Antibacterial Effect of Methanolic Extract of the Root of *Aspilia africana*

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ABSTRACT

The leaf of Aspilia Africana (Pers) C.D. Adams (Asteraceae) is widely used in ethno medicinal practices in Tropical Africa because of its ability to stop bleeding and promote rapid healing of wounds. It is also used in the management of problems related to cardiovascular diseases, lumbago, venereal diseases and parasitic infections. This study was carried out on the root part (which has not been very well exploited in ethno medicinal practices) to determine if it is as potent as the leaf part. The methanolic extract of the root was subjected to preliminary phytochemical screening and it has indicated the presence of saponins, tannins cardiac glycosides, flavonoids, terpenoids and carbohydrates. Acute toxicity test showed that it has LD_{50} of 707.11mg/kg in mice. The in-vitro antibacterial test using agar well diffusion method showed activity against known wound pathogens such as Staphylococcus auerus (clinical isolate), Staphylococcus aureus (ATCC 25922), Staphylococcus aureus (NCTC 8853), Bacillus substiles, Escherichia coli (ATCC 25922), Escherichia coli (NCTC 10418) and Pseudomonas aeruginosa (ATCC 25922). This indicates that the methanolic extract of the root has potentials for use as antibacterial agent in wound care.

KEYWORDS: *Aspilia africana* ; antibacterial; wound pathogens

INTRODUCTION

Aspilia africana is widely used in ethno – medicinal practices in West Tropical and East Africa as a general healing agent, pain killer, sedative, abortifcient, echolic, lactation stimulant and most importantly as a haemostatic agent because of its ability to stop bleeding even from a severed artery. It is also reputed to be used to promote rapid healing of wounds and in the treatment of malaria and eye problems (Dalziel et al., 1960). *Aspilia africana* commonly known as wild sunflower and as hemorrhage plant because of its haemostatic properties is an herb or weed that is grown on land and fallows and widely distributed across tropical Africa. In Nigeria it is grazed by cattle and sheep and is much used in western Nigeria as food for rabbit and hares (Burkill, 1985). The plant belongs to the family Asteraceae. It can grow up to 2 metres in height and is densely cuspid with hairy stem and of perennial woody root stock. The leaves are crowded into capitular heads surrounded by a ring of small green leaves with brilliant yellow star shaped petals. (Akubue, 1983). *Aspilia africana* is known by the Ibibios and Efiks as edemedong, Ibos as orangila, Yorubas as yunyun and Hausa as toozalin- yan- maata. *Aspilia africana* is one of the plants that exhibit a wide range of biological activities including antiviral, fungicide and antibacterial activities (Okwuonu et al., 2008). This work was designed to explore the antibacterial and

antifungal activities of the root part using the extract from methanol.

MATERIALS AND METHODS

Plant Collection and Extraction

Aspilia africana was obtained from Ikot Ntuen village, along Oko Ita – Use Ikot Amama road in Ibiono Ibom Local Government Area of Akwa Ibom State, Nigeria, in November 2010. It was identified and properly authenticated at Department of Pharmacognosy and Drug Development, Faculty of Pharmacy, University of Uyo. The air dried plant material consisting of the root part only was powdered and extracted with methanol by maceration for 72 hours at room temperature. The methanolic extract was concentrated to obtain a reddish brown residue code named 'MER'

Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out on MER using the methods of chemical analysis reported by Trease and Evans (2009), Harbourne (1984) and Sofowora (1993).

Toxicity Test (LD_{50})

The toxicity test (LD_{50}) was carried out using Lorke's method (Lorke, 1983).

In-vitro antibacterial and antifungal studies using agar well diffusion method

The strains of the micro-organisms used for the tests were obtained from the Pharmaceutical Microbiology laboratory of the Faculty of Pharmacy, University of Uyo. The test organisms were characterized and identified using the methods described by Bradshaw (1986). The organisms were cultivated overnight in a nutrient broth and sabouraud dextrose broth and sustained on agar slants at 4 ^{0}C before use. 0.20ml of this overnight broth culture of each organism was dispensed into 20ml of sterile nutrient broth for bacterial organisms and 20ml of sabouraud dextrose broth for fungal organisms and incubated for 4 hours to standardize the culture to 10^6 cfu/ml. (Lovian, 1980)

Preparation of the Extract

Lower and higher concentrations of MER were prepared. For the lower concentrations, 1.0g of MER was dissolved in 10ml of 99% methanol to obtain a concentration of 100mg/ml for the stock. Two fold dilutions were carried out on the stock to get 50mg/ml, 25mg/ml, and 12.5mg/ml concentrations. For the higher concentrations, 5g of MER was dissolved in 10ml of 99% methanol to obtain a stock of 500mg/ml; appropriate dilutions were made to obtain 400mg/ml, 300mg/ml and 200mg/ml concentrations.

Preparation of Muller Hinton Agar

38g of Muller Hinton Agar was suspended in 1000ml of distilled water and boiled to dissolve. This was sterilized by autoclaving at 121^{0}C for 15 minutes.

Preparation of Plates

0.2ml of the 10^6 cfu/ml concentration of the test micro-organism was introduced into the plates (petri dishes) followed by the sterile Muller Hinton Agar and swirled to mix. The mixture was allowed to solidify in the petri dish on bench. 4.0mm sterile cork borer was used to bore five holes aseptically - four holes at the periphery for the different concentrations of MER and labeled appropriately. One hole was made in the centre for the control. Lastly the different concentrations of MER were transferred into

the labeled holes. Methanol (99%) was used as control. The plates were allowed 30 minutes for diffusion and incubated at 37^0C for 24 hours. Microbial sensitivity was determined in triplicate. After the incubation the diameter of inhibition zone was measured horizontally and vertically for each zone and the mean determined.

RESULTS AND DISCUSSION

The phytochemical screening of MER showed high presence of saponins and tannins while other typical plant chemicals namely cardiac glycosides, flavonoids and carbohydrates (reducing sugars) were moderately present. Terpenoids were observed to be faintly present while alkaloids, phlobatannins and anthraquinones were not detected. (Table 1)

Acute Toxicity and lethality (LD_{50}) on MER in mice gave i.p. LD_{50} of 707.11mg/kg weight. (Table 2)

The result in table 3 shows that MER possesses different degrees of antibacterial activity against some wound pathogens like all strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* (ATCC 25922) *and Escherichia coli*. It also has activity against *Bacillus substiles* which is also known to cause skin diseases (Trease and Evans, 2009).

The extent of the activity of MER against micro-organisms seems to be directly related to the type and concentration of the bioactive substances present. These bioactive substances are usually responsible for the pharmacological activities of medicinal plants. El Tantaway et al., (1999) have reported of the presence of α-pinene, a terpenoid in the leaves of *A. africana* known to possess anti-inflammatory activity which might have contributed to the wound healing action of the leaves by suppressing inflammatory reactions invoked by injured tissues. Besides, many other workers have reported the presence of alkaloids, saponins, tannins, cardiac glycosides, terpenoids and carbohydrates in the leaf part of the plant (Okoli et al., 2007; Eweka and Eweka, 2009 and Anibijuwon et al., 2010). But our investigations have shown that these secondary metabolites were present in reduced quantities in MER. The presence of saponins and tannins in the leaves is reported to be partly responsible for the haemostatic activity of the leaves in arresting bleeding from damaged or injured vessels by precipitating proteins to form vascular plugs (Okoli et al., 2007). In particular, tannins which are astringent, help to hasten healing of wounds and inflamed mucous membrane, (Okwu and Josiah, 2007), by binding to the proteins of the exposed tissues and precipitating the proteins to form a mild antiseptic protective coat under which regeneration of new tissue takes place leading to rapid healing of wounds (Okoli et al., 2007).

Preliminary phytochemical screening of the root extract revealed that saponins and tannins are also present in high concentrations in the root part of the plant; therefore the root extract may be expected to have some haemostatic effect as the leaves. We also note that an important antimicrobial secondary metabolite – alkaloid is lacking in the root extract while it is significantly present in the leaves. This may be the cause of the differences in the doses that elicit activity between the leaves and the roots. The concentrations of the root extracts lower than 100mg/ml could not give any antibacterial sensitivity, but doses of the leaf extract as low

as 12.5mg/ml recorded significant antimicrobial inhibitions (Adeniyi, 2000).

Flavonoids which are usually implicated as super antioxidants that provide protection against oxidative cell damage, allergies, virus ulcers and inflammations (Saleh et al., 1995; Del-Rio et al., 1997) have also been detected in the root extract. The evaluation of the potentials of MER as an antimicrobial agent which promotes rapid healing of wounds showed that this activity may be very strong against bacteria only, indicating that MER is basically antibacterial.

The effect of the extract on *Pseudomonas aeruginosa* and *Bacillus substiles* is also significant. *Pseudomonas aeruginosa* is known to have a secondary effect on wounds and skin burns by expanding the affected areas (Oyewale et al., 2004). *Bacillus substiles* are also implicated in skin infections; thus the activity of the extract against this organism justifies the use of this plant to treat skin infections in traditional folk medicine. The activities of *Shigella dysenteriae* (clinical isolate), *Salmonella typhii* (NCTC 8571), *Candida albicans* I and II were not inhibited by MER indicating that it is basically not effective against fungi.

Failure of the extract to show antibacterial activity at the lower concentrations cannot be used to conclude that the extract has no activity against the organisms; neither can it be concluded that the plant part does not contain bioactive substances that can exert antibacterial activity because the potency of the extract depends on the method

Table 1: Result of Phytochemical Screening of Methanolic Extract of Roots of Aspilia africana

Phytochemical constituents	Roots of *Aspilia africana*
Alkaloids	_
Anthraquinones	_
Carbohydrates (reducing sugar)	++
Cardiac glycosides	+++
Digitalis Glycosides	++
Flavonoids	++
Phlobatannins	_
Saponins	+++
Tannins	+++
Terpenoids	+

Key: - = absent; + = faintly present;
++ = moderately present; +++ = highly present.

Table 2: Result of Acute Toxicity and Lethality (LD_{50}) Test of Methanolic Extract of Root of A. africana

Concentration of the extract (mg/kg)	No of death per group of the mice
5000	3/3
4000	3/3
3000	3/3
1000	3/3
500	0/3

$LD_{50} = \sqrt{(D_0 \times D_{100})} = \sqrt{(500 \times 1000)} = 707.11mg/kg$

(D_0 = dose at 0% death; D_{100} = dose at 100% death)

used to obtain the extract (Anibijuwon et al., 2010). It has also been established that the age of the plant when harvested and the season in which the plant is harvested affect the amount of the bioactive ingredients of the plants as these active ingredients vary in quality and quantity from season to season (Sofowora, 1982).

Table 3: Result of Anti Microbial Activity of the crude Methanolic Extract of the Root of A. africana

	MER				Control	MER				Control
Conc. in mg/ml / Test organisms	12.5	25.0	50.0	100.0	99% MeOH	200	300	400	500	99% MeOH
	zones of inhibition in millimetres					zones of inhibition in millimetres				
Staphylococcus auerus(clinical isolate)	–	–	–	–	–	26.33±0.43*	26.67±0.50*	27.33±0.57*	28.00±0.00*	–
Staphylococcus auerus (NCTC 6571)	–	–	–	–	–	21.33±0.44*	21.67±0.65*	23.00±0.00*	23.67±0.55*	–
*Staphylococcus auerus (*ATCC 25923)	–	–	–	–	–	10.67±0.54*	11.33±2.0*	13.00±0.0*	15.00±0.0*	–
Bacillus substiles (NCTC 8853)	–	–	–	–	–	18.00±0.00*	19.67±0.33*	20.00±0.00**	21.00±0.00*	–
Shigella dysenteriae (clinical isolate)	–	–	–	–	–	–	–	–	–	–
Salmonella typhii (NCTC 8571)	–	–	–	–	–	–	–	–	–	–
Pseudomonas aeruginosa (ATCC 25922)	–	–	–	–	–	7.33±0.33*	7.67±0.66*	8.00±0.00*	9.33±0.11*	–
Escherichia coli (NCTC 104418)	–	–	–	–	–	–	–		11.67±0.40*	–
Escherichia coli (ATCC 25922)	–	–	–	–	–	–	–	20.33±0.22*	21.33±0.33*	–
Candida albicans I	–	–	–	–	–	–	–	–	–	–
Candida albicans II	–	–	–	–	–	–	–	–	–	–

*P<0.05 (ANOVA; LSD post hoc); values shown are Mean ± SEM (n = 3).

CONCLUSION

The activity of the methanolic extract of the root of *Aspilia africana* is basically antibacterial and not antifungal as all the strains of the fungi used in this test were not inhibited. This antibacterial activity has confirmed that root part of the plant is potentially useful in the treatment of wound sepsis and mycobacterial infections and can be deployed in wound management because it is able to check the activities of wound contaminants, promote rapid wound healing by eliminating infections and thus allowing the natural tissue repair process to go on unhindered and without side effects as result of toxicity (LD_{50} is high). The study has further provided a rationale for the use of the plant in wound care in ethno-medicinal practices in Africa. This is the first time an antibacterial study has been conducted on the root part of *Aspilia africana.*

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