



Phytochemical activities of *Aspilia africana* leaf using different extractants

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ABSTRACT

Aspilia africana plant is used in herbal medicine for the perceived presence of some bioactive components in the leaves. This study evaluated the phytochemical activities in the meal; aqueous, chloroform and ethanolic extracts of *A. africana* leaves. The anti-microbial activity of the 4 samples of *A. africana* were tested on 9 micro-organisms (6 bacteria and 3 fungal strains) using the agar well diffusion technique. Results of the phytochemical screening and subsequent quantification revealed the presence of high amount of some bioactive compounds; saponins, tannins, alkaloids, flavonoids, terpenoid and phenol, but the absence of steroids (leaf meal and all extracts), phylobatannin (chloroform and ethanolic extracts) and cardiac glycoside (ethanolic extract) in the *A. africana* leaf products. Though the chloroform leaf extract had higher concentrations of these phytochemicals, significant improvements were observed in the chemical composition of the aqueous and ethanolic extracts. The anti-microbial activities indicated that biological activities were dependent on the types of extractants and the concentrations of principles present such as alkaloids and tannins. These activities were comparable to those obtained for ampicillin and gentamycin. The susceptibility of the microorganisms to the different *A. africana* leaf samples demonstrated its anti-bacterial and anti-fungal potentials and validated its use as an anti-microbial agent in ethnoveterinary medicine. Further investigations are required on their efficacies as phytobiotics in animal production.

Key words: Antimicrobials, *Aspilia africana* leaf, Crude extracts, Phytochemistry

The biological activities of phytochemicals have been the focus of many studies (Ushie and Adamu 2010). Several countries have programmes screening indigenous medicinal plants for their antimicrobial activities (Ahmad and Beg 2001, Atai *et al.* 2009, Kumar *et al.* 2010, Thakare *et al.* 2010). Plants from Nigerian biomass were used in indigenous medicines in the treatments of malaria (Ajaiyeoba *et al.* 2006), fungal and bacterial infections (Edeoga *et al.* 2005, Okoli *et al.* 2007) and in livestock management (Oguike and Udeh 2009). The medicinal values of plants lie in some chemical substances that produce definite physiological actions in animals (Ushie and Adamu 2010). The most important of these bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds (Huyghebaert 2003).

Aspilia africana (Bush marigold), sometimes called iodine or haemorrhage plant, an *Asteraceae* species, was evaluated for its potential chemoprotective bioactivities. It is utilized in the feeding of cattle, sheep, goats, rabbits, and hares (Boyejo 1994). Although, aqueous and alcoholic whole plant solutions of *A. africana* are orally or topically administered

in various medications for its wound healing, anti-inflammatory, anti-bleeding and anti-bacterial activities (Page *et al.* 1992), limited scientifically proven information is available on its antimicrobial activities and its efficacy as a potential phytobiotic for animal production.

The antimicrobial efficacy of a plant material is determined by the type of extractant used, method of extraction and the chemical composition of the plant product (Harborne 1993). To determine the efficacy of *A. africana* leaf, the phytochemical profile and the antimicrobial activities of the aqueous, chloroform and ethanolic extracts of the leaves were analysed.

MATERIALS AND METHODS

The leaves used in the experiment were harvested from farms within the vicinity of the University of Calabar's, Teaching and Research Farms. The plant material (*A. africana* leaf) was identified.

The leaves were shroud and oven-dried (40°C) for 24 h. The dried leaves were milled into powder form (*Aspilia africana* leaf meal – AaLM) using an electric-blender and stored in an air-tight container prior to extraction and analyses. Plant extraction was performed using solvents – aqueous, chloroform and ethanol (Smith *et al.* 2001). The

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aqueous and ethanolic extracts of the leaf were prepared by soaking 200 g of AaLM in 400ml of distilled water, 600ml of 80% ethanol and 600ml of 80% chloroform for 24 h. Each slurry was filtered using Whatman filter paper #1. The filtrates were subsequently concentrated *in vacuo* (40°C) and the residues were dried under reduced pressure to determine the dry weight of the leaf residues (Oyewole 2004). The weights of dry residues were recorded and the percentage extraction yield was calculated.

Determination of chemical composition

The phytochemical screening and quantitative chemical analysis of AaLM and its crude extracts were carried out for the presence of anthraquinine, tannins, saponins, cyanogenic glycoside, flavonoids, phenol and terpenoids (Sofowora 1993). The proximate constituents, gross energy and mineral contents of the samples of AaLM and its crude (aqueous, chloroform and ethanolic) extracts were determined using standard (AOAC 2000) methods.

Data obtained were subjected to the one-way ANOVA in a completely randomized design using SPSS (2006) software. The results were presented as mean values of 3 replicates. Means were separated using the Duncan multiple range test at 5% significance level.

Antimicrobial screening: The agar well diffusion technique (Adeniyi and Odunfowora 2000) was adopted in testing the anti-microbial activity of *A. africana* leaf meal and crude extracts on 9 micro-organisms (Table 1) that consisted of 3 fungal and 6 bacteria strains.

The procedure for anti-microbial screening consisted of seeding each of the nutrient agar with 0.1ml of an overnight culture of each bacteria (equivalent to 10^7 – 10^8 cfu/ml). The sabour and dextrose agar plates were also seeded with each fungal strain. The seeded plates were allowed to set, and then dried in the incubator at 17°C for 20 min. A standard cork borer of 8mm diameter was used to drill uniform wells on the surface of the agar and 60ml of sodium was added to each sample. Each sample was resuspended in 50% ethanol at 20mg/ml concentration. Nutrient agar plates with bacterial isolates were incubated at 37°C for 24 h and sabour dextrose agar plates seeded with fungal strains were incubated at 25°C

for 72 h. Since each chloroform and ethanolic extract of *Aspilia africana* leaves was resuspended in 50% ethanol before testing, ethanol was included in each plate as a solvent control aside the chemotherapeutic agents included as positive control. For bacterial strains, gentamycin (10µg/ml) served as the control while trioconazole (1%) was used as negative control for fungi. All assays were carried out in triplicate. The sensitivity of each leaf sample to test bacterial and fungal strains were monitored.

RESULTS AND DISCUSSION

The extraction yield of different leaf extracts of *Aspilia africana* was significantly ($P < 0.05$) higher (Table 2) in the ethanolic extract, followed by the chloroform extract while, the least yield was recorded in the aqueous extract of AaLM. Macroscopic features showed that the aqueous extract, with a coffee-like colour and smell, was most soluble in water while, the yellow-green coloured chloroform extract was insoluble. This was based on the degree of polarity of solvent used. Per cent extract yields were higher than values reported for *Borreria verticillata* leaves in hexane (5.90%), chloroform (5.00%), acetone (2.90%), ethyl acetate (4.5%) and methanol (3.4%) solvents (Ushie and Adamu 2010).

Phytochemical screening of the 4 samples (Table 3) revealed the presence of saponins, tannins, alkaloids, flavonoid, terpenoids and phenol, but the absence of steroids (all 4 samples), phylobatannin (chloroform and ethanolic extract) and cardiac glycoside (ethanolic extract). Quantitatively, the significant ($P < 0.05$) reduction in the levels of the phytochemical concentrations in the 3 crude extract of AaLM (Table 4), came within tolerable levels for, especially, monogastric animals. The presence of these bioactive constituents in *A. africana* leaves and their subsequent reductions under aqueous and ethanolic extractions might explain its use, in traditional medicine, as anti-inflammatory, bactericidal, analgesic and stringent agents (Okwu and Okwu 2004). Marked physiological activity was also reported when administered to animals (Okwu 2004). The chloroform extract of AaLM however, resulted to significant ($P < 0.05$) elevations in phytochemical concentrations comparable to the result of Ushie and Adamu (2010).

Table 1. Micro organisms used in assessing the anti-microbial activity of *A. africana* leaf meal and extracts.

Microorganism	Properties	Source
<i>Ruminococcus flave faciens</i>	-	Laboratory stock
<i>Sarcina ventriculi</i>	-	Laboratory stock
<i>Bacillus subtilis</i>	-	Laboratory stock
<i>Esherichia coli</i> UCH 270	Resistant to ampicillum, terramycin and S&T	Clinical isolation
<i>Streptococcus bovis</i>	-	Laboratory stock
<i>Staphylococcus aureus</i> UCH 560	Sensitive to all antibiotics	Clinical isolation
<i>Aspergillus parasiticus</i>	-	Laboratory stock
<i>Aspergillus fumigatus</i>	-	Laboratory stock
<i>Penicillium notatum</i>	-	Laboratory stock

Table 2. Extraction yield and macroscopic features of crude extracts of *Aspilia africana* leaves

Property	Aqueous extract	Chloroform extract	Ethanol extract
Wt. of AaLM (g)	200	200	200
Wt. of residue (g)	179.60	174.92	166.04
Extract yield (g)	20.40	25.08	33.96
% Extract yield	10.20	12.54	16.98
Macroscopic feature			
Colour	Dark brown	Yellow green	Dark green
Texture	Smooth paste	Smooth paste	Smooth paste
Odour	Coffee-like	Indistinctive	Tobacco-like
Degree of solubility in H ₂ O	Highly soluble	Insoluble	Moderately soluble

Table 3. Phytochemical screening of *Aspilia africana* leaf and crude extracts

Constituent	Leaf	Aqueous extract	Chloroform extract	Ethanol extract
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	+	+
Steroid	-	-	-	-
Phylobatannin	+	+	-	-
Terpenoid	+	+	+	+
Flavonoids	+	+	+	+
Cardiac glycoside	+	+	+	-

+ (presence of constituent); - (absence of constituent).

This partly explains the limited use of chloroform as extractant, since these elevations might cause some adverse physiological conditions (such as toxicity and death) in the animals. Results of proximate and mineral analyses (Tables 5, 6) revealed that the nutritive and mineral availability of *Aspilia africana* leaves improved following extraction. This could have resulted from the possible release of bond nutrients within the plant material. Crude protein content increased up to 2-times the value for the AaLM sample. Calcium was the most abundant element present, followed by zinc and phosphorus while the least was manganese. Trace elements such as copper and lead were not detected in all any of the 4 samples probably due to the relatively uncontaminated environment of the University of Calabar Teaching and Research Farm.

The high calcium and zinc content in the aqueous and ethanolic extracts of AaLM could confirm its value as a useful material for wound treatment and diabetic regulation (Okaka and Okaka 2001). Given the direct relationship between sodium intake and the occurrence of hypertension in humans, the higher sodium content in the chloroform extract, compared to the levels in the aqueous and ethanolic extracts,

Table 4. Quantitative phytochemical constituents of *A. africana* leaf and crude extracts

Constituent	Crude extracts				
	AaLM	Aqueous	Chloroform	Ethanol	SEM
Tannin acid (%)	5.45 ^b	1.33 ^d	9.01 ^a	3.77 ^c	0.42
Phenol (%)	2.41 ^b	0.50 ^d	4.50 ^a	1.65 ^c	0.09
Saponins (%)	6.53 ^b	4.96 ^d	7.44 ^a	5.40 ^c	0.26
Alkaloids (%)	3.56 ^c	3.49 ^c	5.35 ^a	4.27 ^b	0.15
Flavonoids (%)	2.55 ^a	1.85 ^b	0.48 ^c	0.55 ^c	0.05
Cyanide (mg/kg)	1.52 ^b	0.85 ^c	2.20 ^a	1.18 ^b	0.04
Oxalate (mg/kg)	3.27 ^a	0.66 ^c	2.15 ^{ab}	1.89 ^b	0.13
Phytic acid (mg/g)	22.65 ^b	17.30 ^c	27.73 ^a	25.26 ^a	0.07
Phytin phosphorus (mg/g)	6.38 ^b	4.87 ^c	7.81 ^a	7.11 ^{ab}	0.15

Different superscripts (a, b, c, and d) indicate significant (p < 0.05) differences along rows. *AaLM - *Aspilia Africana* leaf meal; SEM, standard error of mean.

Table 5. Proximate composition of *A. africana* leaf and crude extracts

Constituent	Crude extracts				
	AaLM	Aqueous	Chloroform	Ethanol	SEM
Moisture (%)	7.50 ^c	8.42 ^c	10.15 ^b	13.53 ^a	0.88
Crude protein (%)	14.26 ^d	26.88 ^a	25.18 ^b	23.10 ^c	0.15
Crude fat (%)	8.53 ^b	6.59 ^c	9.32 ^a	9.44 ^a	0.05
Crude fibre (%)	6.75 ^b	6.58 ^b	7.37 ^a	6.33 ^c	0.03
Ash (%)	11.99 ^c	11.97 ^c	15.81 ^b	17.91 ^a	0.34
Carbohydrate (%)	51.00 ^a	39.08 ^b	32.18 ^{cd}	29.70 ^d	0.53
Energy (kg/kcal)	1422.34 ^a	1364.98 ^b	1319.96 ^c	1246.11 ^d	2.25

Different superscripts (a, b, c, and d) indicate significant (P<0.05) differences along rows. *AaLM - *Aspilia africana* leaf meal; SEM, standard error of mean.

becomes a disadvantage (Dahl 1972). Kumar *et al.* (2010) reported higher levels of potassium than calcium in chaya leaf.

The anti-microbial activities of *Aspilia africana* leaf meal and its extracts are presented in Table 7. The chloroform extract showed a broader anti-microbial activity compared to the aqueous, ethanolic and leaf meal. The anti-microbial activities observed were comparable to those obtained for ampicillin and gentamycin. Variations observed in anti-microbial activities of the different forms of *A. africana* leaf could be attributed to the presence of some active components as well as the differences in concentration of active ingredients as suggested in literature (Adeniyi and Odufowora 2000, Atai *et al.* 2009). Ushie and Adamu (2010) noted that chloroform and methanol extracts of *Borreria verticillata* leaves exhibited negative activity against

Table 6. Mineral composition of *A. africana* leaf and crude extracts

Mineral (mg/100kg)	Crude extracts				SEM
	AaLM	Aqueous	Chloroform	Ethanollic	
Sodium	203.56 ^c	307.74 ^b	535.34 ^a	320.15 ^b	5.42
Potassium	345.10 ^d	582.10 ^c	1009.45 ^a	619.90 ^{bc}	4.46
Calcium	667.94 ^c	1027.54 ^{ab}	1679.50 ^a	1004.39 ^b	4.22
Magnesium	248.09 ^c	575.84 ^a	209.94 ^c	301.32 ^b	2.16
Zinc	512.88 ^c	872.11 ^b	1163.40 ^a	834.90 ^b	4.75
Iron	11.93 ^c	25.56 ^b	44.79 ^a	50.53 ^a	2.08
Phosphorus	474.51 ^c	440.43 ^c	1211.69 ^a	882.77 ^b	5.44
Cromiumum	0.24 ^{ab}	0.01 ^c	0.17 ^b	0.31 ^a	0.01
Manganese	0.08 ^c	0.31 ^a	0.17 ^b	0.16 ^b	0.01
Copper	ND	ND	ND	ND	-
Lead	ND	ND	ND	ND	.

Different superscripts (a, b, c, and d) indicate significant (P<0.05) differences along rows.

Salmonella spp while, Joseph and Raj (2010), Preethi *et al.* (2010) showed that the aqueous extract of *Ficus religiosa* has high antimicrobial activity against selected organisms especially *Bacillus subtilis* and *P. aeruginosa*.

The susceptibility of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus bovis*, and *Bacillus subtilis* to extracts of *A. africana* leaf strongly supports its anti-bacterial potential and its use as anti-bacterial agent (Akobundu 1987). These anti-bacterial properties exhibited by the plant are likely traceable to the presence of alkaloids and tannins noted for their astringent effects (Adeniyi and Odufowora 2000).

Some of the bacteria that were susceptible to AaLM were implicated in diseases such as diarrhoea and oral, dental and wound infections (Hugo and Russel 1983). Also, the 3 fungi strains studied were susceptible to all *A. africana* leaf samples indicating that *Aspilia africana* leaf possesses strong anti-fungal properties. This observation agreed with the findings of Adeniyi and Odufowora (2000) that *Aspergillus flavus* and *Candida albican* were susceptible to cold extracts of *Aspilia africana* leaf. Variations in the chemical composition and biological activities observed between *Aspilia africana* leaf products could suggest varying *in vivo* physiological effects of each product.

These further confirmed that, the efficacy of a plant material is dependent on the type of extractant used and the concentrations of bioactive substances present as purported by Ellof (1998) and Harborne (1993).

Aspilia africana leaf is rich in crude protein, minerals and beneficial phytochemicals. It has strong anti bacterial and anti fungal properties that validate its applications as an antibacterial agent, for disease prevention and treatment in herbal and ethno-pharmaceutical practices. Aqueous and ethanolic extraction methods improved the bioavailability of nutrients in the plants. Further studies could be conducted to investigate the potentials of *Aspilia africana* leaf meal and

Table 7. Anti-microbial activities of *Aspilia africana* leaf meal and extracts

Microorganism	AaLM	Crude extracts		
		Aqueous	Chloroform	Ethanollic
<i>Ruminococcus flavefaciens</i>	+	-	+	+
<i>Sarcina ventriculi</i>	-	-	-	+
<i>Bacillus subtilis</i>	-	-	+	+
<i>Escherichia coli</i>	+	+	+	-
<i>Streptococci bovis</i>	-	+	-	-
<i>Staphylococcus aureus</i>	-	+	+	-
<i>Aspergillus flavus</i>	+	+	+	+
<i>Fumigatus</i> spp	+	+	+	+
<i>Penicillium notatum</i>	+	+	+	+

AaLM - *Aspilia africana* leaf meal; + microorganism is sensitive to the extract; - microorganism is insensitive to the extract.

extracts as cheap and available phytobiotics in livestock production.

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