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Full Length Research Paper

Screening for antibacterial, antioxidant activity and toxicity of some medicinal plants used in Benin folkloric medicine

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Ten extracts from 10 medicinal plants, *Acanthospermum hispidium*, *Argemone mexicana, Byrsocarpus coccineus, Canthium setosum*, *Croton lobatus, Dichapetalum guineense*, *Nauclea latifolia, Schrankia leptocarpa, Secamone afzelii, Pterocarpus erinaceus,* traditionally used in Benin to treat infectious diseases and were screened for *in vitro* antibacterial activity towards *Staphylococcus aureus*, *Enteroccocus feacalis*, *Escherichia coli* and *Pseudomonas aeruginosa.* The 2, 2-diphenylpicrylhydrazyl radical scavenging activity and toxicity assay using *Artemia salina* were also performed. All extracts were effective against tested microorganisms at different level with MIC ranking from 0.31 to 10 mg/ml. The best inhibition on the growth of tested bacteria was observed with four extracts obtained from *D. guineense, N. latifolia,* and *P. erinaceus* (MIC = 0.313 mg/ml). The species *S. aureus* and *E. faecalis* were more sensitive than the other test bacteria. All extracts showed good radical scavenging activity range from 1.35 to 3.47 µg/ml. The most effective extract was the methanolic extract of *S. leptocarpa*, with an EC_{50} value of 1.35 µg/ml. The lethality test showed that all tested extracts have low toxicity against the shrimps with LC_{50} values ranging from 3.8 to 8.17 mg/ml. The results provide an evidence of the traditional use of same collected plants in the treatment of infective diseases.

Key words: Antibacterial, antioxidant, toxicity, traditional medicine, Benin.

INTRODUCTION

According to World Health Organization, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (WHO, 2008). In developing countries, low income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infectious diseases. These plants are ingested as decoctions, teas or juice preparations (Gonzalez, 1980). The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Making antibacterial therapy effective, safe and affordable has been the focus of interest during recent years (Sharma et al., 2002). There are several reports on antimicrobial activity of different herbal extracts (Adelakun et al., 2001; Camporese et al., 2003; Bonjar, 2004; de Boer et al., 2005; Nair et al., 2005; Varsha et al., 2009).

In recent years, the studies on "oxidative stress" and its adverse effects on human health have become a subject of considerable interest. It is well documented fact that exposure of organism to exogenous and endogenous factors generates a wide range of reactive oxygen species resulting in homeostatic imbalance (Halliwell et

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Table 1. Selected plants for *in vitro* antibacterial activity.

Botanical name/family	Local name	Used part	Voucher No
Acanthospermum hispidium/Asteraceae	Togbama (F), dagouro (Y), tanga (B)	AP	AA6358/HNB
Argemone mexicana/Papaveraceaea	Débio (F, G), Timtonyakourou (B)	EP	AA6359/HNB
Byrsocarpus coccineus/ Connaraceae	Anyomma (F,G), Amodjè (Y)	R	AA6362/HNB
Croton lobatus/Euphorbiaceae	Aloviaton (G, F), Erougali (Y)	AP	Hp 565a
Canthium setosum/ Rubiaceae	Avovoun (G, F), Igielera (Y.N.)	AP	Houngnon 3435
Dichapetalum guineense/Dichapetalaceae	Agbagloma (F. Mi), Alo (Y.N.)	L	Av 1551
Nauclea latifolia/Rubiaceae	Ko (G,F), Igbessi (Y), Ganyèrou (B)	R	AA6363/HNB
Pterocarpus erinaceus/Papilionaceae	-	AP	AA6364/HNB
Schrankia leptocarpa/ Mimosaceae	Ahosibwasa (G), Danhunkan (F), Kpatanwo olokun (Y.N.)	EP	Houngnon 954b
Secamone afzelii/Asclepiadaceae	Zunkuju (G), Ewe ayibu (Y.N.)	AP	Souza 1202a

B: Bariba; F: Fon; G: goun; Y: Yoruba; N: Nago; Mi: Mina; AP: aerial part; EP: entire plant; L: leaves; R: roots.

al., 1992).

Many efforts have been made to discover new antimicrobial and antioxidant compounds from various kinds of sources such as micro-organisms, animals, and plants. Systematic screening of folk remedies is another strategy in the discovery of novel effective compounds (Walter and Memory, 1995).

In our search for natural products with antimicrobial and antioxidant activities, we investigated traditional plants used in Benin to treat infectious diseases. A registry of fourty (40) traditional remedies used to cure diarrhea, stomachache, dysentery and malaria by traditional plants practitioners and sellers in Benin was worked out. In our previous work, we determined the antimalarial activity of some species (Weniger et al., 2004). In the present study we investigate the antibacterial and antioxidant activities of ten species belonging to nine families. The toxicity of extracts was also determinated using *Artemia salina* lethality test.

EXPERIMENTAL

Plant material

The selected species (Table 1) were collected between December 2008 and January 2009 in different area in South Benin: *Secamone afzelii* (Schutt.) K. Schum. (Asclepiadaceae) and *Croton lobatus* L. (Euphorbiaceae) were collected in the Atlantique Department (Southern Benin), *Dichapetalum guineense* (DC.) Keay (Dichapetalaceae), *Canthium setosum* Hiern. (Rubiaceae) and *Schrankia leptocarpa* DC. (Mimosaceae) were collected in Oueme Department (Southeastern Benin), *Nauclea latifolia* (Rubiaceae) was collected in Littoral department (Southern Benin); *Byrsocarpus coccineus* (Connaraceae), *Argemone mexicana* (Papaveraceae), *Acanthospermum hispidium* (Asteraceae) and *Pterocarpus erinaceus* (Papilionaceae) were provided by the plants sellers from the market of Degakon in Cotonou, Department of Littoral (Southern Benin). Botanical determination was performed by taxonomists from the Herbier National of University of Abomey-Calavi in Benin and voucher specimens were deposited at the same herbarium (BENIN).

Preparation of extracts

Collected species was left at room temperature (25°C) in laboratory for two weeks to dry. Samples were chopped into smaller pieces and then ground into powder using GRINDER 3S.S. JARS, EXCELLA. Dry powdered aerial parts obtained (20 g) was exhaustively extracted three time with 200 ml of methanol. The suspension was further filtered through Whatman filter paper (Whatman international Ltd, Maidstone, England). The filtrates were taken to dryness under vacuum and the residues were stored at 4°C until testing.

Antimicrobial screening

The following test organisms were used to determine the Minimal Inhibitory Concentration (MIC) of the plant extracts: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Enteroccocus faecalis* ATCC 29212. These strains are widely used in screening tests. To determine the Minimum Inhibitory Concentration (MIC) of plants extracts against each of these organisms, the microplate dilution method using tetrazolium violet to indicate growth of the bacteria was used (Eloff, 1998). Extracts were reconstituted to 20 mg/ml with a mixture of distilled water/acetone (v/v 1:1). Briefly, 100 µl microliters of the extract solution obtained were serial diluted in 96-well microplates. One colony of each organism was introduced into 5 ml Luria Bertani (LB) broth and incubated for 1 h. 100 µl of the resulting culture (10^6 to 10^7 CFU/ml) where added to each well. The plate was sealed and incubated at 37°C for 18 h. Then, 40 µl of a 0.2 mg/ml solution of p-iodonitrotetrazolium violet (p-INT, Sigma) dissolved in water were added to the microplate wells and the plate was incubated at 37°C for 1 to 2 h. The MIC value, which is the lowest concentration of plant extract, at which bacterial growth was inhibited was then determined. Tests were performed at least in triplicate. Gentamicin was used as positive control.

DPPH radical scavenging activity

Free Radical Scavenging Activity was determined by means, the method previously described by Schmeda-Hirschmann et al. (2003) in which the 2,2-diphenylpicrylhydrazyl (DPPH) was used. The DPPH solution was freshly prepared daily, stored in a flask covered with aluminium foil. The flask was kept in the dark at 4°C between the measurements. 0.75 ml of a methanolic solution of the extract

Table 2. MIC (mg/ml) of methanol extracts of collected ethnomedicinal plants on four bacteria species (*P. aeruginosa, E. faecalis, S. aureus, E. coli*) after 18 h of incubation.

Botanical name	Tested bacteria			
	***P. aeruginosa* ATCC 27853.**	***E. faecalis* ATCC 29212**	***S. aureus* ATCC 25923**	***E. coli* ATCC 25922**
Acanthospermum hispidium	>10	0.313	0.625	5
Argemone mexicana	>10	>10	2.5	>10
Byrsocarpus coccineus	1.25	1.25	1.25	5
Croton lobatus	>10	1.25	>10	>10
Canthium setosum	>10	0.625	1.5	2.5
Dichapetalum guineense	>10	0.313	1.5	1.25
Nauclea latifolia	>10	1.25	0.313	>10
Pterocarpus erinaceus	0.313	1.25	0.313	>10
Schrankia leptocarpa	2.5	0.625	2.5	5
Secamone afzelii	>10	10	1.5	>10
Gentamicin (mg/ml)	0.08	0.02	0.06	0.06

at different concentrations ranging from 1 to 100 µg/ml was placed in a test tube, and 1.5 ml of a DPPH methanolic solution (20 mg/L) was added to each tube. The samples were incubated for 20 min in the dark at 30°C and the decrease in absorbance at 517 nm was measured against a control prepared with methanol and a blank sample, using a spectrophotometer (Genova). The radical scavenging activities or decoloring percentage (DP), which is defined as the concentration of substrate that causes loss of the DPPH activity, were calculated as described by Schmeda-Hirschmann et al. (2003). The experiments were carried out in triplicates. L-ascorbic acid was used as a positive control compound in this assay.

Artemia salina toxicity assay

The *A. salina* (brine shrimp) toxicity assay was performed following a slightly modified version of the method described by Solis et al. (1993). This assay was used to predict the presence, in the extracts, of cytotoxic activity (Meyer et al., 1982). Initial concentration of each extract of plant was 20 mg/ml diluted with two-fold dilutions in 96-well microplates to make 10 concentrations, the lowest being 0.039 mg/ml; Each well containing 15 nauplii. After 24 h incubation at room temperature, the mortality of nauplii was determined.

The number of dead nauplii was plotted against the sample extract concentration, and a logarithmic regression curve was established in order to calculate the IC_{50}, which is the amount of sample necessary to kill 50% of the nauplii. Cyclophosphamide was used as positive control.

RESULTS AND DISCUSSION

Minimum inhibitory concentration (MIC)

The parts traditionally used of each of the ten plants were extracted with methanol. *In vitro* antibacterial activity of the 10 extracts are shown in Table 2. All extracts showed antibacterial activity by inhibiting one or more microorganisms. The highest antibacterial activities were found for the extracts of *Acanthospermum hispidium* and *D. guineense* against *E. faecalis; N. latifolia* against *S. aureus; P. erinaceus* against *S. aureus* and *P. aeruginosa* (MIC = 0.31 mg/ml). The methanolic extract of *A. hispidium, Canthium setosum and S. leptocarpa* all showed MIC value of 0.62 mg/ml. *E. faecalis* and *S. aureus* were the most susceptible bacteria to all plant extracts (0.31 ≤ MIC ≤ 1.25). *P. aeruginosa* exhibited only slight susceptibility with most MIC values beyond 10 mg/ml. This fact could be attributed to the naturally resistance of this bacteria to many antibiotics due to the permeability barrier afforded by its outer membrane (Apak and Olila, 2006). None of the extracts was more active against the tested bacteria than the positive control.

All extracts were active against both gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa, E. faecalis, E. coli*) tested bacteria. Gram-negative bacteria are generally more resistant compared to the gram-positive ones (Cos et al., 2006). This could explain the results obtained with A. *mexicana* and *S. afzelii* which are only effective against Gram+ bacteria (*S. aureus*) with a MIC of 2.5 mg/ml and 1.25 mg/ml respectively. *C. lobatus* showed a specific activity against only one gram– bacteria (*E. faecalis*). The species used in this study had never been evaluated or only partially studied for antibacterial activity. The root extract of *N. latifolia* was found to inhibit the growth of *S. aureus* and *P. aeruginosa*in this study, while Kubmarawa et al. (2007) obtained no activity against both bacteria using the stem bark extract. Kubmarawa et al. (2007) reported good antibacterial activity against *E. coli* (0.1 mg/ml).

DPPH radical scavenging activity

The radical scavenging activities of the different

Table 3. DPPH free radical scavenging activity EC_{50} values (µg/ml) of methanol extract of collected ethnomedicinal plants.

Botanical name	EC_{50} (µg/ml)	R2
Acanthospermum hispidium	nd	nd
Argemone mexicana	1.73	0.92
Byrsocarpus coccineus	nd	nd
Croton lobatus	1.96	0.95
Canthium setosum	3.47	0.99
Dichapetalum guineense	nd	nd
Nauclea latifolia	1.56	0.90
Pterocarpus erinaceus	3.37	0.75
Schrankia leptocarpa	1.35	0.82
Secamone afzelii	1.74	0.99
L-Ascorbic acid	1.1	0.98

nd: not determined.

Table 4. *A. salina* lethality assay of methanol extracts of collected ethnobotanical plants.

Botanical name	LC_{50} (mg/ml)	R2
Acanthospermum hispidium	>20	-
Argemone mexicana	>20	-
Byrsocarpus coccineus	nd	nd
Croton lobatus	8.17	0.76
Canthium setosum	4.22	0.85
Dichapetalum guineense	4.96	0.87
Nauclea latifolia	nd	-
Secamone afzelii	3.8	0.94

extracts are also showed in Table 3. The quantitative DPPH test of the ten extracts revealed that all of them having various degrees of antioxidant properties, with IC_{50} values ranged from 1.35 to 3.47µg/ml. *S. leptocarpa* are the most active extract with an IC_{50} value of 1.35 µg/ml. The extracts of *A. mexicana* and *S. afzelii* also exhibited good activity (1.73 and 1.74 µg/ml, respectively).

Artemia salina toxicity assay

Brine shrimp lethality test results are showed in Table 4. Figure 1 showed the toxicity curve obtained with C. setosum. The LC_{50} values of tested extracts ranged between 3.8 to 8.17 mg/ml. *S. afzelii* extract was the most toxic on the shrimps with LC50 value of 3.8 mg/ml. *C. setosum* and *D. guineense* showed similarly LC50 value of 4.22 and 4.96 mg/ml, respectively. The higher LC50 was exhibited by methanol extract of *C. lobatus* (8.17 mg/ml). According to the results previously obtained by Zakaria et al. (2007), we concluded that all extracts tested in his study exhibited very low or no toxicity, giving LC50 values higher than 100 µg/ml.

Conclusions

The processing of the plants performed in this study was not comparable to the traditional approach when the plants practitioners and sellers used ethanol or/and water for extracts whereas we have used methanol for extraction. In this sense, it is not an exact replication of the traditional knowledge. All extracts showed antibacterial activity by inhibiting one or several microorganisms. The plants with the greatest antimicrobial activity were *A. hispidium*, *D. guineense*, *N. latifolia* and *P. erinaceus*. We have also investigated the antioxidant properties and toxicity of the methanolic extract of some selected plants. The results showed high antioxidant properties without toxicity of the extracts. This results support the traditional use of some of these plants.

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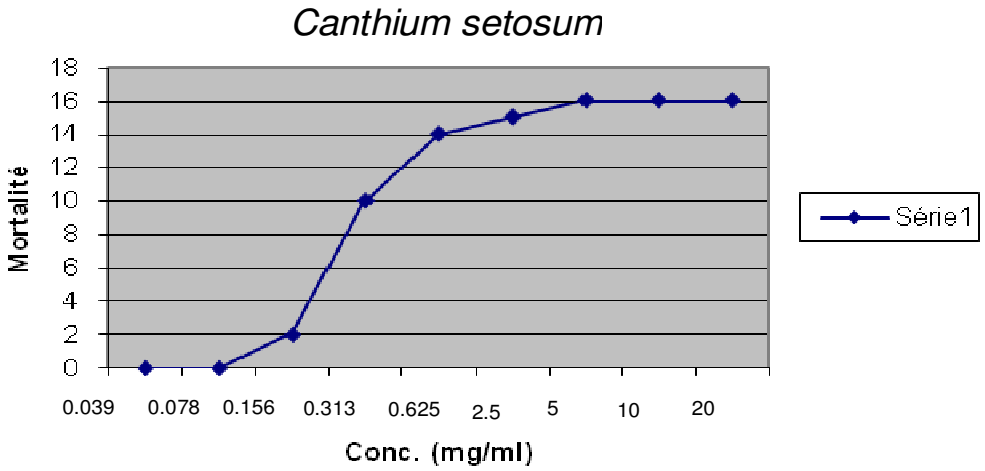

Figure 1. Graph of *C. setosum* LC_{50} determination in *Artemia salina* lethality test.

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