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Full Length Research Paper

Antifungal, antibacterial and antioxidant properties of *Adansonia digitata* and *Vitex doniana* from Bénin pharmacopeia

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The dichloromethane, methanol and hydroethanolic extracts from leaves of *Vitex doniana* and stem bark of *Adansonia digitata* have been investigated for their antibacterial, antifungal, antioxidant activities and toxicity. The antifungal activity has been investigated against six species of *Aspergillus* by measuring the mycelial and sporulation inhibitions. The antimicrobial activity was investigated against six Gram positive and Gram negative strains using the microplate dilution method. The phytochemical study was performed on thin layer chromatography and antioxidant activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging test. The sporulation inhibition percentage was 70% while mycelial inhibition was less than 40%. The minimum inhibitory concentration ranged from 0.039 to 2.5 mg/ml. In DPPH radical scavenging assay, the effect on reducing free radicals increased in a dose dependent manner. Three extracts out of six were very active compared to quercetol, with inhibition percentage ranging from 77.39 to 86.55%. The results supported the utilization of these plants in infectious diseases and also showed these plants as good sources for antioxidants.

Key words: Antifungal, antibacterial, antioxidant, *Adansonia digitata*, *Vitex doniana*.

INTRODUCTION

Diseases due to pathogenic bacteria and fungi represent a critical problem to human health and they are one of the main causes of morbidity and mortality worldwide (WHO, 1998). The emergence of pathogens resistant to antibiotics as a result of their excessive use in clinical and veterinary applications represents a serious public health concern (Keymanesh et al., 2009). In the last three decades, pathogenic resistant bacteria caused major health problems throughout the world although the pharmacological industries produced quantities of antibiotics. Unfortunately, the resistance of bacteria and fungi to these drugs is increasingly important. The search for plants with antibacterial and antifungal activity has gained increasing importance in recent years due to the development of resistance.

On the other hand, it is known that free radicals play a fundamental role in several diseases. The biochemical damages caused by free radicals to cells and tissues, lead to the development of diseases such as arterio-sclerosis, high blood pressure, cancer, inflammation, renal failure, liver disease (Gupta et al., 2007). Medicinal plants are largely used either for the prevention, or for the curative treatment of several diseases. Among the properties behind these virtues, the antioxidant activity holds the first place (Trichopoulou et al., 2007; Rahman, 2008). Plants used in traditional medicine may constitute an important source of new biologically active com-pounds. It is estimated that there are about 2, 500 000 species of higher plants and the majority of these plants have not been studied for their pharmacological activities (Ram et al., 2004). According to World Health

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Organization (WHO), 80% of the world population still relies mainly on substances extract from plants to cure diseases (Kumara et al., 2001).

Vitex doniana, (Verbenaceae) is a tree of 10 to 15 m (32 to 49 feet) in height, commonly called *Fon*, or *Ewe oyi* by traditional healers and plants sellers in Bénin. The leaves consist of 5 sepals. Flowers are white and sometimes stained with purple. Fruits are stone fruits green - dark then black when mature (looking like black olives). *V. doniana* is used by traditional healers alone or in a combination with stem bark of *Adansonia digitata* to treat diarrhea, leprosy and dysentery (Adjanohoun et al., 1989). The leaves are used as antiseptic and anti-diabetic (Muanda et al., 2011). The aerial parts are used in Mali as diuretic, tonifiant, aphrodisiac and bactericide (Ouattara, 2005; Goetz, 2006). *A. digitata* (Bombacaceae) is called *Kpassa*, *Zuntin*, *Oshe* or *Bobodi* in Bénin (Adjanohoun et al., 1989). This plant is traditionally used to treat diarrhea and infectious diseases (Kubmarawa et al., 2007). The fibers of the pod are used as decoction to treat amenorrhea. They can be used as a febrifuge, anti-dysenteric and in the treatment of smallpox and measles (Shahat, 2006; Doughari, 2006). The bark of the trunk is used in the treatment of malaria and also used to bath babies to encourage a smooth skin (Adjanohoun et al., 1989; Kristensen and Lykke, 2003).

The aim of the study is to investigate the phytochemical constituents, the antifungal and antibacterial activities using dichloromethane, methanol and hydroethanolic extracts of leaves of *V. doniana* and stem bark of *A. digitata*, against six species of *Aspergillus* and seven bacteria strains. The antioxidant activity was also performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Our results would strengthen the traditional use of these plants and contribute to the acceptance of traditional medicine.

MATERIALS AND METHODS

The leaves of *V. doniana* and stem bark of *A. digitata* were collected in September 2010 from Ouidah, in the Department of Atlantic, the Southern Commune of Bénin. The collection and identification were carried out by botanists from the University of Abomey-calavi. After identification, the parts collected for each plant were dried for two weeks in laboratory (22°C) and ground to a fine powder using an electric grinder (Excella mixer grinder).

Extraction of plants material

Air dried plant material of each species (100 g) was extracted with dichloromethane (400 ml) at room temperature and after 24 h, filtered through Whatman No. 1 paper. The residue was extracted again twice for 2 h in the same solvent. The extracts were pooled together. After drying under hood extractor, the same plant residue was then extracted with methanol (400 ml) using the same procedure. In brief, 50 g of each plant material were also extracted three times with 20% ethanol for 2 h. The filtrates of each extraction were desiccated under vacuum and the residues were stored in a freezer until use for biological assays.

Phytochemical study

Phytochemical analysis for major constituents was achieved on thin layer chromatography (TLC) plate (Alugram, Silicagel 60 F_{254}) using standard procedures (Wagner and Blat, 2001; Bruneton, 2009). The tests for alkaloids, anthracene derivatives, flavonoids, coumarins, lignans, naphthoquinones, saponins, essential oils, terpenes glycosides, pigments, triterpene and tannins were carried out. Dragendorff, Meyer and iodoplatinate reagents were used for alkaloids detection. Air dried plant material (1 g) is mixed thoroughly with 1 ml 10% ammonia solution and then extracted for 10 min with 5 ml methanol in water bath (50°C). The mixture was filtered through hydrophilic cotton and then concentrated under vacuum to about 1 ml. A volume of 10 µL was used for TLC; chromatographic solvents: chloroform/methanol/ammonia 10% (80:40:15). Alkaloids appear as brown or orange with Dragendorff reagent and blue or brown with iodoplatinate reagent.

The test of anthracene derivatives was also carried out with the filtrate obtained by extracting 0.5 g of powdered plants for 5 min on a water bath (40°C) with 5 ml methanol. A total of 10 µL of the filtrate was used for TLC; chromatographic solvents: ethyl acetate/ methanol/water (100:13.5:10). After spraying of 10% ethanolic potassium hydroxide (KOH), anthracene derivatives appeared as red or yellow spots under UV 365 nm. Intense yellow is also obtained under UV 365 nm with Natural product/ Polyethylene glycol (NP/PEG). Coumarin was identified by extracting 1 g sample with 10 ml methanol for 30 min on the water bath (50°C). The filtrate is evaporated to about 1 ml and 20 µL is used for TLC investigation; chromatographic solvents: ethyl acetate/formic acid/acetic acid/water (100:11:11:26). Coumarin fluoresce pale blue or yellow-brown with 10% ethanolic KOH and blue or blue-green with NP/PEG under UV 365 nm.

Essential oils were detected by dissolving 1 g of powdered plant in 10 ml of dichloromethane for 15 min. The suspension was filtered and the filtrate evaporated to dryness. The residue is dissolved in 1 ml toluene and 20 µL is used for TLC; chromatographic solvents: chloroform/hexane (75:25). Essential oils showed strong blue, green, red and brown coloration after spraying vanillin-sulphuric acid (VS) end heating for 5 min at 105°C. Natural product reagent (NP/PEG) was used for the identification of flavonoids. Briefly, 1 g of powdered plant was extracted with 10 ml of methanol for 5 min in a water bath at 60°C and then filtered. Subsequently, 20 µL of filtrate was used for TLC; chromatographic solvents: ethyl acetate/ formic acid/acetic acid/water (100:11:11:26). Flavonoids appear as orange, yellow or yellow-green zones in visible.

For lignans, 1 g of each powdered plant was extracted with 10 ml methanol by heating on water bath (60°C) for 10 min. The filtrate was concentrate to 3 ml following evaporation and 20 µL was used for TLC; chromatographic solvents: chloroform/methanol/water (70:30:4). For pigments, 1 g of powdered plant was also extracted by shaking for 15 min with 6 ml of a mixture of methanol and 25% HCl (9:1). Overall, 20 µL of the filtrate was used for TLC investigation; chromatographic solvents: ethyl acetate/formic acid/acetic acid/water (100:11:11:26). After spraying vanillin-sulphuric acid and heating (5 min/105°C), lignans show red or brown zones while pigments appears red-violet or blue. Additionally, Liebermann-Burchard reagent (LB) was used for terpene glycosides test. In brief, 1 g of each sample was extracted for 15 min with 15 ml methanol on water bath (60°C). Afterward, 20 µL of filtrate was used for TLC; chromatographic solvents: chloroform/methanol/water (65:25:4). The plate was heated for 5 min at 105°C. Grey or red-brown spots indicated the presence of terpene glycosides. Furthermore, 1 g of powdered material was extracted with 10 ml methanol for 15 min on a water bath (60°C). 20 µl of filtrate is used for TLC and toluene/chloroform/ethanol (40:40:10) for chromatographic solvents. Blue, violet or red color indicated the presence of triterpenes after spraying anisaldehyde sulfuric acid and heating for 5 min at 105°C.

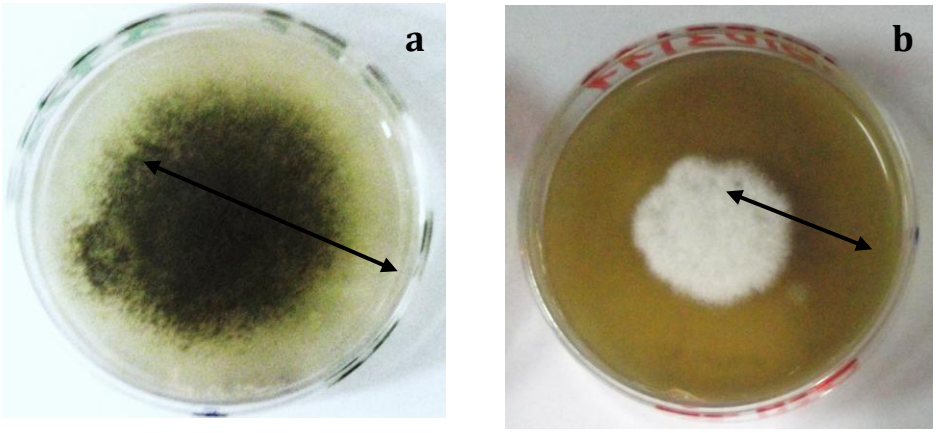

Figure 1. Mycelia development after 5 days in control (potato dextrose agar) and test dish (potato dextrose agar + extract). a: Petri dish with PDA (control); b: Petri dish with PDA + extract (1 mg/ml).

The detection of naphthoquinones was carried out by extracting 1 g of each sample with 10 ml methanol for 10 min on a water bath (50°C). Next, 30 µL of the clear filtrate was used for TLC; chromatographic solvents: toluene/formic acid (99:1). After spraying the plate with 10% methanolic KOH reagent, naphthoquinones show red to red-brown color. For saponin identification, 2 g was extracted by heating for 10 min on water bath (60°C) with 10 ml 70% ethanol. The filtrate is concentrated to 3 ml following evaporation, and 20 µL was used for TLC; chromatographic solvents: chloroform/acetic acid/methanol/water (64:32:12:8). Blue, violet or yellow-brown spots were observed after spraying vanillin-sulphuric acid (vis). Vanillin-hydrochloric acid reagent was used for tannins detection. A quantity of 10 g of each powdered sample was extracted twice with 50 ml methanol for 20 min on a water bath (45°C). The filtrate was taken to dryness under vacuum. Next, 20µL of the extract at 10 mg/ml was used for TLC; ethyl acetate/methanol/water (100:17:13). After heating (5 min, 105°C), tannins appear red color.

Antifungal assay

Six fungi species, *Aspergillus flavus* CMBB75, *A. parasiticus* CMBB20, *A. ochraceus* CMBB91, *A. nidulans* CMBB90, *A. terreus* CMBB94 and *A. fumigatus* CMBB89, were obtained from the Laboratory of Biochemistry and Molecular Biology of the University of Abomey-calavi. These are the most common disease-causing fungi of vegetables, animals and humans. Each fungus was cultured on potato dextrose agar medium (PDA). Antifungal activity was evaluated on mycelia development and sporulation stages as described by Dohou et al. (2004). The mixture of sterilized potato dextrose agar (10 ml) and 10 g of each extract were poured in sterile disposable petri dishes. After solidification, 100 spores prepared in Tween 25% was deposited on the PDA and the petri dishes were left at 25°C for 5 days. Each assay was run in triplicate. Two petri dishes without extract were used as controls (Figure 1). The inhibition percentage (PI) of extracts was determined according to the formula below:

$$PI\ (\%) = \frac{A - B}{A} \times 100$$

Where PI is the inhibition percentage; A is the average diameter of the mycelia or estimated number of spores of control; B is the average diameter of the mycelia or estimated number of spores of tested dishes.

Antibacterial activity

The used bacteria organisms for antibacterial activity were *Escherichia coli* CIP 53126, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* CIP82118, *Salmonella abony* CIP 8039, *Staphylococcus aureus* Methicillin Resistant (SARM) and *Staphylococcus epidermidis* obtained from Laboratoire de Biophotonique et Pharmacologie, University of Strasbourg, France.

Growth inhibition effect of extracts at 10 mg/ml

The aim of this method was to eliminate the extracts, which at 10 mg/ml do not inhibit the growth of bacteria (Eloff, 1998). The extracts were reconstituted to a concentration of 20 mg/ml in acetone/Muller Hinton broth culture. A volume of 100 µL of each extract (20 mg/ml) was introduced in triplicate microplate already seeded with 100 µL of the Muller Hinton broth culture inoculums (10^6 CFU/ml) of the tested bacteria. The microplate was incubated at 37°C. After 18 h of incubation, 40 µL of 0.2 mg/ml solution of *p*-iodonitrotetrazolium (Sigma Aldrich) were added to each well and microplate was incubated at 37°C. Finally, after 30 min, the color change (extract color to red) of mix in each well was examined to select actives extracts. Active extract do not change color.

Minimum inhibitory concentration (MIC)

MIC values of active extracts were determined by slightly modified serial dilution microplate bioassay using specific dye p-iodonitrotetrazolium violet as an indicator of growth (Eloff, 1998). Active extracts were reconstituted to 20 mg/ml with a mixture of acetone/Muller Hinton (v/v). MIC was determined by two-fold serial dilutions of extracts beyond the level where no inhibition of growth of microorganisms were observed. Next, 100 µL of Muller Hinton broth culture of bacteria (10^6 CFU/ml) was added to each well which contains extract. The microplates were incubated at 37°C. After 18 h, 40 µL of *p*-iodonitrotetrazolium (0.2 mg/ml) solution in water were added to each well and the microplates were incubated at 37°C.

Table 1. Phytochemical analysis of *V. doniana and A. digitata*.

Phytochemical	Result			
	Color obtained		+/-	
	Vd	Ad	Vd	Ad
Alkaloid	nc	nc	-	-
Coumarin	nc	nc	-	-
Anthracene derivatives	Yellow	nc	+	-
Flavonoids	Yellow	nc	+	-
Essential oils	Green	nc	+	-
Lignans	nc	nc	-	-
Naphthoquinones	nc	nc	-	-
Pigments	Red-violet	Blue-violet	+	+
Saponin	nc	nc	-	-
Tannins	Red	Red	+	+
Terpene glycosides	Red-brown	Red-brown	+	+
Triterpenes	Red	nc	+	-

-: Absent; +: present; nc: not colored, Vd: *Vitex doniana*; Ad: *Adansonia digitata*.

After 1 h of incubation, the MIC values were recorded. The total activity (TA) values of each extract were determined by dividing the MICs with the quantity extracted from 1 g of the plant material (Eloff, 2004).

Quantitative antioxidant activity

The quantitative antioxidant activity was determined according to the method described previously by Velazquez et al. (2003). Three stock solutions of extracts (300; 30 and 3 µg/ml) were prepared and tested in the final concentrations of 100, 10 and 1 µg/ml. Next, 750 µL of stock solution of each extract and 1500 µL of a 2% solution of DPPH in methanol were introduced into dry and sterile tubes. For each concentration a blank and a negative control are prepared. The Blank consists of 750 µL of extract and 1500 µL of methanol. The negative control consists of 1500 µL of the solution of DPPH (2%) and 750 µL of methanol. Each test was done in triplicate and quercetol was used as positive control. The test tubes were incubated in dark at room temperature. After 20 min, the optical density of each mixture was measured at 517 nm. The inhibitory percentage of DPPH, which means the antioxidant activity of extracts, was calculated as follows (Schmeda-Hirschmann et al., 2003):

$$\% \text{ Inhibition} = [1 - (A_S - A_B) / A_C] \times 100$$

Where % Inhibition is the inhibitory percentage of DPPH; A_S is the optical density (OD) of the sample; A_B is the OD of blank; A_C is the OD of the control.

Brine shrimp lethality bioassay (*Artemia salina*)

This assay was performed as described by Kawsar et al. (2008) and Meyer (1982). To obtain mature naupli larva, the eggs of brine shrimp were hatched in normal seawater for 72 h. The stock solution of each extract (3 mg/ml) was obtained by dissolving 15 mg in 200 µL ethanol and 4.80 ml of seawater. Then, 1 ml of seawater containing 15 living naupli was added to 1 ml of extracts. Six concentrations ranging from 1.5 to 0.075 mg/ml, obtained by a twofold dilution of stock solution, were tested. Each experiment was done in triplicate and control was prepared using only seawater plus 15 living naupli. Survivors were counted after 24 h and the death at each concentration were determined. The percentage of lethality of the brine shrimp was then calculated.

RESULTS

Phytochemical results

The phytochemical screening of *V. doniana* and *A. digitata* on thin layer chromatography (TLC) has been performed. TLC of leaves of *V. doniana* revealed the presence of flavonoids, anthracene derivatives, essential oil, pigments, tannins, terpenes glycosides and triterpenes, while the bark of *A. digitata* revealed the presence of tannins, pigments and terpene glycosides (Table 1).

Antifungal assay

Six extracts from two plants used in Benin folk medicine were tested for their antifungal activity on mycelial growth and sporulation of fungi. The results are compiled in Tables 2 and 3. The inhibitory percentage (IP) of extracts against sporulation of the six species of *Aspergillus* ranged from 20 to 99.85%. Thirty (30) assays out of 36 showed an IP values superior to 60%. The most interesting activity was obtained with *A. fumigatus* with an IP values from 70.7 to 99.85%. *A. flavus, A. fumigatus, A. nidulans* and *A. ochraceus* were the most sensible fungi for extracts. Contrary to sporulation, the inhibition percentage (IP) of extracts against mycelia development ranged from 2.98 to 53.42%. Only dichloromethane extract of *V. doniana* inhibited mycelia development of *A. terreus* with inhibition percentage value of 53.42%. The other extracts showed weaker results.

Table 2. Inhibition effect of extracts against sporulation of fungi.

Extract	Inhibition percentage of sporulation (%)					
	A. flavus	***A. parasiticus***	***A. terreus***	***A. ochraceus***	***A. nidulans***	***A. fumigatus***
Vd DM	68 ± 0,06	77.85 ± 0.11	92.32 ± 0.06	86.81 ± 0.14	83.38 ± 0.02	96.4 ± 0.006
Vd Me	94.4 ± 0.01	60.4 ± 0.08	74.28 ± 0.05	86.44 ± 0.18	84.38 ± 0.019	70.7 ± 0.03
Vd H_2O	88 ± 0.04	58.11 ± 0.37	96.16 ± 0.00	72.74 ± 0.32	91.84 ± 0.014	99.48 ± 0.001
Ad DM	20 ± 0.11	44.08 ± 0.29	69.82 ± 0.02	92.88 ± 0.07	88.33 ± 0.015	98.9 ± 0.001
Ad Me	94 ± 0.02	35.71 ± 0.28	60.71 ± 0.13	57.33 ± 0.10	89 ± 0.008	99.85 ± 0.000
Ad H_2O	95 ± 0.01	51.53 ± 0.08	86.07 ± 0.05	91.55 ± 0.07	95.88 ± 0.007	97.26 ± 0.001

Vd: *Vitex doniana*; Ad: *Adansonia digitata*; DM: dichloromethane; Me: methanol; H_2O: water/ethanol.

Table 3. Inhibition effect of extracts against *Aspergillus mycelia* development.

Extract	Inhibition percentage of mycelia development (%)					
	A. flavus	***A. parasiticus***	***A. terreus***	***A. ochraceus***	***A. nidulans***	***A. fumigatus***
Vd DM	21.42 ± 0.03	47 ± 0.042	53.42 ± 0.0	41.37 ± 0.028	7.46 ± 0.02	11.11 ± 0.02
Vd Me	38.37 ± 0.00	40 ± 0.014	39.72 ± 0.0	44.82 ± 0.026	7.46 ± 0.01	20 ± 0.025
Vd H_2O	16.32 ± 0.03	20 ± 0.042	34.24 ± 0.0	48.27 ± 0.001	41.79 ± 0.0	15.55 ± 0.08
Ad DM	18.36 ± 0.02	36 ± 0.014	47.94 ± 0.0	37.93 ± 0.030	2.98 ± 0.04	8.88 ± 0.065
Ad Me	22.45 ± 0.00	32 ± 0.014	39.72 ± 0.0	41.37 ± 0.028	16.41 ± 0.0	22.22 ± 0.00
Ad H_2O	22.49 ± 0.10	22 ± 0.028	42.46 ± 0.0	39.65 ± 0.043	16.41 ± 0.0	15.55 ± 0.03

Vd: *Vitex doniana*; Ad: *Adansonia digitata*; DM: dichloromethane; Me: methanol; H_2O: water/ethanol.

Table 4: Antibacterial effect of extracts at 10 mg/ml.

Data	Growth inhibition effect of extract at 10 mg/ml					
	Vitex doniana			***Adansonia digitata***		
Extract / Organism	**DM**	**MeOH**	**H_20/EtOH**	**DM**	**MeOH**	**H_20/EtOH**
E. coli (CIP53126)	+	+	+	+	+	+
S. aureus(ATCC6538)	-	-	-	-	-	-
E. faecalis (ATCC29212)	+	+	+	+	-	-
P. aeruginosa (CIP82118)	-	-	+	-	+	+
S. abony (CIP8039)	+	+	+	+	+	+
S. aureus methicillin résistant	-	-	+	-	-	-
S. epidermidis	-	-	-	-	-	-

DM: dichloromethane; MeOH: methanol; H_2O/EtOH: Water/ethanol; -: inhibition; +: no inhibition.

Antibacterial activity

Growth inhibition effect of extracts at 10 mg/ml

The results (Table 4) showed that tested bacteria were sensible to extracts at different levels. The aim of this assay was to eliminate the extracts that did not inhibit the growth of bacteria at 10 mg/ml. All extracts inhibited the growth of *S. aureus*; *S. aureus* methicillin resistant (SARM) and *S. epidermidis*. The dichloromethane and methanol extracts of both plants and hydroalcholic extract of *A. digitata* were the most active extracts by inhibiting the growth of four strains out of seven (4/7). The MIC of active extracts was determined as shown in Table 5. The results showed that antibacterial activity of *V. doniana* extracts was higher than *A. digitata* extracts. The MIC values of *V. doniana* ranged from 0.039 to 2.5 mg/ml.

In general, all extracts inhibited one or more micro-organisms. The dichloromethane extract of *V. doniana* was the most effective against *S. aureus* methicillin resistant (SARM) with MIC value of 39 µg/ml. The methanolic and hydroethanolic extracts of *V. doniana* were also active against SARM with MIC values of 78 µg/ml. *S. aureus* methicillin resistant was found to be

Table 5. MIC values (mg/ml) and the total activity (ml) of extracts.

	Minimum inhibitory concentration (mg /ml)					
Species	***Vitex doniana***			***Adansonia digitata***		
Extract / organism	**DM**	**MeOH**	**H_2O/EtOH**	**DM**	**MeOH**	**H_2O/EtOH**
S. aureus (ATCC6538)	1.25	0.312	1.25	> 2.5	1.25	2.5
E. faecalis (ATCC29212)	na	na	na	na	1.25	0.625
P. aeruginosa (CIP82118)	> 2.5	2.5	na	> 2.5	na	na
S. aureus methicillin resistant	0.039	0.078	na	0.156	0.078	0.078
S. epidermidis	2.5	0.078	nd	> 2.5	1.25	nd
Extract from 1 g (mg)	17.94	108.37	117.5	4.5	52	111.63
Total activity (ml)						
S. aureus (ATCC6538)	14.25	346.78	93.98	>1.8	41.6	44.66
E. faecalis (ATCC29212)	-	-	-	-	41.6	178.6
P. aeruginosa (CIP82118)	>7.2	42.54	-	>1.8	-	-
S. aureus methicillin resistant	459.9	1389.38	-	28.84	666.66	1431.16
S. epidermidis	7.2	173.4	nd	>1.8	41.6	nd

DM: dichloromethane; MeOH: methanol; H_2O/EtOH: Water/ethanol; M: Meticillin; na: not actif; nd: not determined

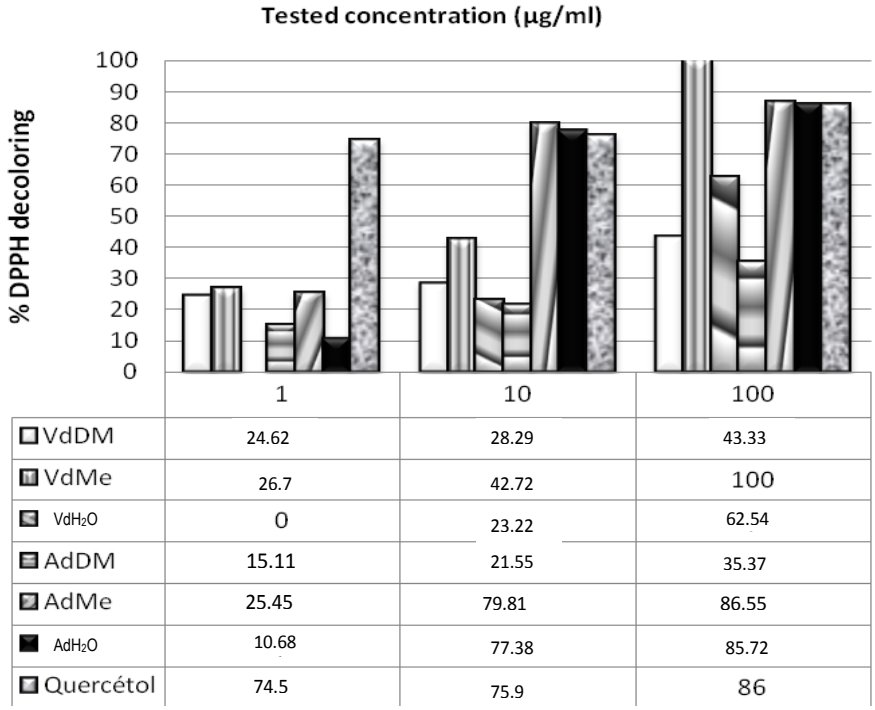

Figure 2. DPPH free radical scavenging activity of extracts.

more sensitive among the tested bacteria while *E. faecalis* the most resistant. The highest total activity was obtained with methanol extract of *V. doniana* and hydroethanolic extract of *A. digitata*, the most interesting with total activity values of 1389.3 ml and 1431.16 ml, respectively (Table 5).

Quantitative antioxidant activity

Figure 2 shows the antioxidant activity of extracts expressed as inhibition percentage (%IP) of DPPH radical in comparison to the positive control (Quercetol). The scavenging activity of all extracts on the DPPH

Table 6. *Artemia salina* lethality assay of extracts.

Species	Extract	DL_{50}	R^2
Vitex doniana	DM	6.27	0.875
	MeOH	54.81	0.428
	H_2O/EtOH	5.69	0.862
Adansonia digitata	DM	nd	nd
	MeOH	5.68	0.771
	H_2O/EtOH	11.14	0.713

DM: dichloromethane; MeOH: methanol; H_2O/EtOH: water/ethanol; nd: not determined.

radical was found to be strongly dependent on the extract concentration. At 1 µg/ml, all extracts showed weak activity with IP values ranging from 0.00 to 26.70%. Moreover, at 10 µg/ml, the most interesting activity was obtained with methanol (79.81%) and hydroethanolic (77.39%) extracts of *A. digitata*. These results compared favorably with that of quercetol with an IP value of 75.9%. At 100 µg/ml, four extracts out of six showed interesting activity (62.24–100%) with IP values higher or comparable for quercetol (86%).

A. salina toxicity assay

Brine shrimp lethality test results are showed in Table 6. Figure 3 shows the toxicity curves obtained for all extracts. The LC_{50} values of tested extracts ranged between 5.68 and 54.81 mg/ml. Hydroethanolic extract of *V. doniana* and methanol extract of *A. digitata* were the most toxic on the shrimps with LC_{50} values of 5.69 and 5.68 mg/ml, respectively. The highest LC_{50} was exhibited by methanol extract of *V. doniana* (54.81 mg/ml).

DISCUSSION

The development of drug resistance in human pathogens against commonly used antibiotics and antifungal has necessitated a search for new antimicrobial substances. Higher plants produce diverse secondary metabolites with different biological activities. These natural compounds may be a source of compounds with antimicrobial effects and therefore possible candidates for the development of new antifungal agents (Barrett, 2002; Abad et al., 2007). In this study, two species were evaluated for qualitative determination of major phytoconstituents. They exhibited different kinds of secondary metabolites. *V. doniana* revealed the presence of flavonoids, anthracene derivatives, essential oil, pigments, tannins, terpenes glycosides and triterpenes. Previous chemical investigations on ethanolic and aqueous extracts indicated the presence of saponins, tannins, anthraquinones, terpenoids and flavonoids (Agbafor and Nwachukwu, 2011; Kubmarawa et al., 2007). Similarities are observed between the results, particularly the presence of tannins and flavonoids. *A. digitata* revealed the presence of tannins, pigments and terpene glycosides. Tannins, phlobatannins, terpenoids, cardiac glycosides and saponins were also found to be present (Masola et al., 2009; Lim et al., 2006).

In this study, methanol and hydroethanol extracts showed interesting antibacterial (0.039 - 2.5 mg/ml) and/or antifungal (IP up to 99.85%) activity by inhibiting one or more microorganisms. Our results were comparable to those obtained by Agbafor and Nwachukwu (2011) with methanol extract of *V. doniana*. Previous investigation on different extracts of *A. digitata* showed weak results with inhibitory percentage ranging from 18 to 78 at 500 µg/ml (Oloyede et al., 2010). The interesting antimicrobial activity against the tested bacteria could be due to the presence of tannins, and flavonoids as these have previously been reported to possess antimicrobial activities (Masola et al., 2008; Finnermore et al., 1988; Erah et al., 1996; Ishida et al., 2009). Tannins are known antimicrobial agents that could inhibit the growth of microorganisms by precipitating the microbial protein and thus depriving them of nutritional proteins needed for their growth and development (Obasi Nnamdi et al., 2010). Flavonoids have been also reported to possess many useful properties, including antimicrobial (Havsteen, 1983), antifungal and antioxidant activities (Cushnie and Lamb, 2005). In our study, tannins and flavonoids were detected in methanol and hydro-ethanol extracts. This could explain the antimicrobial activity of the extracts.

We also observed that the antifungal activity of extracts is more marked on the inhibition of the sporulation than on the mycelia development of the fungi. The higher inhibition percentage of methanol and hydroethanol extracts could be due to the presence of tannins and flavonoids. It has been reported that flavonoids have the ability to inhibit spore germination of plant pathogens (Harborne and Williams, 2000), and tannins inhibit the germ-tube formation and stimulus of phagocytosis by macrophages (Ishida et al., 2006), and extracellular microbial enzymes through inhibition of oxidative phosphorylation (Scalbert, 1991). Galangin, a flavonol, has been shown to have inhibitory activity against *Aspergillus tamarii*, *A. flavus*, *Cladosporium sphaerospermum*, *Penicillium digitatum* and *Penicillium italicum*, *Candida neoformans* (Ishida et al., 2009; Afolayan and Meyer, 1997). This could explain the results obtained on inhibition of sporulation.

The results from the present investigation suggested that *A. digitata* and *V. doniana* have interesting activity by inhibiting DPPH radical. Our results were comparable to those obtained by Muanda et al. (2011) with methanol extract of *V. doniana*. Previous investigation on different extracts of *A. digitata* showed higher activity with inhibitory percentage ranging from 18 to 78 at 500 µg/ml

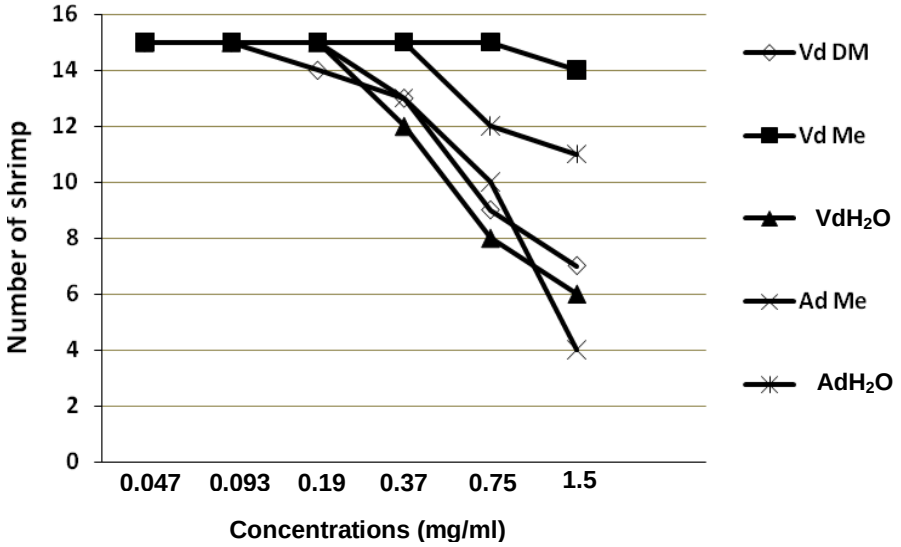

Figure 3. Lethal effect of extracts on brine shrimp nauplii at different concentrations.

(Oloyede et al., 2010). The scavenging activity obtained here is dose dependent. It has been shown that the scavenging effects on the DPPH radical increase with increasing concentration of samples and standards to a certain extent (Motalleb et al., 2005). The antioxidant activity obtained here could be due to the presence of flavonoids and tannins. These chemical compounds have been reported to possess antioxidant activity and several reports indicated that the antioxidant potential of medicinal plants may be related to the concentration of their phenolic compounds which include phenolic acids, flavonoids, anthocyanins and tannins (Djeridane et al., 2006; Wong et al., 2006). These species could contain compounds with strong radical scavenging activity and could possess therapeutic effects such as inflammatory diseases and cardiovascular protection.

Antioxidants are also believed to provide protection against cancer (Anani et al., 2000; Ames, 1983). Several epidemiological studies suggest that plants rich in antioxidants play a protective role in health and against diseases (Muanda et al., 2011) and their consumption lowered the risk for cancer, heart disease, high blood pressure and stroke (Vinson et al., 2001; Wolfe et al., 2003). It is also interesting to note that preliminary toxicity test against *A. salina* showed that LC_{50} of all extract are superior to most of the concentrations of extract which inhibits studied pathogens. According to the results previously described by Zakaria et al. (2007), we concluded that all tested extracts exhibited very low or no toxicity, giving LC_{50} values higher than100 µg/ml.

Therefore, the demonstration of antibacterial, antifungal, antioxidant activities and low toxicity of methanol and hydroethanolic extracts provides the scientific basis for the use of these plants in traditional medicine, since most traditional medicine use water and alcohol as their solvent in which the decoctions are prepared.

Conclusion

All the extracts showed various degrees of biological activity on the tested pathogens. The chance to find compounds with antifungal, antimicrobial activities was more apparent in the two species. These findings give a scientific basis to the traditional uses of *A. digitata* and *V. doniana.*

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