.82[b]	8.876 ± 0.05[a]	6.116 ± 0.24[b]	23.141 ± 2.61[c]
C. grandiflorum	6.317 ± 0.36[c]	4.015 ± 0.09[b]	3.103 ± 0.53[c]	3.407 ± 1.77[d]
D. pinnata	9.874 ± 0.28[b]	8.071 ± 0.76[a]	4.197 ± 0.26[d]	44.709 ± 7.68[b]
Z. golungensis	11.674 ± 0.97[b]	5.787 ± 0.05[c]	6.453 ± 0.18[b]	47.181 ± 9.52[b]
Ascorbic acid	-	-	-	1626.54 ± 7.76[e]
BHT	-	-	-	62.35 ± 3.01[f]
Catechin	-	-	-	971.55 ± 1.07[g]

n = 3, X ± SD. Means along the same column with different superscripts are significantly different at P < 0.05. Values are expressed per g of plant dry weight.

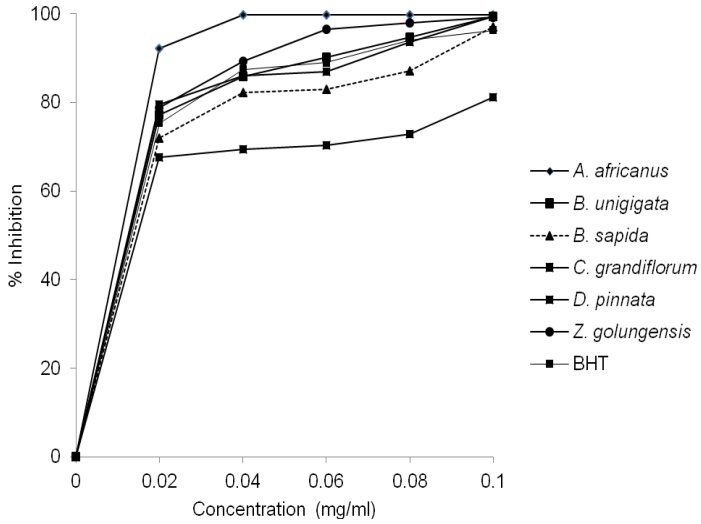

Figure 1. ABTS radical scavenging activities of methanol extracts of six members of the Sapindaceae family.

of *A. africanus* while *C. grandiflorum had* the least. Although considerable variability in the content of all polyphenolic classes within these species was observed, the differences between *B. unijugata*, *B. sapida, D. pinnata* and *Z. golungensis* were not statistically significant. However, the total phenolics in these plants were higher than that of previously studied medicinal and aromatic plants such as *Salvia officinalis*, *Salvia pratensis, Juglans regia, Echinacea purpurea* and *Thymus froelichianus* (Milliauskas et al., 2004; Vitalini et al., 2006). It has been reported that the phenolic content of plants are influenced by a number of intrinsic (genus, species, cultivar) and extrinsic (agronomic, environmental, handling and storage) factors (Thomas-Barberan and Espin, 2001).

The extracts contained lower total flavonoid and proanthocyanidin contents than the total phenolics. The content of flavonoids varied from 4.0 to 8.9 mg quercetin/g of the plants dry weight. The highest amount of flavonoids was found in the extract of *B. sapida* and was not significantly different from that of *A. africanus*, *B. unijugata* and *D. pinnata* while *C. grandiflorum* and *Z. golungensis* contained significantly lower amounts. The concentrations of proanthocyanidins varied considerably amongst the different plant species and range between 3.1and 7.6 mg catechin/g, with the highest in *A. africanus* and the least in *C. grandiflorum* (Table 2).

Antioxidant activity

ABTS radical scavenging activity

The plant species namely; *A. africanus*, *B. sapida, B. unijugata C. grandiflorum*, *D. pinnata,* and *Z. golungensis* showed varying inhibition of ABTS absorption (Figure 1). At 0.02 mg/ml, the interaction of the plant extracts with ABTS radical gave between 62.7 to 92.2% inhibitions. The strongest was detected in *A. africanus* while *C. grandiflorum* had the least activity. It was also observed that very low concentrations of the plant extracts resulted in significant scavenging of the ABTS radicals. This percentage inhibitions did not however change significantly up till 0.1 mg/ml, which was the highest concentration used in this study.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH scavenging activity of the extracts, BHT and ascorbic acid is shown in Figure 2. The results showed a concentration- dependent scavenging activity of the

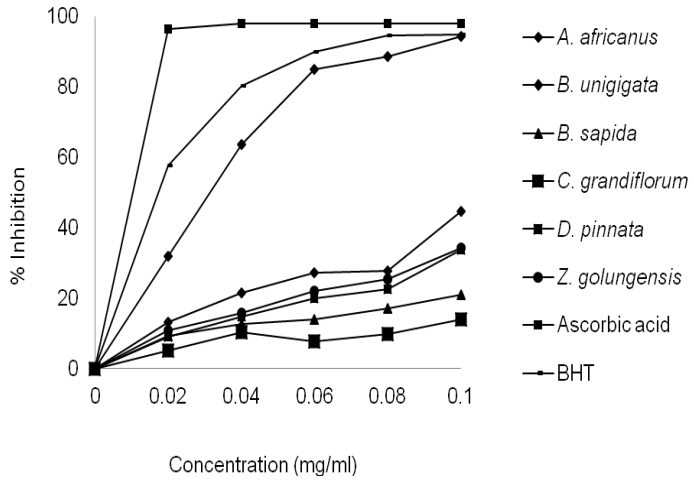

Figure 2. DPPH radical scavenging activities of methanol extracts of six members of the Sapindaceae family.

DPPH radical which varied significantly amongst the species. At 0.1 mg/ml, the DPPH radical scavenging activity of the extracts decreased in order of *A. africanus*> *B. unijugata* > *Z. golungensis*> *D. pinnata*> *B. sapida*> *C. grandiflorum*. The % inhibition values for *A. africanus* and BHT at the same concentration were similar, with 94.4 and 94.8, respectively. Other species studied exhibited lower activity than that of ascorbic acid and BHT.

Total antioxidant power (FRAP)

The antioxidant potential of the different plant species were therefore further examined by their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) and compared with standard antioxidants such as BHT, ascorbic acid and catechin. *A. africanus* had the highest reducing ability while *C. grandifolium* had the least (Table 2). The reducing ability of *A. africanus* was significantly higher than that of BHT while the reducing ability of the other plant species was slightly below that of BHT. Generally, the activity exhibited by catechin and ascorbic acid were higher than all the extracts.

Numerous methods have been proposed for the evaluation of the antioxidant activity of a test compound based on free radical chain breaking, metal chelation and reducing capacity amongst others (Amarowic et al., 2000). One of the mechanisms involved in antioxidant activity is the ability of a molecule to donate a hydrogen atom to a radical and the propensity of hydrogen donation is the critical factor that involves free radical scavenging (Hu et al., 2000). It is possible that, the ABTS and DPPH radical scavenging activities exhibited by these plant species may be due to their hydrogen donating abilities.

Comparing the results of the two radical scavenging tests, the activity of the extracts in ABTS test was found to be higher than in the DPPH test. Factors like stereo selectivity of the radicals or the solubility of the extracts in different testing systems have been reported to affect the capacity of the extracts to react and quench different radicals (Yu et al., 2002). Similarly, Wang et al. (1998) reported that some compounds which gave $ABTS^{\cdot+}$ scavenging activity did not show DPPH scavenging ability; suggesting that the kinetics of radical scavenging reactions in these two systems differ. In addition, by simple regression analysis, total phenolic content positively correlated with DPPH radical scavenging activity of these species (R^2 = 0.9359), suggesting phenolic constituents played a major role in the scavenging activity of these plants. Weak correlation (R^2 = 0.3473) was observed for their ABTS radical scavenging activity. This probably implies that non-phenolic compounds may also be responsible for the observed antioxidant activity of these plants (Yam et al., 2008).

It has been reported that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Mier et al., 1995). The correlation analysis showed a strong relationship between total reducing content as measured by FRAP and the phenolic content (R = 0.9343), suggesting that phenolic compounds in the plant species may determine the ferric ion reducing antioxidant power of the extracts.

Antibacterial activity

The results of antibacterial activity of methanolic extracts from six sapindaceae plant species are presented in Table 3. All plant extracts showed activity against at least one microorganism except *B. sapida* and *B. unijugata*. The strongest inhibitory activity was found in *A. africanus* and *C. grandiflorum* extracts. Generally, the activity of the extracts was found to be higher on Gram-positive bacteria than the Gram-negative strains. The observed low activity of the extracts against the Gram negative bacteria is in agreement with other studies (Rabe and Van Staden, 1997; Grierson and Afolayan, 1999; Velazquez et al., 2007; Ruiz-Bustos et al., 2009). Moreover, according to Suffredini et al. (2006), Gram-negative bacteria are hardly susceptible to plant extracts in doses as low as 200 mg/ml.

B. cereus was the most sensitive bacterium to the extracts with 50% of plant extracts showing in vitro activity with MIC ranging from 0.1 to 1.0 mg/ml. The inactivity of the six extracts against *S. marcescens, K. pneumonae* and *P. aeruginosa* was particularly noteworthy. The lack of sensitivity of these extracts to these organisms, and in particular, *P. aeruginosa* could be attributed to the fact that the bacterium is naturally resistant to many antibiotics due to the permeability barrier offered by its outer membranes (Wagate et al., 2010). Further studies need to be conducted to elucidate the molecular and chemical basis of bacterial

Table 3. Antibacterial activity of six members of the Sapindaceae family.

Bacteria	Minimum inhibitory concentration (mg/ml)								
	Gram +/-	Aa	Bs	Bu	Cg	Dp	Zg	Str	Chl.
Bacillus cereus	+	0.1 [a]	-[c]	-	1.0	-	1.0	1.0	0.13
Staphylococcus epidermidus	+	-	-	-	-	-	-	1.0	0.25
Staphylococcus aureus	+	0.5	-	-	2.0[b]	-	-	1.0	1.0
Micrococcus kristinae	+	0.5	-	-	2.0	2.0	-	0.5	0.5
Streptococcus pyrogens	+	1.0	-	-	1.0	-	-	1.0	1.0
Escherichia coli	-	-	-	-	-	2.0	-	>1.0	1.0
Salmonella pooni	-	0.5	-	-	2.0	-	-	1.0	0.13
Serratia marcescens	-	-	-	-	-	-	-	1.0	0.13
Pseudomonas aeruginosa	-	-	-	-	-	-	-	<2.0	<2.0
Klebsiella pneumonae	-	-	-	-	-	-	-	>1.0	0.63

[a], minimum; [b], Maximum concentration of extract tested; [c], not active at 2 mg ml^{-1}. Aa- *A. africanus;* Bs- *B. sapida*; Bu- *B. unijugata; Cg- C. grandiflorum; Dp- D. pinnata*; Zg- *Z. golungensis;* Str: streptomycin; Chl: chloramphenicol.

susceptibility to these plant extracts.

Among the six Sapindaceae species investigated in this study, *A. africanus* had the best antioxidant and antibacterial activities. There is a report about the antioxidant and antibacterial properties of other Allophylus species (*A. rubifolius*) (Marwah et al., 2007). The plant was reported to show very good DPPH scavenging activity with % inhibition values >70% at an effective test concentration of 50 µg/ml. It is well known that plant species from same genus contain similar chemical constituents; therefore, they could show comparable biological activities (Ruiz-Bustos et al., 2009). However, the chemical and biological characterizations of *A. africanus* extract will provide a better understanding of its effects. The results of this study indicated the potential of *A. africanus* among the other species investigated in this study, for the treatment of bacterial infections as well as free radical implicated diseases and strongly support the beneficial use of its extracts in folk medicine. *In vivo* studies are warranted to investigate *A. africanus* as an antioxidant in various oxidative complications.

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