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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Antibacterial Activity and Phytochemical Study of Six Medicinal Plants used in Benin

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Abstract: The ethanol extracts obtained from *Psidium guajava*, *Flacourtia flavescens*, *Boswellia dalzielii*, *Ficus exasperata*, *Pavetta corymbosa* and *Hybanthus enneaspermus*, six species traditionally used in Benin to treat several infectious diseases, were evaluated for their *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The minimum inhibitory concentration of extracts was determined using the microplate dilution method. The presence of major phytoconstituents was detected qualitatively. The diphenylpicrylhydrazine radical scavenging activity was also performed. The extracts exhibited antibacterial activities against the tested bacteria. *Boswellia dalzielii*, *Psidium guajava*, *Pavetta corymbosa* and *Flacourtia flavescens* exhibited the lowest minimum inhibitory concentration values (0.313-2.5 mg mL⁻¹). *Pseudomonas aeruginosa* was the least sensitive microorganism with MIC values higher than 10 mg mL⁻¹. In antioxidant assay the crude extracts of *B. dalzielii* and *P. corymbosa* appeared to be as potent as quercetol with an inhibition percentage of 83 and 75.3% at 10 µg mL⁻¹ which is comparable to 75.9% for quercetol at the same concentration.

Key words: Antimicrobial, antioxidant, DPPH, phytochemical study, medicinal plants

INTRODUCTION

Plants have been used for centuries to treat infections and other illnesses in native communities but controlled clinical studies are scarce. The use of medicinal plants as a source for relief from illness can be traced back over five millennia. The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents (Mahesh and Satish, 2008). Many plants especially those used by traditional healers produce pharmaceutically active compounds that have antimicrobial, antihelminthic, antifungal, antiviral, anti-inflammatory and antioxidant activity, antioxidant activity and anti-diabetic activity (Rabah *et al.*, 2007; Ansari and Sitaram, 2011; Gupta *et al.*, 2007; Karim *et al.*, 2011).

Now-a-days, the problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Nascimento *et al.*, 2000). Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms *in vitro* (Cowan, 1999). Unfortunately, development of effective antibacterial agents has been accompanied by the emergence of drug-resistant organisms due to the irrational and overuse of antibiotics, failure to complete a course of treatment, genetic versatility of microbes and horizontal transfer of resistant genes among bacterial species. All the mentioned factors diminish the clinical effectiveness of antibiotics (Amit and Shailendra, 2006; Aibinu *et al.*, 2007).

In recent times, there has been renewed interest on plants as sources of antimicrobial agents due to their use historically and the fact that a good portion of the world's population, particularly in developing countries, rely on

plants for the treatment of infectious and non-infectious diseases (Ayoola *et al.*, 2008). Scientists from divergent fields are investigating plants anew with an eye to their antimicrobial (Seal, 2011; Philip *et al.*, 2009; Elizabeth, 2005; Srinivasan *et al.*, 2001).

In other hand, the studies on “oxidative stress” and its adverse effects on human health have become a subject of considerable interest. Free radical reactions have been implicated in the etiology of several human diseases including atherosclerosis, ischemic heart disease, the ageing process, inflammatory lesions, diabetes mellitus, different immunosuppressive disorders, metabolic disorders, neuromuscular degenerative conditions (Gupta *et al.*, 2007).

Many botanical and some common dietary supplements are good sources of antioxidants and anti-inflammatory compounds. The importance of these antioxidant constituent of plant material is the maintained of health and protection from coronary heart disease and cancer (Khalil *et al.*, 2007). Several researches on the phenolic constituent and antioxidant activities in various plants have been conducted by Siddiq *et al.* (2005), Aher *et al.* (2009) and Ullah *et al.* (2009).

In Bénin, medicinal plants are usually easier to obtain than pharmaceutical products by population. A wide range of medicinal plant parts is used to treat numerous diseases. To improve treatment of disease, the ethnomedicine can be useful in the elaboration of new agents. Before the development of antimicrobials and antioxidants agents based on plants, the knowledge of traditional medicine requires scientific studies to confirm the properties of medicinal plants. In a preliminary study, we tested ethnomedicinal plants extracts used against infectious diseases for their inhibitory effect on the growth of beta-lactamase producing clinical isolates of *Escherichia coli* (Anago *et al.*, 2006).

In this study, we investigated the antibacterial and antioxidant activities of six plants which showed high inhibition of a multiresistant *E. coli* strain. This species *Hybanthus enneaspermus* (Violaceae), *Boswellia dalzielii* (Burceraceae), *Ficus exasperata* (Moraceae) *Flacourtia flavescens* (Flacourtiaceae), *Psidium guayava* (Myrtaceae) and *Pavetta corymbosa* (Rubiaceae) belonging to six different families were chosen. The tested bacteria are reference strains of

Staphylococcus aureus, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Phytochemical screening was also carried out to identify major biologically active compounds.

MATERIALS AND METHODS

Plant material: The selected species (Table 1) were collected in 2007 in different areas in Benin. In the Ouémé region (Southeastern Benin) for *Pavetta corymbosa* (Rubiaceae), *Psidium guayava* (Myrtaceae) and *Hybanthus enneaspermus* (Violaceae). In the Atlantique region (Southeastern Benin) for *Ficus exasperata* (Moraceae) and *Flacourtia flavescens* (Flacourtiaceae). *Boswellia dalzielii* (Burceraceae) was acquired by plants sellers in the market of Godomey (department of Atlantic, Southeastern Benin). Botanical determination was performed by taxonomists from the Herbarium National of University of Abomey-Calavi in Benin and voucher specimens were deposited at the same Herbarium.

Extraction: A sample (100 g) of powdered air-dried plant materials was soaked in 98% ethanol (500 mL) and the mixture was left on a shaker (IKA KS260 Basic) for 72 h. At the end of the extraction, the suspension was filtered using Whatman filter paper (Whatman international Ltd, Maidstone, England). The filtrate was concentrated to dryness using a Buchi rotary evaporator then stored at 4°C until further use.

Microorganisms used: 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 *Enterococcus faecalis* ATCC 29212 obtained from Département de Génie de Biologie Humaine, University of Abomey-Calavi.

Preparation of inoculum: A loop full of bacteria from the stock culture was streaked on Mueller-Hinton Agar (MHA) plates which were kept for incubation at 37°C for 24 h. Active cultures for experiments were prepared by introducing one colony of bacteria into 5 mL of Mueller-Hinton Broth (MHB). The suspensions were incubated for 1 h at 37°C under agitation, to achieve the concentration of $1-3 \times 10^6$ colony forming units (cfu mL⁻¹).

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

Botanical name (Family)	Voucher N°	Used part	Ethnopharmacological use
<i>Hybanthus enneaspermus</i> (Violaceae)	Houngnon 3708	Leaves	Jaundice, urinary tract infections
<i>Boswellia dalzielii</i> (Burceraceae)	APS/CA/02/CAN° 6	Stembark	Syphillis
<i>Ficus exasperata</i> (Moraceae)	AA6360/HNB	Leaves	Conjunctivitis, gonococcie,
<i>Flacourtia flavescens</i> (Flacourtiaceae)	AA6275/HNB	Leaves	Dysentery, diarrhoea, skin infections
<i>Psidium guayava</i> (Myrtaceae)	AA6361/HNB	Stembark	Diarrhoea, dysentery
<i>Pavetta corymbosa</i> (Rubiaceae)	Houngnon 4870	Leaves	Wound infection, malaria

Minimum Inhibitory Concentration (MIC): 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⁻¹ with a mixture of water/acetone (v/v 1:1). The final extracts concentrations tested were 10; 5; 2.5; 1.25; 0.625; 0.315; 0.156 mg mL⁻¹. Gentamicin was used as positive control.

Phytochemical screening: The phytochemical screening to identify the major compounds of the extracts was performed based on the methods described previously (Houghton and Raman, 1998).

Mayer and Dragendorff tests (Bruneton, 1999) are used for the identification of alkaloids. The powdered sample (5 g) was extracted with 25 mL of a mixture of ether/chloroform (2:1) after adding 5 mL ammonia. The suspension was macerate 24 h and the filtrate was extracted with 5% HCL. The Mayer reagent was added to the aqueous phase. For the Dragendorff test, a maceration of 10 g of powdered sample in 50 mL H₂SO₄ (10%) was used. The Dragendorff reagent was added to the filtrate. The presence of tannins was detected by boiling 1 g powdered sample in 10 mL distilled water, followed by addition of 1% FeCl₃ to the filtrate. Flavonoids were revealed by the Shinoda test. The 15 mg of dry extract was dissolved in 1 mL of a mixture of 50% ethanol/ concentrated HCl (2:1 v/v). Magnesium turnins were added.

The test for anthocyanins was carried out with the filtrate of the boiled powdered sample (1 g in 10 mL distilled water) by adding drops of 1% HCl and 50% ammonia. The plants were also tested for the presence of anthracene-derivate by boiling 1 g of the powdered sample in 10 mL chloroform for 3 min at 90°C. Ammonia was added to the filtrate to reveal unbounded anthracene-derivate. Quinones were identified by extracting 2 g sample with 20 mL chloroform after adding 2 mL 5% HCl. The solution was filtrated and 5 mL of 50% ammonia was added.

Cyanogenic components were identified by subjecting 0.5 g powdered sample in 10 mL sterile water and filtrated. Sodium picrate paper was added to the filtrate and heated to boil. The Fehling test was performed to detect the presence of reducing sugar. Saponins were detected on the basis of froth upon vigorous shaking.

Mucilages are determined by observing the viscosity after addition of absolute ethanol. The detection of coumarins was carried out by extracting 0.5 g of sample in 10 mL ether. The suspension was filtrated, evaporated and

2 mL distilled water were added. The observation of fluorescence was performed by UV light at 365 nm.

DPPH radical scavenging activity: Antioxidant activity of the different extracts was studied by free radical scavenging by DPPH, as reported by Schmeda-Hirschmann *et al.* (2003). Each experiment was repeated three times and quercetol use as control.

RESULTS AND DISCUSSION

The botanical name, family, plant parts used together with the traditional therapeutic practice for the 6 ethnomedicinal plants tested are showed in Table 1. The antibacterial activities of all six extracts are shown in Table 2. These results are interesting then the ethanolic extracts of all the selected plants showed antibacterial activity by inhibiting the growth of the tested microorganism. *Boswellia dalzielii*, *Psidium guajava*, *Pavetta corymbosa* and *Flacourtia flavescens* exhibited the lowest MIC values (2.5 to 0.313 mg mL⁻¹). The most interesting activity was obtained by *P. guajava* against *S. aureus* with a MIC value of 0.313 mg mL⁻¹. Previous pharmacological investigations indicated that the bark, fruit and leaves possess hypoglycemic, antiinflammatory, analgesic, antipyretic, spasmolytic and antimicrobial activities (Begum *et al.*, 2002; Sanches *et al.*, 2005; Ndukwe *et al.*, 2005). This result should be explained by the presence of alkaloids, tannins and flavonoids (Sanches *et al.*, 2005; Ali and Shamsuzzaman, 1996). *Pseudomonas aeruginosa* was the less sensitive microorganism then the MIC values of all studied extracts were equal or higher than 10 mg mL⁻¹. *Enterococcus faecalis* and *Staphylococcus aureus* were the most sensitive bacteria with MIC values ranging from 0.313 to 2.5 mg mL⁻¹ excepted *Hybanthus enneaspermus* and *Ficus exasperata* extracts. The tested plant extracts were most active against *S. aureus*, a gram-positive bacteria with a MIC of 0.313 mg mL⁻¹ (*Psidium guajava*). *E. Faecalis* was the most sensitive among the gram-negative microorganisms.

The powdered samples were evaluated for qualitative determination of major phytoconstituents. All the plants exhibited different kinds of secondary metabolites.

Phytochemical analysis showed the presence of alkaloids, tannins, flavonoids, anthocyanins, quinones, mucilages, saponins and coumarins and the data has been presented in Table 3.

Presence of tested secondary metabolites of *Hybanthus enneaspermus* is in line with earlier reports, (Majumder *et al.*, 1979) no data are available concerning its biological activities. Sahoo *et al.* (2006) reported

Table 2: Antibacterial activity of ethanol extracts of collected ethnomedicinal plants

Botanical name (Family)	Minimum Inhibitory Concentration (MIC) mg mL ⁻¹			
	<i>P. aeruginosa</i> ATCC 27853	<i>E. Faecalis</i> ATCC 29212	<i>S. Aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922
<i>Hybanthus emeaspermus</i> (Violaceae)	>10	>10	>10	>10
<i>Boswellia dalzielii</i> (Burceraceae)	>10	2.5	2.5	>10
<i>Ficus exasperata</i> (Moraceae)	>10	>10	>10	>10
<i>Flacourtia flavescens</i> (Flacourtiaceae)	>10	0.625	1.5	2.5
<i>Psidium guajava</i> (Myrtaceae)	>10	1.25	0.313	>10
<i>Pavetta corymbosa</i> (Rubiaceae)	10	1.25	2.5	>10
Control				
Gentamicin (mg mL ⁻¹)	0.08	0.02	0.06	0.06

Table 3: Qualitative phytochemical detection

Class of phytochemicals	Species					
	<i>Hybanthus emeaspermus</i>	<i>Flacourtia flavescens</i>	<i>Boswellia dalzielii</i>	<i>Pavetta corymbosa</i>	<i>Ficus exasperata</i>	<i>Psidium guajava</i>
Alkaloids	+	-	-	+	-	-
Tannins	-	++	++	nd	+	++
Flavonoids	+	-	-	+	+	-
Anthocyanins	nd	+	-	-	-	-
Quinones		-	-	-	-	-
Anthracene derivates	nd	-	-	-	-	-
Cyanogenic derivates	nd	-	-	-	-	-
Mucilages	nd	+	+	nd	-	++
Saponins	-	-	-	nd	+	-
Coumarins	nd	+	+	-	-	+
Reducing sugar		-	-	nd	-	-

-: Not found, +: Moderate concentration, ++:Marked concentration, nd: Not determined

significant activity of the ethanolic extract against the same pathogen, with maximum inhibition against *E. faecalis* and minimum against *E. coli* (Sahoo *et al.*, 2006).

For *Flacourtia flavescens*, the qualitative phytochemical analysis showed the presence of tannins, anthocyanins, mucilages and coumarins. Little is known about the antibacterial activity of *Flacourtia flavescens*. The aqueous extract of this specie have revealed early antibacterial activity by inhibiting the growth of *E. coli*, *S. aureus*, *P. aeruginosa* and *E. faecalis* among other microorganisms (Djikpo-Tchiboza, 2004). These results are in accordance with those obtained in present study.

The phytochemical screening of the stem bark of *Boswellia dalzielii* revealed that tannins, mucilages and coumarins were present. These results were in accordance with that obtained by Nwinyi *et al.* (2004) about the presence of tannins and the absence of alkaloids, saponins and anthraquinones (Nwinyi *et al.*, 2004). The tannins and coumarins could be responsible for the antimicrobial activity. In present study the growth of *P. aeruginosa* and *E. coli* was not inhibited at a concentration of 10 mg mL⁻¹. This finding is in agreement with the data of Nwinyi *et al.* (2004) who obtained no inhibition at 2 mg mL⁻¹ (Nwinyi *et al.*, 2004). In other hand the MIC obtained for *S. aureus* were similar (2 mg mL⁻¹ by Nwinyi and 2.5 mg mL⁻¹ in present study). According to Kubmarawa *et al.* (2007), ethanol leaves extract of the same specie showed also antibacterial activity by

inhibiting the growth of *E. coli* (0.50 mg mL⁻¹) while no inhibition was observed with *S. aureus* (Kubmarawa *et al.*, 2007).

For *Pavetta corymbosa*, alkaloids and flavonoids have been found in present study. Alkaloids and polyphenols have been also described in the genus (Arbain *et al.*, 1989; Balde *et al.*, 1990). Few reports existed about the biological activities of *Pavetta corymbosa*. Present results indicated that the ethanol extract inhibited the growth of *E. faecalis* and *S. aureus* with MIC values of 1.5 and 2.5 mg mL⁻¹, respectively. These activities are consistent with those found by Agassounon *et al.* (2001). Alkaloids have exhibited activity against a number of bacterial strains (Matsunaga *et al.*, 1999; Karou *et al.*, 2005). Flavonoids have also been reported to possess antibacterial activity which could be attributed to their ability to form complex with extracellular, soluble proteins and bacterial cell walls (Tsuchiya *et al.*, 1996). In agreement with present results, other studies on leaves extract of *Ficus exasperata* showed the presence of flavonoids, sapononins and tannins (Ayinde *et al.*, 2007; Adebayo *et al.*, 2009). Despite the presence of this phytochemicals, Macfoy and Cline (1990) observed no effect against *Escherichia coli* and *Staphylococcus aureus* (Macfoy and Cline 1990). According to Adebayo *et al.* (2009) the stem bark extract had minimal inhibition at 100, 75 and 50 mg mL⁻¹ against *E. coli*, *S. aureus* and *P. aeruginosa*, respectively (Adebayo *et al.*, 2009). These differences may be due to

the concentration of extracts used to determinate the antibacterial activity. In present preliminary study, *Ficus exasperata* showed antibacterial activity at 100 mg mL⁻¹.

Finally, *Psidium guajava* has extensively been studied. According to Tanaka *et al.* (1992) and Lopes *et al.* (1998) tannins have been isolated in the stem bark and leaves of this plant. The ethyl acetate fraction of the leaves of *P. guajava* contained also tannins (Geidam *et al.*, 2007). In contrary to present results and the results obtained by Geidam *et al.* (2007) and Ali and Shamsuzzaman (1996) Ali reported the presence of alkaloids. The antibacterial activity of the stem bark extract has been demonstrated against several microorganism (Sanches *et al.*, 2005; Ali *et al.*, 1997). Present results indicated a very low susceptibility of *E. coli* and *Pseudomonas aeruginosa*. Ndukwe *et al.* (2005) reported no activity against both bacteria and the data obtained by Sanches *et al.* (2005) were similar to Sanches *et al.* (2005).

DPPH radical scavenging activity of the extracts and the control quercetol are presented in Fig. 1. In this study, the antioxidant activity of the ethanol extracts of the selected plants were investigated by using DPPH radical scavenging assay. The DPPH antioxidant assay is based on the stable free radical DPPH ability, to deize in the presence of antioxidants. The absorbance measure indicates the radical scavenging (antioxidant) power of the extracts.

The tested extracts exhibited a significant dose dependent inhibition of DPPH activity. At 1 µg mL⁻¹, *B. dalzielii* was the most potent with an inhibition percentage of 38% where as quercetol showed 74.57%. The crude extracts of *B. dalzielii* and *P. corymbosa* appeared to be as potent as quercetol with an inhibition percentage of 83 and 75.34% at 10 µg mL⁻¹ which is comparable to 75.9% for quercetol at the same concentration. Except *F. exasperata* which presented a weak activity at the three tested concentrations (15 to 23%), all the tested extracts showed a strong inhibition

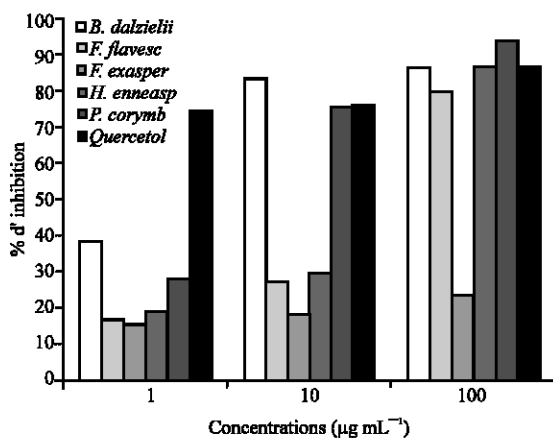


Fig. 1: Free radical scavenging activity (µg mL⁻¹)

percentage (79.55 to 93.71%) comparable to that of quercetol (86%) at the concentration of 100 µg mL⁻¹. These results suggest that these plants possess antioxidant activities which can counteract the oxidative damage in human.

CONCLUSIONS

Antimicrobial and antioxidant studies of Beninese plants, selected base on their use in the folkloric medicine has provided various extracts with strong activity. The plants with the greatest antimicrobial activity, *Boswellia dalzielii*, *Psidium guajava*, *Pavetta corymbosa* and *Flacourtia flavescens*, seem to be of particular interest for further investigations, as they showed high *in vitro* activities towards one or many of the four tested bacteria. *B. dalzielii* and *P. corymbosa* showed also the high scavenging activity with the radical DPPH. The results of this study support the folkloric use of some of these plants. It is still unknown which compounds are responsible for their biological activity; bioassay-guided isolation and identification of the active metabolites of these plants will be process.

ACKNOWLEDGMENTS

The authors wish to thank the traditional healers and traditional plants sellers from Benin for their willingness to share with us their knowledge about plants. The authors wish to thank International Foundation for Sciences (IFS) and the Organization for the Prohibition of Chemical Weapons (OPCW) for financial support for solvents and equipments.

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