Research Article

Evaluation of Antibacterial and Antioxidant Activities of the Crude Extracts of *Albizia ferruginea* (Fabaceae)

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ABSTRACT

Albizia ferruginea is a plant used in traditional medicine in Cameroon for the treatment of microbial and non-microbial diseases. Due to the lack of scientific data to support the ethnopharmacological use, the present work focused on investigating the antioxidant and antibacterial activities of stem and root barks' crude extracts of this plant. Phytochemical screening of different extracts has been performed and these extracts were evaluated for their antioxidant activities using ABTS**,** DPPH and FRAP assays, while the antibacterial assays were evaluated by diffusion method. The phytochemical screening showed the presence of coumarins, saponins, tanins, glycosides, lipids, sugars, alkaloids, resins and phenolic compounds. The results of the antioxidant tests revealed that whatever the solvent, the extract of the stem bark of *A. ferruginea* showed greater activity compared to the roots' extract. Among all the extracts, hydroalcoholic extract of root (AFR3) was found most active against *E. coli* ATCC 25922, *E. coli* ATCC 11775, *S. aureus* BAA 997, *S. aureus* ATCC 25923 and *K. pneumonia* ATCC 700603, with average zone of inhibition of 20 mm. The ethyl acetate extract of root (AFR2) was also active against *K. pneumonia* ATCC 700603 (21 mm). In the case of stem extracts only hydroalcoholic extract (AFS3) exhibited higher activity against *S. aureus* ATCC 25923 (21 mm).

Keywords: Phytochemical screening, Antibacterial, free radical scavenging, *Albizia ferruginea.*

INTRODUCTION

In Africa, the use of remedies derived from plants permit to treat many infectious diseases such as malaria, cholera chlamydia etc.[1]. McGaw et al (1997) reported that about 80 % of the African population consults traditional healers and uses folk medicine for the treatment of various diseases.[2] A scientific evaluation of the plants used by healers is essential before traditional medicine can be incorporated into the officinal healthcare system of Africa. Medicinal plants constitute a major source of bioactive substances.[3] In fact, plants produce a diverse array of secondary metabolites many of which have antibacterial activity. The screening of plant for antimicrobial and antioxidant compounds is therefore very important especially due to the emergence of drug resistant pathogenic strains of microorganisms.[4]

Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms. *Albizia ferruginea* is a plant of the Fabaceae (Leguminosae) and *Albizia* genus, deciduous tree, up to 45 m high.[5] Called 'Evouvous' by Ewondo tribe in the center region of Cameroon, the decoction of its barks mixed with those of Ongokea gore and *Piptadeniastrum africanum* is used as traditional remedy to cure infertility.[6] Leaves decoctions are used externally to treat headache, and as a wash or steam inhalation against fever (including malaria) and toothache. Previous bio-guided studies reported that methanol extract of another species of *Albizia* (*Albizia lebbeck*) exhibited diuretic effect in rodents and had good antibacterial activities.[7-8]

Previously, saponin, flavonoids and diterpenoids were reported as secondary metabolites identified from different species of *Albizia*.[9-11]The aim of this study is to evaluate the antibacterial and antioxidant activities of the crude extracts of AF trunk and root barks.

MATERIALS AND METHODS

Plant Material

Albizia ferruginea was harvested in Yaoundé-Cameroon, (November 2017) and identified at the Cameroon National Herbarium (HNC), where a voucher specimens was deposited (49871/HNC).

Extraction of Plant Material

The powdered root (80 g) and stem bark (70 g) of *Albizia ferruginea* were macerated successively in n-Hexan (n-Hex, 4 L), ethyl acetate (AcOEt, 4 L), ethanol-water (7:3, v/v) (EtOH-H_2O, 4 L), ethanol (EtOH 4 L) and distilled water (H_2O, 4L) at room temperature for 72h. The macerate was filtered and evaporated under reduced pressure to obtain ten extracts (AFR1: 2.6 g), (AFR2: 9.4 g), (AFR3: 11.3 g), (AFR4: 7.1 g), (AFR5: 41.1 g); (AFSB1: 3.0 g), (AFSB2: 6.4 g), (AFSB3: 9.3 g), (AFSB4: 8 g), (AFSB 5: 15.1 g) respectively.

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Phytochemical Screening

The extracts were subjected to phytochemical screening to detect the presence of alkaloids, tannins, saponins, flavonoids, glycosides, sterols, triterpenes, anthraquinones, phenols, cardiac glycosides, sugars, lipids anthocyanins, coumarins and polyphenols using protocols described by Sofowora in 1993.[12]

Antimicrobial activity

Microorganisms Tests

The microorganisms used in this study were isolates provided by the bacteriology laboratory of the University Hospital Center of Yaoundé. Among these strains we have 7 Gram-negative (*Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 11775, *Klebsiella pneumoniae* ATCC 700603, *Neisseria gonorrhoeae* ATCC 49226, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella choleraesuis*, *Proteus mirabilis*) and 4 Gram-positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC BAA 977, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 51299). These strains were stored at 37 ° C on nutrient agar in the Galenic Pharmaceutical Laboratory Microbiology Section of the Department of Galenic Pharmacy and Legislation of the Faculty of Medicine and Biomedical Sciences of the University of Yaounde I.

Stock Solutions and Disc Preparation

One hundred mg of the various extracts to be tested were diluted in 1 mL of dimethylsulfoxide (DMSO) at 1 %. Whatman No. 39 sterile paper disks of 6 mm diameter were impregnated with 2 µL, 10 µL and 20 µL of each stock solution, corresponding respectively to 200 µg, 1000 µg and 2000 µg of extract per disc. Discs impregnated with DMSO were also prepared and they served as negative controls. All the disks were dried in an oven at 37 ° C for. Ciprofloxacin and gentamicin discs have also been used as reference antibiotics for gram positive and gram‾negative microorganisms respectively.

In vitro Antibacterial Screening

The antibacterial activity of the various plant extracts is evaluated by the diffusion method in agar medium as described by Bauer and repeated by Barry.[13-14] From an 18-24 hours' pure culture inoculated on a non-selective solid medium (Mueller Hinton), 1-3 well-isolated colonies were harvested with a platinum loop and suspended in a few milliliters of sterile solution. NaCl 0.9 % for each strain. The turbidity of each suspension is adjusted to 0.5 Mac Farland. After adjusting the turbidity of the inoculum suspension, a sterile cotton swab was soaked in the suspension and then wrung out by pressure spinning. Then the agar surface was seeded by swabbing, i.e., the inoculum was banded three times over the entire agar surface by a three-fold 60 ° rotation of the petri dish. To ensure a homogeneous distribution of the inoculum. The impregnated discs of the various extracts are then gently deposited on the surface of the agar using a pair of pliers. It is the same for the discs of ciprofloxacin and gentamicin. The dishes are first left for 30 minutes at room temperature for diffusion of the substances, before being incubated at 37 ° C for 24 hours.

Antibacterial activity was determined by measuring with a vernier caliper the diameter of the zone of inhibition around each disc.[15] The sensitivity to the various extracts is classified according to the diameter of the zones of inhibition as follows: non-sensitive (-) for the diameter less than 8 mm; sensitive (+) for a diameter between 9-14 mm; very sensitive (+ +) for a diameter between 15-19 mm and extremely sensitive (+++) for the diameter more than 20 mm. The activity of extracts was classified as follows: 0 - 12 mm low activity; 13 – 17 mm moderate activity and > 17 mm high activity.[16]

Antioxidant Activity

ABTS radical activity

The ABTS radical scavenging capacity of the samples was measured with modification to a 96-well microtitre plate format as described by Re et al. (1999) with slight modifications.[17]ABTS radical was generated by reacting 7 mM solution of ABTS and 2.45 mM solution of potassium persulfate at room temperature for 12 h. The ABTS radical stock solution was adjusted to 7.00 ± 0.02 at 734 nm before use. The test samples (40 µL) were made in a concentration range of 0.78 to 100 µg /mL by two fold serial dilutions and 160 µL of ABTS radical solution was added. Absorbance was measured after 6 min at 734 nm. Trolox and ascorbic acid were used as positive controls, methanol as negative control and compound without ABTS as blank. Percentage of ABTS•+ inhibition was calculated using formula:

Scavenging capacity (%) = 100 - [(absorbance of sample - absorbance of sample blank) × 100/(absorbance of control) - (absorbance of control blank)]. The IC_{50} values were estimated from the percent inhibition versus concentration plot derived from the percentage scavenging activity.

DPPH assay

The DPPH radical-scavenging activity was determined using the method proposed by Brand-Williams et al. (1995), with some modifications to 96-well microtitre plate.[18] Various concentrations of compounds in methanol were prepared (7.81 to 1000 µg /mL). Ascorbic acid and trolox were used as a positive control at concentration of 100 to 0.78 µg/mL. Then, 160 µL of DPPH (0.037 mg/mL) in methanol was added to 40 µL of the test solution, or standard, and allowed to stand at room temperature in a dark for 30 min. Methanol was used as a blank. The change in colour from deep violet to light yellow was then measured at 517 nm using a Versamax microplate reader. Results were expressed as percentage reduction of the initial DPPH absorption in relation to the control. The concentration of compound that reduced DPPH colour by 50 % (IC_{50}) was determined as for ABTS•+.

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Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was carried out according to the procedure of Benzie and Strain (1996) with slight modifications.[19] Briefly, the FRAP reagent was prepared from acetate buffer (pH 3.6), 10 mmol 2,4,6-tripyridyl-S-triazine (TPTZ) solution in 40 mmol HCl and 20 mmol iron (III) chloride solution in proportions of 10:1: 1 (v/v), respectively. The FRAP reagent was freshly prepared and was warmed to 37 º C in a water bath prior to use. Fifty microliters of sample were added to 1.5 mL of the FRAP reagent. The absorbance of the reaction mixture was then recorded at 593 nm after 4 min. The standard curve was constructed using $FeSO_4$ solution (0.1-2 mM), and the results were expressed as µmol $FeSO_4$/g dry weight of compound. All the measurements were taken in triplicate and the mean values were calculated.

RESULTS

Phytochemical screening

The preliminary phytochemical screening of different crude extracts revealed the presence of phenols, steroids, flavonoids, coumarins, saponins, tanins, and lipids (Table 1). This result can be explained by the fact that during maceration, secondary metabolites are separated according to their affinity and solubility with extraction solvent.[20] The presence of all these classes of secondary metabolites in extract of root and stem back of *Albizia ferruginea* is an indication of its pharmacological importance.

Antibacterial assay

The bacterial inhibition zone diameters of crude extracts are summarized in table 2. The results below indicated that the inhibition zone diameters vary from 0.00 ± 0.00 to 21.00 ± 0.00 mm.

The activity of extracts was classified as follows: 0-12 mm low activity; 13-17 mm moderate activity and > 17 mm high activity.[16] Following this classification, the extract AFR1 showed moderate activity on six bacteria strains with inhibition diameter ranging between 14-16 mm. Extract AFR2 exhibited moderate activity on two bacterial strains with inhibition diameter 14 mm and high activity on *K. pneumonia ATCC* 700603 with inhibition diameter 21.0±0.0 mm. The hydroethanolic extract (AFR3) exhibited the high activity on five bacteria strains (*E. coli* ATCC 25922, *S. aureus* ATCC 25923, *K. pneumonia ATCC* 700603, *E. coli* ATCC 11775, *S. aureus* BAA 997) with inhibition diameter ranging between 18-20 mm. Similarly, the extract (AFR4) showed moderate activity on seven bacteria strains with inhibition diameter 14-17 mm. Whereas the extract (AFR5) showed moderate activity only on four bacteria strains with the same inhibition diameter (14-17 mm). AFSB1 and AFSB2 extract showed moderate activity on four and five bacteria strain respectively with inhibition diameter range 13-15 mm. AFSB3 extract exhibited high activity on *S. aureus* ATCC 25923 with inhibition diameter 21.0±0.0 mm and moderate activity on five another bacteria strains with inhibition diameter ranging between 14-15 mm. The extract AFSB4 showed moderate activity on three bacteria strains with inhibition diameter ranging between 16-17 mm (Table 2).

Antioxidant activity

The use of at least two different assays in evaluating antioxidant activity of plant products has been recommended by Moon and Shibamoto, (2009) [21]. From the dose-response activities, the IC_{50} values were obtained and presented in Table 3. The IC_{50} values for the different extracts ranged from 0.48 µg/mL to 2.87 µg/mL in DPPH assay, from 2.53 µg/mL to 17.04 µg/mL in ABTS assay and from 5.09 µg/mL to 76.01µg/mL in FRAP assay (Table 3).

Table 1: Phytochemical constituents of different extracts

Secondary metabolites	Extract from root bark									
	AFSB1	AFSB2	AFSB3	AFSB4	AFSB5	AFR1	AFR2	AFR3	AFR4	AFR5
Terpenoids	-	-	-	+	+	-	-	-	-	+
Steroids	++++	-	-	-	-	++++	++++	++++	+++	-
Alkaloids	++	-	-	-	-	+	+	+	-	-
Free flavonoids	++++	++++	+	++++	+	+++	+	-	++++	+
Phenols	+++	++++	+	++++	-	+	-	-	++++	++
Coumarines	-	++++	-	-	-	++++	++++	-	++++	-
carbohydrates	+	+	+	-	-	-	-	-	+	+
Tanins	+++	-	++++	++++	-	++	-	-	++++	-
Saponosides	-	+++	++++	++++	+	-	-	-	++++	++
Resines	++++	-	-	-	+++	-	-	-	-	-
Anthocyanes	-	-	-	+	+	-	-	-	-	-
Cardiotonicheterosides	-	-	-	-	-	-	-	-	-	-
Lipids	+++	++++	++++	+++	-	-	-	-	+++	-

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Table 2: Bacteria inhibition zone diameters of different extracts

Strain/Extracts	Microorganisms and inhibition zone diameter ± S.D (mm)											
	AFR1	***AFR2***	***AFR3***	***AFR4***	***AFR5***	***AFSB1***	***AFSB2***	***AFSB3***	***AFSB4***	***AFSB5***	**Genta**	**Cipro**
E. coli ATCC 25922	14.0±0.0	11.0±0.0	20.0±0.0	15.0±0.0	0.0±0.0	15.0±0.0	14.0±0.0	15.0±0.0	9.0±0.0	10.0±0.0	/	25.0±0.0
S.aureus ATCC 25923	9.0±0.0	14.0±0.0	20.0±0.0	14.0±0.0	0.0±0.0	13.0±0.0	15.0±0.0	21.0±0.0	16.0±0.0	00.0±0.0	30.0±0.0	/
K.pneumonia ATCC 700603	15.0±0.0	21.0±0.0	20.0±0.0	17.0±0.0	14.0±0.0	0.0±0.0	12.0±0.0	11.0±0.0	16.0±0.0	9.0±0.0	28.0±0.0	/
E.coli ATCC 11775	14.0±0.0	11.0±0.0	20.0±0.0	15.0±0.0	5.0±0.0	15.0±0.0	14.0±0.0	15.0±0.0	9.0±0.0	10.0±0.0	/	26.0±0.0
S.aureus BAA 997	16.0±0.0	10.0±0.0	18.0±0.0	15.0±0.0	5.0±0.0	11.0±0.0	9.0±0.0	14.0±0.0	17.0±0.0	11.0±0.0	23.0±0.0	/
S.aureus ATCC 29213	8.0±0.0	11.0±0.0	0.0±0.0	0.0±0.0	17.0±0.0	12.0±0.0	9.0±0.0	0.0±0.0	10.0±0.0	9.0±0.0	26.0±0.0	/
Salmonella choleraesis	14.0±0.0	14.0±0.0		14.0±0.0	14.0±0.0	0.0±0.0	13.0±0.0	11.0±0.0	9.0±0.0	0.0±0.0	/	40.0±0.0
Proteus mirabilis	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	14.0±0.0	14.0±0.0	14.0±0.0	9.0±0.0	0.0±0.0	/	20.0±0.0
Enterococcus ATCC51229	14.0±0.0	14.0±0.0		14.0±0.0	14.0±0.0		12.0±0.0	14.0±0.0	11.0±0.0			
Pseudomonas aeruginosa ATCC 10145	6.0±0.0	2.0±0.0	7.0±0.0	0.0±0.0	6.0±0.0	8.0±0.0	5.0±0.0	0.0±0.0	6.0±0.0	0.0±0.0	/	25.0±0.0

Extracts (Root) : AFR1 : n-hexan ; AFR2 : Ethyl acetate ; AFR3 : Water-ethanol ; AFR4 : Ethanol ; AFR5 : Aqueous Extracts (Stem back): AFSB 1: n-hexan ; AFSB 2 : Ethyl acetate ; AFSB 3 : Water-ethanol ; AFSB 4 : Ethanol; AFSB 5 : Aqueous; Genta: Gentamicine; Cipro: Ciprofloxacine;

Table 3: Antioxidant Activity

Extracts	**DPPH IC_{50} (µg/mL)**	**ABTS IC_{50} (µg/mL)**	**FRAP IC_{50} (µg/mL)**
AFR1	1.12±0.10	4.13±0.10	72.41±2.02
AFR2	-	-	22.98±4.29
AFR3	-	-	6.43±4.21
AFR4	0.48±0.02	2.53±0.49	10.98±13.70
AFR5	-	-	17.43±4.25
AFSB1	0.9±0.06	3.53±0.29	76.01±1.10
AFSB2	2.08±0.19	17.04±0.29	7.34±1.06
AFSB3	-	-	5.09±0.40
AFSB4	1.12±0.10	4.13±0.10	8.41±2.02
AFSB5	-	-	22.98±4.29
Trolox	5.36±0.10	3.71±0.21	Nd
Ascorbic acid	2.80±0.03	2.61±0.08	Nd

DISCUSSION

The observed antimicrobial activity was certainly due to the presence of various classes of secondary metabolites within the crude extracts and these metabolites may exert their inhibitory effect through different mechanisms. In fact, it is known that tannins exert their antimicrobial activity by binding with proteins and adhesins, inhibiting enzymes, complexation with the cell wall and metal ions, or disruption of the plasmatic membrane.[20] On the other hand, saponins have the ability to cause leakage of proteins and certain enzymes from the cell.[22] The sensitivity of steroids and the membrane lipids indicate their specific association that causes leakage from liposomes.[23] Flavonoids have the ability to complex with proteins and bacterial cells forming irreversible complexes mainly with nucleophilic amino acids. This complex often leads to inactivation of the protein and loss of its function.[24-25] Hence, the presence of these compounds in *Albizia ferruginea* may explain the antibacterial activities observed. However, further studies are needed to isolate and characterize the active ingredients responsible for the efficacy of the most active extracts. The antibacterial effects of *Albizia ferruginea* extracts against 7 gram negative (*Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 11775, *Klebsiella pneumoniae* ATCC 700603, *Neisseria gonorrhoeae* ATCC 49226, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella choleraesuis*, *Proteus mirabilis*) and 4 Gram-positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC BAA 977, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 51299, *E Coli* ATCC 25922 suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhoea in man, skin diseases and fungal infections.[26] The high potency of *Albizia ferruginea* against these bacteria gives scientific basis for its use in folk medicine in the treatment of abscesses, bilious conditions, cough, dysuria, diarrhea and

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candidiasis scurvy.[27-28] Successive isolation of active compounds from plants depends upon the plant part and type of solvent used in extraction procedure.[29] Our results showed that the activity is mainly concentrated in the extracts from hydroalcoholic extracts of root and stem bark, indicating that the potential antibacterial compounds were in the high polarity extract.

The antioxidant activity of extracts can be determined *in vitro* by hydrogen atom transfer (HAT) method and single electron transfer (SET) method. HAT methods measure the capacity of an antioxidant to scavenge free radical by hydrogen donation to form a stable compound. SET methods determine the ability of the antioxidant to transfer one electron to reduce compound including metals, carbonyls and radicals.[30] FRAP assay involves SET method, while DPPH and ABTS assay involve both method predominantly via SET method.[31] In this study, the antioxidant activity of extracts was determined using the free radical 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the ferric reducing antioxidant power (FRAP) assays.

The IC_{50} values AFR4, AFBS2 and AFBS4 were significantly different from the IC_{50} of ascorbic acid and trolox, standard antioxidant agents used as a positive control. The capacity of flavonoids to act as antioxidants *in vitro* has been previously studied.[32] AFR4, AFSB2 and AFSB4 exhibited the highest DPPH inhibitory activity among the extracts while AFR2, AFR3, AFR5, AFSB3 and AFSB5 were not potent in scavenging the DPPH radicals. Almost similar results were obtained with ABTS radical activity unless that AFR4 had the highest values while AFSB2 was less effective one. The scavenging of the ABTS+ radical by the extracts was found to be much higher than that of DPPH radical. Many factors such as the stereoselectivity of the radical, the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals.[33] The IC_{50} values in the FRAP assay of the different extracts of the roots and stem bark of *A. ferruginea* were significantly lower than that of ascorbic acid and trolox. This result indicated that *A. ferruginea* may be useful therapeutic agents for treating radical-related pathological damage.

CONCLUSION

In this study, we studied for the first time the phytochemical composition, antimicrobial and antioxidant activities of the ethanolic, hydroalcoholic and aqueous extracts of the roots and stem bark of *Albizia ferruginea.* This research showed that roots of this plant may possess considerable antioxidant and antimicrobial activities compared to the rest of the entire plant. In the near future, we intend to use the potent extract for the isolation of active compounds that may be valorized as alternative source of antibacterial or antioxidant potential.

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