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Evaluation of antibacterial properties of flavonoid fraction from *Antiaris africana* (Engl)

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ABSTRACT

Objective: To investigate the antibacterial properties of the flavonoid fraction of stem bark extract of *Antiaris africana* used ethnobotanically in Nigeria to relieve rheumatic, respiratory and stomachic pains.

Methodology and results: The stem bark of *Antiaris africana* and clinical isolates of *Bacillus subtilis*, *Streptococcus pyogenes* and *Escherichia coli* were used in this study. The extract from *A. africana* was screened for phytochemical properties. The extracted flavonoid fraction was assayed using the agar diffusion method. The zones of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were recorded. The flavonoid fraction exhibited antibacterial activity against *B. subtilis, S. pyogenes* and *E. coli*. The MIC against *B. subtilis*, *S. pyogenes* and *E. coli* were 0.04, 0.050 and 0.050 mg/ml, respectively.

Conclusions and application of findings: The extract of *Antiaris africana* (stem bark) was found to contain flavonoids that exhibited antibacterial activity. This justifies the ethnobotanical use of stem bark extract of *Antiaris africana* to relieve respiratory and stomach pains. The flavonoid fraction from *A. africana* could be used as source of antibacterial agents.

Key words: *Antiaris africana*, antibacterial properties, ethnobotanical use, flavonoid fraction, phytochemical properties

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INTRODUCTION

Plants are sources of bioactive phytocompounds and they are used directly as therapeutic agents, as well as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (Cowan, 1990). There is growing interest in the use of natural substances, generally known as bioactive phytocompounds, as antimicrobial agents (Fabricant & Farnsworth, 2001). This need is prompted by various reasons including inadequate access to drugs, incidents of epidemic drug resistant microorganisms and emergence of hitherto unknown diseases (Mann, 2007). Antimicrobial resistance is one of the biggest challenges facing global public health (Ibezim, 2005). The emergence of multidrug-resistant isolates in tuberculosis, acute respiratory infections, and diarrhea and epidemic of HIV/AIDS has had the greatest toll in developing countries (Mann, 2007).

Infectious diseases account for approximately one-half of all deaths in tropical countries (Iwu *et al.,* 1999). These challenges

have aroused interest and intensified the search for new antimicrobial drugs of plant origin to manage infectious diseases. The screening of plant extracts for antimicrobial activity has shown that higher plants represent potential sources of novel antimicrobial agents (Kinghorn, 1994; Butler, 2005). Phytomedicines have shown great promise in the treatment of intractable infectious diseases (Hostettmann *et al*., 2000; Cragg & Newman, 2005). Some natural products have been approved as new antimicrobial drugs, but there is an urgent need to identify novel substances that are active against pathogens with high resistance (Cragg *et al.,* 1997).

Flavonoids are hydroxylated phenol substances that occur as a C6 – C3 unit linked to an aromatic ring. They are 15-carbon compounds which occur naturally and are widely distributed in the plant kingdom appearing in flowers, fruits, stems, leaves, roots and plant derived beverages such as tea and wine (Whiting, 2001). These are ubiquitous in occurrence in nearly all plants; the ease with which they are isolated and identified even from small amounts of plant materials as well make this chemical the most used for medicinal purposes. Flavonoids occur naturally in plants as agents of protection against external pathogens, thus possess pharmacological activities (Whiting, 2001). The most important class of flavonoids include: anthocyanidins, flavones, flavonoids, flavanones, flavan-3-01(also known as catechins) (Kuhuau, 1976; Whiting, 2001). Flavonoids possess anti-inflammatory properties, e.g. Miski *et al*. (1983) reported the antibacterial activity of flavonoids from *Salvia palestina*, and they also act as modulators of the immune system in several biological systems. This stems from the fact that they are powerful antioxidants protecting biosystems against damaging effects of free radicals. For instance, green tea contains a mixture of catechin compounds which exert antimicrobial activity (Sakanada *et al*., 1992). Catechin compounds are known to inhibit *Vibrio cholerae* 01 *in vitro* (Borris, 1996) and *Streptococcus mutans* (Batista *et al*., 1994). Most flavonoids belong to a group of chemicals, called polyphenols, and their antioxidant properties are dependent on this polyphenolic chemical structure.

Africa is endowed with enormous biodiversity resources, some of which contain flavonoids that have been found to be active against a wide variety of microorganisms (Malcolm & Sofowora, 1969). Atawodi (2005) reviewed the antioxidant potentials of numerous plants of African origin and found that many natural antioxidant compounds are present in African foods and medicinal plants.

Antiaris africana belongs to the family Moraceae, the plant is commonly called “Oro” among the “Yoruba” speaking people of Western Nigeria and “Ojianwu” among the “Ibo” speaking people of Eastern Nigeria. The plant is a large tree with heavy flat crown and blotchy grey and white bark. The small, greenish white flowers yield red, velvety fruits (Gill, 1992). The stem bark of *Antiaris Africana*, when ground with fresh pepper (*Capsicum annum*) and alligator pepper (*Aframomum melegueta*) into a paste is ethnobotanically used in Nigeria to relieve rheumatic, respiratory and stomachic pains (Gill, 1992; Mann *et al*., 2003).

The objective of this study was to investigate the antibacterial properties of the flavonoid fraction extracted from *A. africana*.

MATERIALS AND METHODS

Plant material: The stem bark of *Antiaris africana* was collected at Doko village, Niger State, Nigeria. The plant was authenticated at the Department of Plant Science, University of Ilorin, Nigeria where a Voucher specimen was deposited in the Herbarium with the Herbarium number UNILORINH146.

Test microorganisms: Clinical isolates of *Bacillus subtilis*, *Streptococcus pyogenes* and *Escherichia coli* used in this study were obtained from the Medical

Microbiology Laboratory of the Federal Medical Centre, Bida, Niger State, Nigeria. The organisms were maintained on nutrient agar slants in a refrigerator.

Standardization of microorganisms: Overnight cultures (0.2 ml) of each test microorganism was dispensed into 20 ml of sterile nutrient broth and incubated for 4 h to standardize the culture to10^6 cfu/ml. A loopful of the standard culture was used for the antimicrobial assay (Collins *et al.*, 1995).

Preparation of plant extract: The stem bark of *Antiaris africana* was air-dried at room temperature (28 °C) for 5 weeks and pulverized to powder using a clean electric blender (Model Phillips 190). A 50g sample of the pulverized stem bark of *A. africana* was soaked in 200 ml of ethanol and allowed to stand for 72 h with intermittent stirring. This was filtered through a whatman No. 1 filter paper and the filtrate obtained was evaporated to a dry mass using a rotary rotavaporator at 35 °C. The dried extract was exposed to UV light for 24 h and checked for sterility by streaking on nutrient agar plate. The extract was assayed against the test bacteria to determine the antibacterial properties (Bauer *et al*., 1966).

Determination of flavonoids: A small piece of magnesium ribbon was added to ethanolic extract of stem bark of *Antiaris africana*. This was followed by dropwise addition of concentrated hydrochloric acid. Colours ranging from orange to crimson are indicative of the presence of flavonoids.

Extraction of flavonoid fraction: A 5 ml sample of the ethanol stem bark extract of the plant was transferred into a conical flask and hydrolyzed by heating on a water bath with 10 ml of 10% H_2SO_4 for 30 min. The mixture was then cooled on ice for 15 min (Mahmoud *et al*., 1994). The precipitate (Flavonoid aglycone) formed was recovered by filtration and used for further tests.

Antibacterial test: The antibacterial test was performed using the agar diffusion method of Nair *et al*. (2005). The test organisms were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Wells of 5 mm diameter were made on the nutrient agar plates and spread uniformly using a sterile cork borer. The cut agar disks were carefully removed using forceps that had been sterilized by flaming. To each well were introduced different concentrations (0.01 - 0.05 mg/ml) of the flavonoid fraction separated from the plant extract. Control experiments comprising bacteria inoculum without flavonoid fraction were set up. The plates were allowed to stand for 1 h at room temperature (25 ± 2 °C) for diffusion of the substances to proceed before the growth of organisms commenced. The plates were incubated at 37 °C for 24 h to observe inhibition zones.

Determination of Minimum Inhibitory Concentration (MIC): Various concentrations of flavonoid fraction from *Antiaris africana* ranging between 0.01 - 0.05 mg/ml were introduced into different test tubes containing nutrient broth; each tube was inoculated with an overnight culture of *Bacillus subtilis, Streptococcus pyogenes* and *Escherichia coli* diluted to give a final concentration of 10^6 cells/ml. The tubes were incubated at 37 °C for 24 h. The least concentration of flavonoid fraction that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the minimum inhibitory concentration (MIC) in each case (Collins *et al*., 1995).

Determination of Minimum Bactericidal Concentration (MBC): After culturing the test organisms separately in nutrient broth containing various concentrations of flavonoid fractions, the broth was inoculated onto freshly prepared agar plates to assay for the bactericidal effect. The culture was incubated at 37 °C for 24 h. The lowest concentration of flavonoid that did not yield any colony growth on the solid medium after the incubation period was regarded as the minimum bactericidal concentration (Alade & Irobi, 1995).

RESULTS

The extracted flavonoid fraction exhibited antibacterial activity against *B. subtilis, S. pyogenes* and *E. coli* (Table 1). No zone of inhibition was recorded when 0.02 mg/ml of the flavonoid fraction was assayed against *S. pyogenes* and *E. coli*; though a zone of inhibition of 7.0 ± 0.2 mm was recorded against *B. subtilis* at this concentration. Zones of inhibition of 9.5 ± 0.1 mm and 4.0 ± 0.1 mm were recorded when 0.03 mg/ml of the flavonoid fraction was assayed against *B. subtilis* and *S. pyogenes,* respectively (Table 1), while 0.05 mg/ml of the flavonoid fraction produced zones of inhibition measuring 11.5 ± 0.2 mm, 8.5 ± 0.4 mm and 9.5 ± 0.1 mm against B. *subtilis*, *S. pyogenes* and *E. coli,* respectively.

Table 1: Antibacterial effect of the flavonoid fraction from *Antiaris africana*

Concentration (mg/ml)	Mean diameter of zone of Inhibition (mm) ± SD		
	B. subtilis	*S. pyogenes*	*E. coli*
0.01	0	0	0
0.02	7.0 ± *0.2*	0	0
0.03	9.5 ± 0.1	4.0 ± 0.1	0
0.04	10.0 ± 0.3	6.5 ± 0.3	7.5 ± 0.5
0.05	11.5 ± 0.2	8.5 ± *0.4*	9.5 ± 0.1

SD = Standard deviation; Control treatment consisted of the test bacteria without the flavonoid fraction; No zone of inhibition was observed in the control.

The minimum inhibitory concentrations of flavonoid fraction of *Antiaris africana* against *B. subtilis*, *S. pyogenes* and *E. coli* were 0.04, 0.050 and 0.050 mg/ml, respectively (Table 2). The lowest MIC was recorded against *B. subtilis* while a higher MIC (0.050mg/ml) was recorded against both *S. pyogenes* and *E. coli*. Minimum bactericidal concentration of 0.045 mg/ml was recorded against *B. subtilis* while a higher MBC of 0.050mg/ml was recorded against *S. pyogenes* and *E. coli*.

Table 2: Minimum inhibitory concentration of flavonoid fraction from *Antiaris Africana.*

	Concentration of flavonoid fraction (mg/ml)										
Organism	0.055	0.050	0.045	0.040	0.035	0.030	0.025	0.020	0.015	0.01	MIC
B. subtilis	-	-	-	-	+	+	+	+	+	+	0.04
S. pyogenes	-	-	+	+	+	+	+	+	+	+	0.05
E. coli	-	-	+	+	+	+	+	+	+	+	0.05

+ = presence of growth; - = absence of growth; MICs were presented as the lowest concentrations that did not permit any visible growth of test organisms in broth culture.

Table 3: Minimum bactericidal concentration of the flavonoid fraction from *Antiaris africana*

	Concentration of Flavonoid fraction (mg/ml)										
Organism	0.055	0.050	0.045	0.040	0.035	0.030	0.025	0.020	0.015	0.01	MBC
B. subtilis	-	-	-	+	+	+	+	+	+	+	0.045
S. pyogenes	-	-	+	+	+	+	+	+	+	+	0.050
E. coli	-	-	+	+	+	+	+	+	+	+	0.050

+ = presence of growth; - = absence of growth; MBCs were regarded as the lowest concentrations that prevented growth of test organisms on nutrient agar.

DISCUSSION

The antibacterial test of the flavonoid fraction from *Antiaris africana* indicated that the plant has activity against *B. subtilis, S. pyogenes* and *E. coli.* This observation agrees with the reports of Leven *et al* (1979) and Scherbonvaski (1971), both reports linked the antibacterial properties of plants to the presence of phytocompounds such as tannins, alkaloids, flavonoids and saponins. It was reported that flavonoid inhibited *in vitro* growth of *Vibrio cholerae* 01 (Borris, 1996) and *Streptococcus mutans* (Batista *et al*., 1994). An isoflavone found in West African legume prevents schistosomol infection when applied topically (Perrett *et al*., 1995). In this study, the antibacterial activity of flavonoid fraction from *A. africana* was enhanced by increase in the fractional concentration, as reported by of Banso and Mann (2006).

The large size of the zones of inhibition produced by the flavonoid fraction from the plant material used in this study against the test organisms is indicative of the potency of flavonoid fraction from *A. africana*. The antibacterial activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Tsuchiya *et al*., 1996).

The minimum inhibitory concentration values of the flavonoid fraction from *A. africana* against the test organisms showed that bacteria vary in the degree of their susceptibility to antibacterial agents. As

expected, antibacterial agents with low activity against organisms have a high minimum inhibitory concentration, and vice versa (Mann *et al*., 2008). The MIC and MBC are the parameters used to evaluate the efficacy of agents such as antiseptics, disinfectants and chemotherapeutic agents (Croshow, 1983). The results of this study show that flavonoid fraction from *A. africana* has activity against several bacteria, and thus it could be useful as a source of antibacterial agents.

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