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Full Length Research Paper

Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*

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Acacia nilotica **was assessed for active principles. The results showed that the stem bark extract of the plant possessed the active principles e.g. terpenoids, tannins, alkaloids, saponins and glycosides. The antimicrobial activity of the extracts was assayed against** ***Streptococcus viridans*****,** ***Staphylococcus aureus*****,** ***Escherichia coli*****,** ***Bacillus subtilis*** **and** ***Shigella sonnei*** **using the agar diffusion method. The plant extract exhibited antimicrobial activity against all the test microorganisms.** ***B. subtilis*** **was the most susceptible to the plant extract while** ***Candida albicans*** **was the most resistant. The minimum inhibitory concentration of the stem bark extract of the plant ranged between 35 and 50 mg/ml while the minimum bactericidal concentration ranged between 35 and 60 mg/ml.** ***A. nilotica*** **could be a potential source of antimicrobial agents.**

Key words: *Acaacia nilotica*, active principles, microorganisms.

INTRODUCTION

Plant compounds are of interest as a source of safer or more effective substitutes than synthetically produced antimicrobial agents. Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals. These procedures have shown that many substances originally thought to be rather rare in occurrence are of almost universal distribution in the plant kingdom.

The drugs contained in medicinal plants are known as active principles. The active principles are divided chemically into a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes (Mitcher et al., 1988; Habtermariam, 1993). Miski et al. (1983) reported the antibacterial activity of flavonoids from *Salvias palatine*. The authors identified ten aglycones and six glycosides of luteolin and apigenin from the leaf extracts. It was reported that cirsimaritin among the compounds identified showed antimicrobial activity against *Streptococcus aureus* , *Staphylococcus epidermitis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa.* Clark et al. (1981) showed that phenolic constituents of *Maynolia grandiflora* have some antibacterial activity. In an investigation carried out by Mitcher et al. (1988) on *Halianthus annuus*, some phenollics with antimicrobial property were isolated.

Acacia nilotica commonly called Acacia belongs to the family *Mimosaceae*. It is known as “Bagaruwa” among the “Hausa” speaking people of northern Nigeria. The plant is a tree with yellow mimosa-like flowers and long grey pods constricted between seeds. The bark and branches are dark with fissures. The branches bear spikes about 2 cm long. The leaves are five and densely hairy with 3 - 6 pairs of pinnae consisting of 10 - 20 pairs of leaflets that are narrow with parallel margins that are rounded at the apex and with a central midrib closely crowded. The inflorescence consists of bright yellow flowers in auxillary head on stalks that are half way up. The flowering period of the plant is between November and March (Mann et al., 2003). The powdered bark of the plant with little salt is used for treating acute diarrhea (Gill, 1992). Hence the aim of this study is to determine the phytochemical constituent and to investigate the antimicrobial properties of *A. nilotica* to ascertain the rationale for its use in traditional medicine.

MATERIALS AND METHODS

Collection of plant material

Stem bark of the plant was collected from Forestry Research Institute, Ibadan, Nigeria. The plant was authenticated at the institute as *A. nilotica.*

Test microorganisms

Clinical isolates of *S. viridans*, *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli* and *Shigella sonnei* used in this study were obtained from Federal Medical Centre, Bida, Nigeria.

Preparation of plant extract

Ethanolic extract of the stem bark of the plant was extracted according to the method described by Okogun (2000) with slight modifications. A 50 g sample of the stem bark of the plant was air-dried, ground into powder using an electric blender (National MX4911V, Matsushita electronics). The blended material was transferred into a beaker and 10 ml of 95% ethanol was added at ambient temperature (28 ± 2°C). The mixture was extracted by agitation on a rotary shaker. Extraction was allowed to proceed for 48 h. The mixture was decanted and the solvent was removed by evaporation at room temperature (28 ± 2°C) to obtain the extract.

Phytochemical analysis of extract

The methods described by Harborne (1978) with slight modifications were used to test for the presence of the active ingredients in the test sample.

Test for steroids

A 10 ml of chloroform extract of the test plant leaves was evaporated to a dry mass and the mass dissolved in 0.5 ml of chloroform.

Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds.

Test for terpenoids

The presence of terpenoids was determined as described for steroids except that red, pink or violet colour indicates the presence of terpenoids.

Test for tannins

i.) $\frac{1}{3}$ cm^3 of freshly prepared 10% KOH was added to 1 cm^3 of the extract. A dirty white precipitate indicated the presence of tannins.

ii.) Powdered stem bark of the test plant (1.0 g) was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% $FeCl_3$ were then added. Production of greenish precipitate indicated the presence of tannins.

Test for flavonoids

A small piece of magnesium ribbon was added to ethanolic extract of the plant material, this was followed by the drop wise addition of concentrated hydrochloric acid. Colours varying from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavonones (Harborne, 1978).

Test for alkaloids

The extract of the plant stem bark sample (0.5 g) was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract (Harborne, 1978).

Test for saponins

Stem bark of the test plant was ground into powder form and 0.5 g of the powdered stem bark was introduced into a tube containing 5.0 ml of distilled water, the mixture was vigorously shaken for 2 min, formation of froth indicated the presence of saponins.

Test for glycosides

Coarsely powdered stem bark (1 g) was added into two separate beakers. To one of the beakers was added 5 ml of dilute sulphuric acid while 5 ml of water was added to the other beaker. The two beakers were heated for 3 - 5 min and the contents filtered into labeled test tubes. The filtrate was made alkaline with 5% sodium hydroxide and heated with Fehling's solution for 3 min. The presence of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides (Harborne, 1978).

Antimicrobial assay

The antimicrobial assay was performed using the agar diffusion method of Collins et al. (1995) with slight modifications. 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 using a sterile cork borer. The cut agar disks were carefully removed by the use of sterilized forceps. To each well was introduced various concentrations (5, 10, 15, 20, 25 and 30 mg/ml) of the extracts. Control experiments comprising inoculum without plant extract were set up. The plates were allowed to stand for one hour 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. The zones of inhibition were then recorded.

Table 1. Phytochemical analysis of stem bark extract.

Active principle	Extract
Steroids	-
Terpenoids	+
Tannins	+
Flavonoids	-
Alkaloids	+
Saponins	+
Glycosides	+

+ = Present
- = Absent

Table 2. Antimicrobial activities of stem bark extract.

Concentration (mg/ml)	Mean diameter of zone of inhibition (mm) ± SD				
	Sv	Bs	Sa	Ec	Ss
5	0	0	0	0	0
10	0	0	0	0	0
15	7.5±0.1	10.0±0.01	6.5±0.02	0	0
20	10.5±0.05	14.0±0.05	8.5±0.02	5.0±0.04	0
25	13.0±0.1	16.5±0.2	11.5±0.02	9.5±0.01	5.0±0.01
30	15.5±0.02	18.0±0.05	15.0±0.01	13.5±0.1	8.5±0.02

Sv = *Streprococcus viridans*, Bs = *Bacillus subtilis*,
Sa = *Staphylococcus aureus*, Ec = *Escherichia coli*,
Ss = *Shigella sonnei*,
SD = Standard Deviation

Determination of Minimum Inhibitory Concentration (MIC)

Various concentrations of the plant extract ranging between 10 and 60 mg/ml were introduced into different test tubes, each tube was inoculated with an overweight culture of *S. viridans*, *B. subtilis*, *S. aureus*, *E. coli* and *S. sonnei* diluted to give a final concentration of 10^6 cells per ml. The tubes were incubated at 37°C for 24 h. The least concentration of the plant extract that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the 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 concentration of the stem bark extract of the plant, 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 the plant extract that does not yield any colony growth on the solid medium after the incubation period was regarded as MBC (Alade and Irobi, 1993).

RESULTS AND DISCUSSION

Phytochemical screening of the stem bark of *A. nilotica* revealed that the plant contain terpenoids, alkaloids, saponins and glycosides (Table 1). Negative results were recorded for steroids and flavonoids which confirm the absence of these active principles (Table 1). The active principles identified in this study exhibited antimicrobial activity against all the test organisms (Table 2). Several plants, which are rich in alkaloids, tannins and glycolsides, have been shown to possess antimicrobial activity against a number of microorganisms. For example, Adebajo et al. (1983) investigated the antimicrobial activity of leaf extract of *Eugenia uniflora* and reported that tannins, glycosides and alkaloids were detected and that the ethyl acetate and methanolic leaf extract of the plant were active against *E. coli*, *P. vulgaris*, *K. pneumoniae* and *Aspergillus niger*. Saponins are a special class of glycolsides which have soapy characteristic and facilitate the absorption of foods and medicine. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Fluck, 1973). It therefore suggests that the medicinal plant used in the present study may have a general antimicrobial activity.

The large size of the zones of inhibition indicated the

Table 3. Minimum inhibitory concentration (MIC) of stem bark extract.

Organism	MIC (mg/ml)
Streprococcus viridans	40
Bacillus subtilis	35
Staphylococcus aureus	40
Escherichia coli	45
Shigella sonnei	50

Table 4. Minimum Bactericidal Concentration (MBC) of stem bark extract.

Organism	MBC (mg/ml)
Streprococcus viridans	45
Bacillus subtilis	35
Staphylococcus aureus	40
Escherichia coli	50
Shigella sonnei	60

potency of the active principles of the plant (Table 2). It was recorded that an increase in the concentration of the extract yielded higher activity as shown by the diameter of zone of inhibition (Table 2). The fact that organisms may need higher concentrations of extracts to inhibit or kill them may be due to their cell wall components.

The results (Table 3) showed that the MIC of the stem bark extract of the plant ranged between 35 and 50 mg/ml. The effect of the plant extract on the MIC for the test microorganisms correlate with the report that micro-organisms varied widely in the degree of their susceptibility (Emeruwa, 1982). Antimicrobial agents with a low activity against an organism have a high MIC while a highly active antimicrobial agent gives a low MIC. The minimum bactericidal concentration (MBC) of the stem bark extract of the plant ranged between 35 and 60 mg/ml (Table 4). The MIC and MBC which is normally used to evaluate the efficacy of the agents such as antiseptics, disinfectants and indeed chemotherapeutic agents (Croshaw, 1983) under standard conditions also support the sensitivity test results.

Conclusion

Ethanolic stem bark extract of *Acacia nilotica* produced antimicrobial activity against *S. viridis*, *B. subtilis*, *S. aureus*, *E. coli* and *S. sonnei*. The extract contains the active principles – terpenoids, tannins, alkaloids, saponins and glycosides. This study observes that *A. nilotica* has useful antimicrobial properties.

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