Ethnobotanical Leaflets 14: 390-401. 2010.

Antimicrobial Activity of Leaf of *Aspila africana* on some Pathogenic Organisms of Clinical Origin

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Issued April 1, 2010

Abstract

The antimicrobial activities and Minimal Inhibitory Concentration (MIC) of the extracts of *Aspilia africana* were evaluated against five bacteria (*Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli*). History of the organisms used indicated pathogenicity during laboratory analysis of specimen collected. Leaf of the plant was collected from the farm. The ethanolic and hot aqueous extracts were obtained by standard methods. The antimicrobial activity was conducted using a modified agar well diffusion method. The results showed that the ethanolic extract of *Aspilia africana* exerted antimicrobial effect on the test organisms at 12.5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml concentrations, while the hot aqueous extract exerted no antibacterial activity against any of the tested organisms at the same concentrations. The ethanolic extract of *Aspilia africana* showed the highest antibacterial activity with diameter of zone of inhibition of 31.5mm against *Pseudomonas aeruginosa*, 25.0mm against *Klebsiella pneumonia*, 21.5mm against *Staphylococcus aureus* and 17.5mm and least effective against *Streptococcus faecalis* and *Escherichia coli* at 100mg/ml concentration. The Minimum Inhibitory concentration (MIC) of the ethanolic extracts was at a concentration 1.325mg/ml for all the organisms. The antibacterial activity of the extract could be enhanced if the components are purified. This plant therefore holds a promise as a potential source of new drug for treating infections caused by these clinical pathogens.

Key words: *Aspilia africana,* Antimicrobial activity, Antibacterial activity, Microorganisms.

Introduction

In most developing countries, low income people such as farmers, people of small isolated villages and native communities use folks medicine, extracts from the leaves, seeds, fruits, barks and roots of plants in the preparation of syrups and infusions in traditional medicine for the treatment of common infections. These preparations have been used to treat cases ranging from the common cold to malaria, liver cirrhosis, hypertension, and so on. The active constituents contributing to these protective effects are the phytochemicals, vitamins and minerals (Okwu and Ekeke; 2003). Extracts from the roots, barks, seeds and fruits of these plants are used in the preparation of syrups and infusions in traditional medicine for the treatment of various ailments. One of such

plants is *Aspilia africana.*

Antimicrobial substances are substances that inhibit the growth and existence of microorganisms (Paul and Sainburg, 1994). These microorganisms could be pathogenic or non pathogenic, hence, antimicrobial substances are used in the treatment of various ailments. Quite a number of antimicrobial substances exist and they are gotten from diverse sources such as microbial, plant, animal and chemical sources (Ganellin and Roberts, 1999).

Medicinal uses of these plants ranged from the administration of the plant's roots, barks, stems, leaves, fruits and seeds, to the use of extracts from the whole plant (Akujobi *et al*., 2004). Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants (Jigna and Chanda, 2006). This search for new antimicrobial properties of natural products cannot be ignored because this can be found in the most remote parts of the world where medical doctors are not present (Olukemi and Kandakai, 2004).

Among the diseases that have been managed successfully by traditional (herbal) medicine include malaria, epilepsy, infertility, convulsion, diarrhoea, dysentery, gonorrhoea, flatulence, tonsillitis, bacterial and fungal infections, mental illness and worm infections (Sofowora, 1996). Health for all by year 2000 as proposed by the World Health Organization (WHO) has helped to focus attention on the system of traditional medicine which was used extensively in all countries in developing world. More co-operation has been achieved between practitioners of orthodox and traditional medicine following the incorporation of traditional medicine into WHO's programme in 1976 (Sofowora, 1993; Akinyemi *et al*., 2005). It has been said that the knowledge of medicinal plant was gained by accident. Although, this theory has been refuted by a number of traditional medicinal practitioners who claimed that information on such plant were communicated to their ancestors in various way (Akpata, 1979.). However early man could have gained some scientific knowledge by watching the effects produced by various plants when eaten by domestic animals (Sofowora, 1993).

Materials and Methods

Collection and identification of plant sample

Fresh leaf sample of *Aspilia africana* was obtained from Agbada Farm, Kabba, in Kabba/Bunu Local Government Area of Kogi State. It was identified properly and authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Source and maintenance of test organisms

Pure culture of test organisms used in the project; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Streptococcus faecalis* were obtained from the Medical Laboratory of the Microbiology and Parasitology Unit of the University of Ilorin Teaching Hospital and properly identified.

Preparation of extract

The fresh leaf sample was properly sundried and ground with mortar and pestle obtained from the Department of Microbiology, University of Ilorin. The finely grounded powder was kept in a polythene bag until use.

Extraction

Aqueous and ethanolic extraction of the plant material was prepared as described by Oyagade *et al*., 1999. The extractions of the plant material were carried out by suspending 25g of the finely ground leaf in 125ml of 95% ethanol and 250mls of distilled water respectively. A preliminary test has shown that the extract shared greater activity at 80^0C than at 28^0C, so the aqueous extraction was done at 80^0C in a water bath for $1^1/2$ hours. The ethanolic extraction was done at 28 ± 1^0C for 120 hours by subjecting it to agitation on rotator shaker at 200 rpm. The resulting aqueous extract suspension were filtered with Whatman filter paper and evaporated to dryness at 45^0C in an oven.

Sterilization of materials

All glassware used in this research were washed with detergent, rinsed with distilled water, air dried and sterilized on a hot air oven at 121^0C for 2 hours. Each of the materials was wrapped with aluminium foil before sterilization. Distilled water and all prepared media were sterilized in the autoclave at 121^0C for 15 minutes. Cork borers and glass rods were sterilized by dipping into 70% alcohol prior to flaming in a Bunsen burner. The working bench was swabbed with 75% alcohol before and after each experiment.

Reconstitution and sterilization of extract

The dried residue was weighed into McCartney bottles and appropriate volume of distilled water was added to make a stock solution of 100mg/ml, for example 1000mg in 10mls of distilled water. The stock solution was then sterilized using 0.65 membrane filter by suction pump. The sterilized extract were stored inside McCartney bottle and kept in a refrigerator.

Standardization of inoculums

Five inoculums of the organism growing as pure culture in a nutrient agar plates were inoculated into 10mls of nutrient both in a test tube aseptically. The mouth of the tube was covered with cotton wool and wrapped with aluminum foil followed by incubation at 37^0C for 24 hours.

Antimicrobial Sensitivity Testing

Agar well diffusion method

15ml of molten sterile nutrient agar was poured into Petri dishes. After solidification, an overnight broth culture of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Streptococcus faecalis* were introduced into the surface of the sterile plate and a sterile glass spreader was used for even distribution of the molecular. Holes were mad aseptically with a 7mm sterile cork borer and 0.1ml of the test solution of different concentration were introduced into well. The extract was allowed to diffuse into the medium i.e used for 1 hour and then incubated aerobically to diffuse into the medium kept for 1 hour and then incubated

aerobically for 24 hours at 37^0C.

One well containing extractant serves as control in each plate. The plates were examined for zones of inhibition, which indicate the degree of susceptibility of the test organism. The antimicrobial activity of water and ethanolic extract were measured with an outer and compared with the control well (well containing only water and ethanol).

Determination of Minimum Inhibitory Concentration (MIC)

MIC of the aqueous and ethanolic extracts of this plant was determined by solution of the extract to various concentrations of 12.5, 25, 50 and 100 mg/ml. 9ml of sterile peptone water was dispensed into each test tube, then 1ml of each of the extract at different concentrations were introduced and mixed in a test tube 0.1ml of inoculums was added to each tube. The tubes were incubated aerobically at 37^0C for 24 hours. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and the growth medium without the inoculums) and organism control (the tube containing the growth medium and the inoculums). The lowest concentration (higher dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tube was regarded as MIC

Determination of Minimum Bactericidal Concentration (MBC)

MBC was determined by sub culturing test solution which showed no detectable growth (no turbidity) after 24hours incubation onto fresh drug free nutrient agar and incubated further for 24hours to determine the MBC of the extract required to kill the organism. These concentrations were indicated by the failure of the test organism to grow on the recovery media to grow on the plate after incubation indicated a bacteriostatic effect, while the plates that do not show growth after incubation indicated a bactericidal effect.

Phytochemical screening of ethanolic extract

Phytochemical screening for major constituents was carried out using standard qualitative methods as described by Sofowora (1993). The method described by Odebiyi and Sofowora (1979) was used for the presence of Saponins, Tannins, Phenolics, and Alkaloids. The Lieberman-Buirchard reaction as described by Herburne (1978) was used to test for steroids and triterpenes.

Results and Discussion

The results of the medicinal plant (*Aspilia africana*) tested in this study is shown in this chapter. Table 1 shows the profile of test organism (*Aspilia africana)*. The MIC and MBC of both the ethanolic and hot aqueous extract of the plant are shown in Table 3. Generally the ethanolic extract showed greater antibacterial activity compared to its corresponding extract in the aqueous extract. The ethanolic extract showed the highest activity against *Pseudomonas* and *Klebsiella*, followed by *Staphylococcus aureus* and least on *Streptococcus faecalis* and *E. coli*. *All* the ethanolic plant extract have antibacterial activity at 100mg/ml and in the descending order at double dilutions; 50mg/ml, 25mg/ml and 12.5mg/ml.

The data obtained in Table 2 shows the antibacterial activity of the crude hot aqueous and ethanolic extracts of

Aspilia africana S. aureus, Streptococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumonia, and *E. coli*. The ethanolic extract of *Aspilia africana* showed the highest antibacterial activity with the diameter of zone of inhibition of 31.5mm against *Pseudomonas aeruginosa*, 25.0mm against *Klebsiella pneumoniae* and 17.5mm against *Streptococcus faecalis* and *Escherichia coli*, and least effective against *Staphylococcus aureus* at 100mg/ml concentration, while the cold extract had no effect. This is due to the failure of the active ingredient to dissolve in it and all the sensitive extracts were more at higher concentrations than lower concentration.

Table 3 shows the MIC and MBC of the sensitive extracts against concentration. Table 4 shows the antibacterial effectiveness of the extracts of leaf or plant samples at concentrations of 100mg/ml, 50mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml as compared with the activity of some selected antibiotics namely Chloramphenicol, Tetracycline and Ampicilin of same concentration. The antibacterial activities of the conventional antibiotics are higher than that of the plant extracts at the same concentration.

The data obtained from this study indicates that the ethanolic extract of the leaf of *Aspilia africana* possesses antibacterial activity against *Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumonia*, and *Escherichia coli*. It was shown in this study that the ethanolic extract of *Aspilia africana* is superior to that of hot aqueous extract**.** The antibacterial activity of the crude plant extracts (Table 2) on the test organisms justifies the active principle or ingredient observed in herbal physician in their preference for the local gin "ogogoro" as extract in the preparation of crude drugs from medicinal plant materials (Brain and Turner, 1975). Local gin obtained from fermented palm wine distillation is known to contain a high concentration of alcohol. When these solvents are used as herbal extractants, it may be possible that bioactive substances that are less soluble in water would then be dissolved by the solvent (Oyagade *et al*., 1999). In the antibacterial sensitivity test, ethanolic extract of *Aspilia africana* exhibited the most outstanding antibacterial activity against *Pseudomonas aeruginosa* with an inhibiting effect of 31.5mm at 100mg/ml concentration (Table 2) is higher than others at the same concentration.

The ethanolic extract of *Aspilia africana* showed growth inhibitory effect for all the concentrations (i.e 12.5, 25.0 ,50 and 100mg/ml) against *Klebsiella pneumoniae* in which it has its diameter of zone of inhibitory effect of 25mm at 100mg/ml concentration (Table 2 and 3), followed by *Staphylococcus aureus*. The ethanolic extract of *Aspilia africana* showed the same growth inhibitory effect against *Streptococcus faecalis* and *E. coli* at 100m g/ml. The cold extract of all these plant did not exert antibacterial effect on the organisms, due to the failure of the organisms due to the failure of the active ingredient to dissolve in it and all the sensitive extracts were more at higher concentrations than lower concentration.

Failure of some of the extract to exert antibacterial effect or the test organism is not enough to conclude that the leaf does not contain substances that can exert antibacterial activity against the test organism because the potency of extract depends on the method used to obtain the extract (Unaeze *et al*., 1989). Research has shown that the age of plant when harvested and the season of plant determine the amount of the amount of the active constituents and since the active ingredients of plants can vary in quality and quantity from season to season (Sofowora, 1982).

The sensitive extract as shown in Table 3; was only able to inhibit the growth of the organisms but did not exert a killing effect on the test organism and suggests that the ethanolic extract was bacteriostatic but not bactericidal.

The comparison of the activity of the plant extract with conventional antibiotics confirmed reports by other workers (Emerawa, 1982) that constitutional antibiotics are more active than plant extracts (Table 5). Phytochemical screening of the extract revealed presence of Saponins, Tannins, Phenolics, and Alkaloids, Steroids and Triterpenoid (Table 5). Various studies have shown that plants that are rich in phenolic compounds possess antimicrobial activity against a number of microorganisms (Adebayo *et al*., 1989).

In conclusion, the higher activity of the ethanolic extract may not be without the extraction solvent. ethanol has been shown to be a stronger extractant than water. The antibacterial activity of the extract could be enhanced if the component are purified caused. Research laboratories are therefore enjoined to work hand in hand with traditional herbal practitioners so that while the traditional healers from their historic knowledge provide preliminary information on the uses of medicinal plant, the scientific basis for the efficacy of the extracts and so that, proper advice can be given on how the drugs should be prepared and administered. This plant therefore holds a promise as a potential source of new drug for treating infections caused by these clinical pathogens.

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Table 1: Profile of Test plant.

Botanical Name	Family	Yoruba name	Igbo name	Hausa name	Plant part used
Aspilia africana	Asteraceae	"Yun-yun"	"Orangila"	"Tozalin"	Leaves

Table 2: Antibacterial activity of the crude plant extracts on the test organisms.

Diameter Of Zone Of Inhibition (mm)*										
	95% Ethanolic Extract (mm)					**Hot Aqueous Extract (mm)**				
Conc. mg/ml. / Test Orgs.	12.5	25	**50**	100	Control	12.5	25	50	100	Control
S. aureus	10.5	12.8	17.2	21.5	Nil	-	-	-	-	Nil
S. faecalis	10.3	12.5	13.0	17.5	Nil	-	-	-	-	Nil
K. pneumoniae	11.5	12.8	24.0	25.0	Nil	-	-	-	-	Nil
P. aeruginosa	21.0	24.5	26.8	31.5	Nil	-	-	-	-	Nil
E. coli	8.5	12.5	14.0	17.5	Nil	-	-	-	-	Nil

PH			6.8	NID					6.7	7.0

*The wider the diameter of zone of inhibitions, the higher the antibacterial activity of the extract. - : No zone of inhibition. NIL: No inhibition

Table 3: The MIC and MBC of the sensitive extracts against concentration.

Test organism	MIC (mg/ml)	MBC (mg/ml)*
S.aureus	1.325	-
Strep. faecalis	1.325	-
Pseudosomas aeruginosa	1.325	-
Klebsiella	1.325	-
E. coli	1.325	-

* - : No killing effect

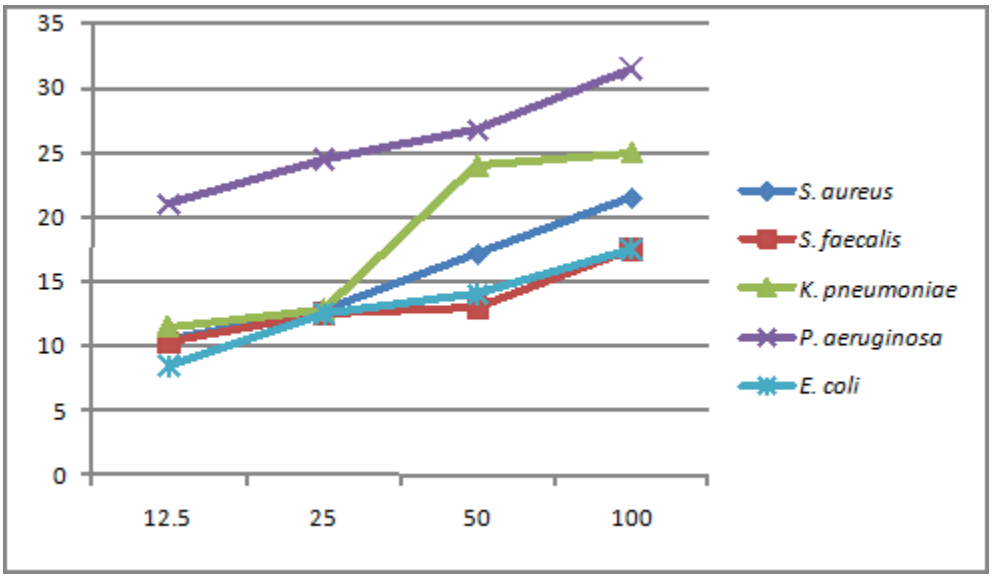
Table 4: Comparison of ethanolic extract of plant extract with Conventional Antibiotics.

Test organisms	Diameter of zone of inhibitors (mm)						
	Concentration of ethanolic extracts mg/ml						
	12.5	25	50	100	Chloramphenicol	Tetracycline	Ampicillin
S. aureus	10.5	12.8	-	21.5	25.0	41.0	33.0
Streptococcus faecalis	10.3	12.5	13.0	17.5			
P. aeruginosa	21.0	24.5	-	31.5	35.0	34.0	38.0
Klebsiella spp	11.5	12.8	24.0	25.0			
E. coli	8.5	12.5	14.0	17.5	23.0	35.0	28.0

Table 5: Result of Phytochemical screening of ethanolic extract of plant sample.

Chemical constituents	*Aspilia Africana*
1. Saponins	++
2. Tannins and Phenolics	++
3. Alkaloids	+
4. Steroids and Triterpernoid	+/-
5. Glycoside	_

++ - Strongly positive + - Positive +/- - Weakly positive- Not detected



Organisms Concentration (mg/ml)

Figure 1: Graph of Test organisms against Concentration of Plant Extract.