

Original Article

ACACIA ATAXACANTHA (BARK): CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE EXTRACTS

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ABSTRACT

Objective: The present work aimed to investigate the phytochemical constituents and antibacterial activity of several extracts of *Acacia ataxacantha* bark on pathogenic microbes.

Methods: The phytochemical study was performed using tube test and Thin layer chromatography methods. The growth inhibitory effect and the Minimum Inhibitory Concentration of hexane, dichloromethane, ethyl acetate, methanol and hydroalcoholic extracts were determined using the microplate dilution method.

Results: Phytochemical screening revealed the presence of alkaloid, tannins, lignan, triterpenoids, anthracene derivatives, flavonoids, saponins and coumarins at different level. The extracts exhibited different effect against the tested bacteria. The minimum inhibitory concentrations of extracts ranged from 325 µg/ml to 5 mg/ml. Ethyl acetate extract was more potent than other extracts with the MIC values of 325 µg/ml against *S. aureus* and 625 µg/ml against all other tested bacteria. *Escherichia coli* was resistant to all extracts.

Conclusion: These findings suggest that the rich phytochemical content of *Acacia ataxacantha* and its good antibacterial activity may be responsible for its popular and wide traditional use.

Keywords: *Acacia ataxacantha* bark, Phytochemical constituent, Antibacterial activity.

INTRODUCTION

Infectious diseases are becoming a crisis as a major cause of mortality and human and animal morbidity [1]. They cause massive mortality in developing countries due to the extreme poverty of the population compared to developed countries. This situation is aggravated by the lack of appropriate vaccine, inaccessibility and/or the absence of antibiotics and the emergence of antibiotic resistant strains. Many efforts have been made by researchers to discover new antibacterial compound. One important source of discovery of the antibacterial drugs is medicinal plants through traditional medicines. Traditional treatments have been investigated and a significant success was achieved. In addition, it is known that, unlike synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to cure many infectious diseases [2, 3].

The use of herbal medicine in Benin is a long history of human interaction with the environment. The plants used in traditional medicine contain a wide range of substances that can be used to treat chronic and infectious diseases. *Acacia* species have had a long history of medicinal use in the treatment of diarrhea, urinary infections, throat inflammation, gastritis, tuberculosis and headaches [4].

Acacia Ataxacantha (Fabaceae) is widespread in much of sub-Saharan Africa. This species is a very thorny shrub scandent 5 to 8 m in height. The leaves are alternate, pinnate with spine that carries 5 to 12 pairs of pinnae. On twigs, spines are short, clearly pointing down. The white flowers with a long transition axillary 4 to 5 cm long and arranged on stem 10 to 15 mm are sometimes isolated in pairs. The fruit pods are flattened, brownish red in the dry state. The dough sheet is used topically in the treatment of abscesses. The leaf decoction is used orally in febrile convulsions. Its bark is used against tooth decay, by inhalation in case of bronchitis and cough [5]. In Nigeria, pods and seeds of *Acacia ataxacantha* were also used against dysentery [6]. The roots are used in Kenya in the treatment of joint and back pain [7]. *Acacia ataxacantha* is also known for the treatment of pneumonia [8].

In the best of our knowledge, there is no scientific information concerning the in vitro antibacterial activity of this medicinal plant.

In our search for natural products with antibacterial activity, we investigated a traditional plant used in Benin (West Africa) to treat infectious diseases. Polar and apolar extracts were prepared from selected plant and then evaluated for in vitro antibacterial activity against four Gram positive and two Gram negative bacteria. First, by assessing the ability of each extract to inhibit the growth of bacteria at a concentration of 10 mg/ml, secondly, by determined the Minimum Inhibitory Concentration and total activity of extracts. The chemical constituent of the plant was also determined.

MATERIALS AND METHODS

Plant material

The barks of *Acacia ataxacantha* were collected in September 2012 from Ouidah, Department of Atlantic, South Bénin. Botanical determination was performed by taxonomists from the Herbar National of Abomey-Calavi University in Benin and voucher specimens were deposited at the same herbarium (AA 6509/ HNB). The collected material was dried for two weeks in the laboratory (22°C) and ground to a fine powder using an electric grinder (Excella mixer grinder).

Preparation of the extracts

Two hundred and fifty grams (250 g) of dry powder of the barks of *Acacia ataxacantha* were successively extracted by maceration with hexane, dichloromethane, ethyl acetate and methanol for 72 h stirring. Fifty grams (50 g) of dry powder was also extracted with a mixture of ethanol-water (20:80). Each extraction is repeated three times. The macerates were filtered and concentrated using a rotary evaporator (RE 300, stuart) and the extracts were stored at 4° C until biological assay.

Phytochemical analysis

Phytochemical screening of the plant was carried out according to the methods described by Wagner and Blat [9] and Bruneton [10]

for the detection of plant secondary metabolites. Tannins, alkaloids, flavonoids, steroids, coumarins, saponins, naphthoquinones, triterpenes, lignans, pigments, anthracene derivatives have been investigated using tube test. Each extract (10 mg/ml) were deposited on TLC plate to confirm the results.

Antibacterial activity

Bacterial strains

Several extracts of *acacia ataxacantha* bark were individually tested against a panel of bacteria including four Gram-positive: *Staphylococcus aureus* (ATCC 6538), *S. epidermidis* (CIP8039), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* Methicillin Resistant (SARM) and two Gram-negative: *Escherichia coli* (CIP 53126) and *Pseudomonas aeruginosa* (CIP82118) obtained from Laboratoire de Biophotonique et Pharmacologie, University of Strasbourg, France.

Growth inhibition effect of extracts at 10 mg/ml

Sensitivity of different bacterial strains to various extracts was determined using 96-well microplate. The aim of this method was to eliminate the extracts, which at 10 mg/ml do not inhibit the growth of bacteria [11]. 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 (10⁶CFU/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 active extracts. Active extract do not change color.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentrations (MICs) of antibacterial (sensitive bacterial) activity were determined by the method of broth microdilution using *p*-iodonitrotetrazolium (INT) as an indicator of bacterial viability [12]. To determine the MIC of extracts, 100 µL of Mueller Hinton broth (DIFCO) were added to each wells of a 96-wells microplate and then 100 µL of plant extract (20 mg/ml) were added to the first well (A) of the plate. A two-fold successive dilution was carried from well (A) to the last wells (H) of the plate. Then, 100 µL of bacterial broth at 10⁶CFU/ml were finally added into all the wells. The plate was covered and incubated at 37°C. After 18 h of incubation, 40 µL of *p*-iodonitrotetrazolium (0.2 %) was added in all wells and the plates were incubated again at 37°C. After 1 h of incubation, wells were examined and the MIC values were recorded.

Total activity

To select the extracts that can be used for further testing, the determination of the total activity is important because since the MIC value is inversely proportional to the amount of antimicrobial extracts. The total activity of each extract was calculated by dividing the MICs with the amount of extract obtained from 1 g of plant material [13]. This value indicates the volume in which the active principle (extract) present in 1 g of dry plant material can be diluted to always have inhibitory activity against organisms [14].

Table 1: Phytochemical constituents of bark extracts from *A. ataxacantha*

Phytochemical component	Plant extracts				
	Hex	CH ₂ Cl ₂	AcOEt	MeOH	H ₂ O/Et
Coumarin	+	+	+	+	+
Flavonoid	-	+	++	+	+
Naphthoquinone	-	++	++	-	-
Alkaloid	-	+	+	+	+
anthracene derivative	-	+	+	+	+
saponin	-	-	+	+	+
lignan	+	+	+	++	+
triterpene	-	++	+	+	+
tannin	-	+	+	+	+
Pigment	+	+	+	+	-

Hex: hexane, CH₂Cl₂: dichloromethane, AcOEt: ethyl acetate, MeOH: Methanol, H₂O/Et: hydroalcolic; (+) = present in moderate concentration, (++) = present in high concentration, (-) = indicates the absence of the compound tested.

Table 2: Antibacterial activity of bark extracts from *A. ataxacantha* at 10 mg/ml

Extracts	Growth inhibition effect of extract at 10 mg/ml					
	Gram (+)			Gram (-)		
	<i>S. aureus</i>	<i>S. a. m. r.</i>	<i>S. epidermidis</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Hexane	-	-	-	-	-	-
Dichloromethane	-	-	-	+	-	+
Ethyl acetate	+	+	+	+	-	+
Methanol	-	-	-	+	-	-
Hydroalcolic	-	-	-	+	-	-

+: sensitive; -: not sensitive; *S. a. m. r.*: *Staphylococcus aureus* methicillin resistant

Table 3: Minimum Inhibitory Concentration and total activity of various extracts from *A. ataxacantha*

Extracts	Minimum Inhibitory Concentration (mg/ml)				
	Gram (+)			Gram (-)	
	<i>S. aureus</i>	<i>S. a. m. r.</i>	<i>S. epidermidis</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>
Dichloromethane	-	-	-	0.625	0.625
Ethyl acetate	0.325	0.625	0.625	0.625	0.625
Methanol	-	-	-	2.5	-
Hydroalcolic	-	-	-	2.5	5
Total activity (ml)					
Dichloromethane	-	-	-	8.02	8.02
Ethyl acetate	43.32	22.53	22.53	22.53	22.53
Methanol	-	-	-	16.99	-
Hydroalcolic	-	-	-	41.36	20.68

-: not sensitive; *S. a. m. r.*: *Staphylococcus aureus* methicillin resistant

RESULTS

Phytochemical analysis

Phytochemical Constituents extracts of *A. ataxacantha* are shown in Table 1. On the whole, coumarins, flavonoids, alkaloids, derivatives of anthracene, lignans, tannins and triterpenes were identified in all extracts with the exception of the hexane extract. The ethyl acetate extract gave a positive result for all groups secondary metabolites investigate. Phytochemical tests have also shown the absence of saponins and naphthoquinone respectively in dichloromethane and methanol extracts.

Antibacterial activity

Growth inhibition effect of extracts at 10 mg/ml

The purpose of this test was to eliminate the extracts did not inhibit the growth of bacteria at 10 mg/ml. In this study, both polar as well as non polar solvents were used for the extraction of active components from the bark of *Acacia ataxacantha* plant. The results (Table 2) showed that the tested bacteria are sensitive to one or more extracts. However, the dichloromethane, ethyl acetate, methanol and hydroalcolic extracts inhibited the growth of *E. faecalis*. It was noticed that the ethyl acetate extract inhibited the growth of all strains tested except *E. coli* while dichloromethane extract inhibited *P. aeruginosa*. The hexane extract of *A. ataxacantha* showed no inhibition of bacterial growth. The growth inhibitory activity exercised by dichloromethane and ethyl acetate extracts on the most studied strains (five germs out of six or 80% of cases for ethyl acetate extract and two germs on 6 or 35% of cases for dichloromethane extract) reflects the reality of an inherent antimicrobial property to *A. ataxacantha* bark with an antibacterial spectrum of action that affects indiscriminately Gram positive and Gram negative bacteria

Minimum Inhibitory Concentration (MIC) and total activity

The bacterium growth inhibition produced by *A. ataxacantha* extracts varied in relation to the type of extract and to the bacterium strain used. The Minimum Inhibitory Concentrations (MICs) and total activity of extracts are recorded in Table 3. The extracts showed MIC values ranging from 0.325 to 5 mg/ml. Ethyl acetate extract showed interesting activity against *S. aureus* with MIC values 325 µg/ml. A MIC of 625 µg/ml was obtained with the same extract on the following strains: *S. aureus* meticillin resistant, *S. epidermidis*, *E. faecalis* and *P. aeruginosa*. The dichloromethane extract of *A. ataxacantha* inhibits the growth of *E. faecalis* and *P. aeruginosa* with a concentration of 625 µg/ml. Regarding the methanolic and hydroalcolic extracts, the minimum inhibitory concentration was respectively 2.5 mg/ml on *E. faecalis* and 5 mg/ml on *P. aeruginosa*.

The extracts with higher total activity (TA) values are considered the best. The most interesting total activities of *A. ataxacantha* were obtained with ethyl acetate (43.32 ml) and hydroalcolic (41.36 ml) extracts against *Staphylococcus aureus* and *E. faecalis* respectively.

DISCUSSION

Medicinal plants are the basis of therapeutic treatments in developing countries. Recent years have also seen an increase in the use of herbal medicines in developed countries. The plants are used medicinally in different countries of the world and are a good source of many potent and powerful drugs [15]. The resistance of pathogens to antibiotics commonly used, the increase in opportunistic infections and the effect of toxicity due to the continued use of several drugs have led to increased attention paid to the search for new therapeutic agents from various sources, including plants, which are good starting materials for the discovery of new antimicrobial agents [16, 17]. Secondary metabolites produced by plants constitute a source of bioactive substances. All over the world, plants scientist interest has increased due to the search for new drugs from plant origin. Secondary metabolites of plant have been reported to serve as defense mechanisms against predation by many microorganisms, insects and herbivores [18].

In the present study, phytochemical investigation and antibacterial activity were carried out on the barks of *Acacia ataxacantha*, an herb

use traditionally in Benin to treat numerous diseases. Several species of genus *Acacia* have been studied and it has been demonstrated that they have hypoglycemic [19] anti-aggregation platelet effect [20], antigenotoxic and antimicrobial [21], antihypertensive, antispasmodic [22, 23] and anti-inflammatory activity [24].

The phytochemical examination of *Acacia ataxacantha* extracts indicated the presence of tannins, flavonoids, triterpenes, coumarins, lignans, pigments, saponins, alkaloids, anthracene derivatives and naphthoquinones. Some secondary metabolites have been reported in the literature to have interesting pharmacological activities.

Our results showed that MIC values of extracts range from 0.325 to 5 mg/ml. Ethyl acetate extract was the most active with MIC value of 325 µg/ml against *S. aureus*. The activity of the ethyl acetate extract is higher compared to other extracts. This may be due to the capacity of ethyl acetate to extract compounds which have antibacterial properties. The dichloromethane extract of *A. ataxacantha* inhibited the growth of *Pseudomonas aeruginosa* with a MIC of 625 µg/ml. This means potent antibacterial activity of the dichloromethane extract considering the multidrug resistance of *P. aeruginosa* (gram negative bacteria) which is responsible for many nosocomial infections. The development of multidrug-resistant of *P. aeruginosa* is currently one of the biggest challenges to the effective management of infections [25]. The variation in the effectiveness of the extracts against different microorganisms may be attributed to the phytochemical composition of the extracts and/or membrane permeability of the microbes for the chemicals and their metabolism.

Phytochemical study of ethyl acetate and dichloromethane extracts revealed the presence of tannins, flavonoids, triterpenes, coumarins, lignans, pigments, saponins, alkaloids, anthracene derivatives and naphthoquinones except saponins in dichloromethane extract. These results could justify the antibacterial activity of these extracts. Several studies have demonstrated the antibacterial potential of these secondary metabolites. Tannins inhibit the microbial growth by causing the bacterial colonies to disintegrate which probably results from their interference with the bacterial cell wall [26]. They precipitate proteins covering the surface of the cell or tissue, which acts as a barrier between tissue and irritants, and the underlying tissue is therefore soothed and protected from further damage, so that healing can take place [27]. Flavonoids have been found to exhibit antimicrobial activity through various mechanisms like inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function and energy metabolism [28]. Plants containing tannins and flavonoids are used in the treatment of diarrhoea and dysentery [29, 30]. The phytochemical analysis of extracts also revealed the presence of naphthoquinones in dichloromethane and ethyl acetate extracts while the methanol and hydroalcolic extracts did not contain. The antibacterial effect of dichloromethane and ethyl acetate extracts could be due or accentuated by the presence of naphthoquinones. Some studies confirm the antibacterial effect of naphthoquinones [31, 32]. Generally, naphthoquinones are activated inside the microbial cells and become covalently attached to the cellular nucleophiles such as proteins and basic parts of DNA, often leading to inactivation of proteins and loss of function [33]. Others authors have also reported the antibacterial effect of triterpenes [34, 35]. Their antimicrobial activity has associated with among other bacterial cell membrane disruption by the lipophilic compounds [36].

The presence of saponins in the ethyl acetate and hydroethanolic extracts and its absence in dichloromethane extract indicates that saponins do not play an essential role in inhibiting bacterial growth. Indeed, the mode of action of antibacterial effects of saponins seems to involve membranolytic properties [37]. They possess detergent like activity and might increase the permeability of the bacterial cell membrane without destroying them. In theory, this activity might facilitate antibiotic influx through the bacterial cell wall membrane [38].

The presence of active chemical groups such as tannins, naphthoquinones, triterpenes and flavonoids in the studied extracts could justify interesting results obtained and the indications of this plant in traditional medicine, particular for its antibacterial properties. Our results also confirm the antibacterial activity of *A. ataxacantha* as

a broad spectrum antimicrobial agent, since it inhibits the growth of Gram-positive and Gram-negative bacteria. Thus, these results support the use of *A. ataxacantha* in traditional medicines for the treatment of infections caused by bacteria tested in our study.

CONCLUSION

The acacia species used in this study had never been evaluated or only partially studied for antibacterial activity. At least, this plant seems to be of particular interest for further investigation, as it is effective against both gram-negative and gram-positive bacteria. It is an indication that the plant can be a source of bioactive substances. In addition, our results support the idea that herbal medicines with medicinal value have a promising future in regard to the discovery of new substances able to participate in the development of pharmaceutical products based drugs or improved traditional plant medicines (MTA). Bioguided fractionation of the corresponding extracts is under process.

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CONFLICT OF INTERESTS

Declared None

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