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Antimicrobial and anthelmintic Evaluation of Nigerian Euphorbiaceae Plants 3: *Acalypha wilkesiana*

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ABSTRACT: Methanolic extracts of leaf, stem and root of *Acalypha wilkesiana* were investigated with the goal of establishing its acclaimed potency as an anthelmintic and antimicrobial agent. Antimicrobial assay was carried out using the agar cup plate diffusion method. The extracts were tested against 8 human pathogenic microorganisms (5 bacteria and 3 fungi). The leaf and stem methanol extracts exhibited antibacterial and antifungal activities. The leaf methanol extract showed antibacterial activity against all the test organisms namely, *Staphylococcus aureus, Bacillus subtilis, Salmonella typhii*, *Eschericia coli* and *Pseudomonas aeruginosa* and displayed marked antifungal properties against *Aspergillus niger, Candida albicans* and *Dermatophyte sp*. exhibiting higher antifungal activity than the reference drug tioconazole. The methanolic extract of the stem on the other hand inhibited the growth of all the test microorganisms except *Salmonella typhii* and *Dermatophyte sp*. and displayed greater antibacterial activity with two of the microorganisms namely, *Bacillus subtilis and Escherichia coli* than the reference compound ampicillin. Antibacterial and antifungal activity was concentration- dependent except for *Dermatophyte sp* where activity was independent of extract concentration. All the extracts exhibited *in vitro* anthelmintic activity against *Fasciola gigantica, Taenia solium* and *Pheritima pasthuma. T. solium* was most sensitive to the ethyl acetate extract of the leaf while *P. pasthuma* was more sensitve to most of the extracts than the reference compound, piperazine citrate. The extracts contain important secondary metabolites - cardiac glycosides, tannins, saponins and alkaloids. These results justify the ethnomedicinal uses of the plant.

Keywords: *Acalypha wilkesiana*, antimicrobial, anthelmintic, Euphorbiaceae.

Introduction

Acalypha wilkesiana Mull. Arg. (Euphorbiaceae), a lush ornamental tallish hedge shrub known for its antimycotic, antibacterial, anti-inflammatory, hemostatic and analgesic activities. The plant is widely distributed in West Africa and tropical Asia. The juice of the leaf is smeared on fungal skin infections and decoction of leaves when drunk is reported to be antimycotic and antibacterial in action (Oliver-Bever 1986). Leaf poultice is used for headache, swelling, cold and wound dressing. Chopped pieces of the dried stem and root were steeped in alcohol and used for stomach ache and as worm expellant in man in the Delta region of Nigeria (Iwu 1993; Burkill 1985).

A flavonoid, liquiritigenin, condensed and hydrolysable tannins (Maran *et al*., 1993) have been isolated from the leaves. Phytochemical and biological examinations of the leaves against seven organisms have also been reported (Adesina *et al*., 1980; 2000). In continuation of our antimicrobial evaluation of Nigerian Euphorbiaceae plant (Onocha *et al*., 2003), the anti-microbial and anthelmintic evaluation of Nigerian Euphorbiaceae plants 3: *Acalypha wilkesiana* (Mull. Arg.) was undertaken and the findings hereby presented.

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Materials and Methods

Plant Material

The root, stem and leaves of *A. wilkesiana* were collected from Amina Way, University of Ibadan and authenticated by Mr Felix Usang of the Forest Research Institute of Nigeria (FRIN) where a voucher specimen (FHI - 106454) was deposited.

Preparation and Extraction of Plant Material

Air dried and coarsely powdered leaves (500 g), stem (1 kg) and root (1.5 kg) were extracted successively with hexane (yielded 3.9, 1.4 and 1.8g of leaves, stem and roots extracts, respectively), ethyl acetate (gave 4.2, 3.2 and 4.2g of leaves, stem and roots extracts, respectively) and methanol (afforded 5.9, 4.5 and 3.9g of leaves, stem and root extracts, respectively), using a soxhlet apparatus. The extracts were stored in the refrigerator until required.

Antimicrobial Assay

Nutrient broth, nutrient agar, sabouraud dextrose agar (SDA), tryptone soya agar (Oxoid Laboratories, U.K.) were used in the assays. Methanol (Merck) was also used in solubilising the extracts\drugs and as a negative control in the assays. Ampicillin, 12.5 mg\ml (Lab Oftalmiso, Spain), tioconazole cream 12.5 mg\ml (Pfizer Inc., New York) were included as standard reference drugs in the study.

Clinical strains of human pathogenic microorganisms made up of 5 bacteria and 3 fungi namely; *Staphylococcus aureus, Bacillus subtilis, Salmonella typhii*, *Eschericia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger, Candida albicans* and *Dermatophyte sp.* (listed in Table 1) were obtained from the Microbiology laboratory unit of the Pharmaceutical Microbiology Department, University of Ibadan. The cultures were maintained on nutrient agar slants and preserved at 4-5^{o} C until needed for antimicrobial activity. Antimicrobial activity was carried out by the cup agar broth diffusion method (Kavanagh 1972).

An overnight broth culture of 1-2 x 10^{7} CFU of each bacterium and fungus was used to seed sterile molten agar medium maintained at 45^{o}C. Sterile tryptone soya agar plate was similarly seeded with fungi. Seven wells were bored in each plate (7mm, diameter) with an aseptic cork borer when seeded plates had solidified. Different concentrations of the extracts (1ml portions) were introduced into the wells with the aid of a Pastuer pipette and controls were set up containing solvent and ampicillin solution (12.5 mg / ml). Diameters of zones of inhibition were determined after incubating plates at 37^{o} C for 24 h (bacteria) and at 25^{o}C for 72 h (fungi). When seeded with bacteria, each plate had wells filled with methanol as well as ampicillin and for fungi; tioconazole was filled in one of the wells also. Diameters of zones of inhibition ≥10mm were considered active (Kavanagh 1972). Antimicrobial studies were done in triplicates and diameters of zones of inhibition (mm) are expressed as the mean and standard errors of means. Student's 't' test was used to test probability at $P<0.05$.

Anthelmintic Assay

Helminths used for the assay include *Fasciola gigantica* (liver fluke, mean weight of 0.05-0.07g), *Taenia solium* (tapeworm, 2.4-2.8g) and *Pheritimia pasthuma* (earthworm, 0.5-0.6g). *P. pasthuma* was collected from the Awba Dam and the water logged areas of the Staff School, both within the campus of University of Ibadan (UI) while the other two worm types were obtained from freshly slaughtered cows in the Bodija abattoir, in Ibadan metropolis. All three worm types were authenticated at the Parasitology Research Unit, Zoology Department of the University of Ibadan. Anthelmintic assay was carried out as previous described (Ajaiyeoba *et al*., 2001).

Two worms (same type) were both placed in 9 cm petri dishes in solution of crude extracts of six different concentrations (10, 20, 30, 50, 80 and 100 mg/ml in distilled water), respectively. Mean times for paralysis (P, in minutes) were recorded when no movement of any sort could be observed, except when the worms were shaken vigorously. Times of death of worms (D, in minutes) were also recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^{o}C). Piperazine citrate (10 mg/ml) was included as standard. The assay was done in triplicates for each concentration of the extract, standard and control. Six extracts namely, ethyl acetate extracts of leaves (LE), root (RE) and stem (SE) respectively and methanolic extracts of leaves (LM), root (RM) and stem (SM) respectively of the plants (as listed in Table 2) were used in this study.

Results

Antimicrobial activity

Concentrations ranging from 25 mg/ml to 125 mg/ml (in triplicates) of the various extracts were tested against 8 human pathogenic micro organisms as shown in Table 1, the methanol extract of the leaf showed remarkable inhibition of all the test organisms.

The mean zones of inhibition produced by the methanol extract of the leaf was found to be 9-17 mm (bacterial) and 20-28 mm (fungi) while ampicillin was found to be 13-27 mm and tioconazole 12-16 mm. Antibacterial and antifungal activity was concentration-dependent except against *Dermatophyte sp* where activity was independent of extract concentration. The methanolic extract of the leaf displayed marked antifungal properties against *Aspergillus niger, Candida albicans* and *Dermatophyte sp*. exhibiting higher antifungal activity than the standard/reference drug, tioconazole. It is worthy to note that *Dermatophyte sp*. used in the study was resistant to tioconazole but the methanolic extract of the leaf inhibited their growth. The mean zones of inhibition exhibited by the methanolic extract of the stem ranged from 8 to 19 mm (bacterial) and 8 to 20 mm (fungi) while that of ampicillin and tioconazole ranged from13 to 25 mm and 12 to 17 mm respectively. The methanolic extract of the stem inhibited the growth of all the test microorganisms except *Salmonella typhii* and *Dermatophyte sp*. and displayed greater antibacterial activity with two of the microorganisms, *Bacillus subtilis* and *Eschericia coli* than the reference compound ampicillin.

Table 1. Antimicrobial and anthelmintic Evaluation of Nigerian Euphorbiaceae Plants 3: *Acalypha wilkesiana*

Plant Extract	Mean Diameters of zones of Inhibition of bacteria in mm (+SEM[b])					Mean Diameters of zones of Inhibition of fungi in mm (+SEM[b])		
Leaf extract	*B. subtilis*	*S.typhii*	*S.aureus*	*E.coli*	*P. aeruginosa*	A..niger	C.albicans	*D.spp*
25	13±0.5	13±0.4	-	-	13±0.2	20±0.5	22±0.9	20±0.2
50	13±0.1	13±0.3	-	9±0.5	14±0.6	20±0.2	22±0.5	20±0.1
75	13±0.5	15±0.1	9±0.8	10±0.2	14±0.5	20±0.1	25±0.5	20±0.3
100	15±0.2	17±0.2	-	11±0.3	14±0.3	25±0.1	25±0.1	20±0.3
125	17±0.5	17±0.2	15±0.4	11±0.3	17±0.3	25±0.2	28±0.9	20±0.2
Amp/Tioconazole (12.5)	15±0.5	23±0.1	27±0.2	13±0.2	18±0.1	12±0.5	16±0.8	-
Stem extract								
25	10±0.5	-	12±0.1	-	-	-	-	-
50	12±0.3	-	14±0.8	-	-	8±0.1	15±0.1	-
75	16±0.1	-	16±0.6	12±0.1	8±0.1	8±0.1	20±0.6	-
100	17±0.6	-	17±0.4	15±0.5	11±0.3	12±0.4	20±0.1	-
125	19±0.1	-	18±0.2	16±0.6	13±0.1	12±0.5	20±0.2	-
Amp/Tioconazole	15±0.4	20±0.5	25±0.1	13±0.1	17±0.9	12±0.5	17±0.1	-

Anthelmintic activity

Anthelmintic activity is reported in Table 2. All the extracts exhibited *in-vitro* anthelmintic activities against *Fasciola gigantica, Taenia solium* and *Pheritima pasthuma.* Interestingly, *T. solium* was more sensitive to the ethyl acetate extract of the leaf than the reference drug piperazine citrate while *P. pasthuma* was more sensitve to most of the extract than piperazine citrate.

Table 2 :Anthelmintic activity of Extracts of *Acalypha wilkesiana*

S/N	Plant Extracts	Yield (%)	Conc. (mg/ml)	Time of Paralysis (P) and Death (D) of worms in minutes (+SEM)					
				T. solium		***F. gigantica***		***P. pasthuma***	
				P	D	P	D	P	D
1.	LE	5.9	10	5.4±0.5	8.4±0.3	5.1±0.1	8.6±0.2	23±0.1	60±0.2
			20	4.3±0.3	8.3±0.2	4.3±0.1	7.5±0.9	22±0.3	57±0.7
			30	2.3±0.2	7.0±0.6	3.5±0.2	7.0±0.5	20±0.8	55±0.1
			50	1.7±0.5	5.0±0.5	3.2±0.5	5.5±0.5	18±0.2	52±0.3
			80	1.6±0.4	4.0±0.2	3.0±0.2	5.3±0.1	15±0.5	49±0.2
			100	1.5±0.9	2.0±0.1	2.3±0.1	5.1±0.9	13±0.1	46±0.1
2.	LM	4.2	10	23±0.5	70±0.2	43±0.1	60±0.5	45±0.2	60±0.2
			20	20±0.3	67±0.6	40±0.3	60±0.2	42±0.5	55±0.5
			30	18±0.2	65±0.5	38±0.8	58±0.1	40±0.3	50±0.3
			50	15±0.5	58±0.2	36±0.2	55±0.1	38±0.8	44±0.8
			80	18±0.9	50±0.1	28±0.5	40±0.2	31±0.5	45±0.5
			100	10±0.4	40±0.2	28±0.3	58±0.2	32±0.9	60±0.5
3.	RE	3.9	10	29±0.5	52±0.2	36±0.5	30±0.3	33±0.9	45±0.2
			20	25±0.4	49±0.1	27±0.7	24±0.6	28±0.5	42±0.1
			30	23±0.5	46±0.3	25±0.2	18±0.5	26±0.3	40±0.3
			50	19±0.2	38±0.3	21±0.5	18±0.3	23±0.8	36±0.3
			80	18±0.1	31±0.2	20±0.4	15±0.2	22±0.2	35±0.2
			100	15±0.1	26±0.2	17±0.5	13±0.3	20±0.1	30±0.3
4.	RM	4.2	10	7.1±0.9	38±0.2	4.5±0.5	8.1±0.1	39±0.1	45±0.5
			20	6.1±0.5	30±0.2	4.3±0.7	7.1±0.5	30±0.2	38±0.3
			30	3.4±0.3	31±0.3	3.3±0.2	7.0±0.3	26±0.5	32±0.2
			50	1.2±0.8	26±0.6	3.0±0.5	6.6±0.5	24±0.3	27±0.5
			80	1.0±0.2	24±0.5	2.6±0.4	6.0±0.4	15±0.1	20±0.9
			100	1.0±0.1	18±0.5	2.0±0.4	3.9±0.1	9±0.1	15±0.1
5.	SE	3.2	10	23±0.1	60±0.1	8.3±0.9	23±0.5	80±0.2	105±0.1
			20	22±0.3	57±0.5	7.1±0.5	22±0.5	75±0.1	100±0.3
			30	20±0.8	55±0.2	4.5±0.5	20±0.4	70±0.3	98±0.8
			50	18±0.2	52±0.7	4.3±0.1	18±0.3	70±0.3	90±0.5
			80	15±0.5	49±0.3	4.2±0.9	15±0.2	68±0.4	90±0.2
			100	13±0.1	46±0.2	3.1±0.5	14±0.1	62±0.2	90±0.1
6.	SM	4.5	10	34±0.9	57±0.5	15.4±0.2	18.5±0.2	39±0.5	45±0.5
			20	31±0.5	40±0.8	11.5±0.5	16.5±0.6	30±0.7	35±0.3
			30	28±0.3	37±0.2	8.0±0.5	15.0±0.5	26±0.2	32±0.2
			50	13±0.8	15±0.3	5.4±0.1	13.5±0.2	21±0.5	27±0.5
			80	10±0.1	12±0.2	5.3±0.1	11.5±0.2	15±0.4	20±0.1
			100	5±0.2	8±0.2	3.5±0.2	7.4±0.3	9±0.1	15±0.9
7.	Piperazine citrate		10	1.6±0.05	40±0.05	1±0.2	3±0.05	20±0.2	60±0.5

$P < 0.05$

[a] control worms (in distilled water) for:

(i) *T. Solium* lived till the next 24 hours.

(ii) *F. gigantica* were alive for 5 hours.

(iii) *P. pasthuma* were alive up till 48 hours.

[b] N =3

Discussion

Acalypha wilkesiana is widely used in various classical and herbal formulations worldwide. Apart from its diverse uses, it has been reported to be antimycotic and antibacterial in action (Oliver-Bever 1986). Leaf poultice is used for headache, swelling, cold and wound dressing. Chopped pieces of the dried stem and root is steeped in alcohol and used for stomach ache and as worm expellant in man in the Delta region of Nigeria (Iwu 1993; Burkill 1985).

To the best of our knowledge, there is no previous ethno-medical report on the anthelmintic activity of all the parts of *Acalypha wilkesiana* or the antimicrobial activity of the stem. Our decision was based on the observation of its use as a worm expellant, an antimycotic agent and in the treatment of wounds and swellings. This is thus, the first report on the anthelmintic assay of different parts of the plant as well as the antimicrobial activity of the stem. The results of present study indicated that the methanolic extract of the stem of *A. wilkesiana* is antimicrobial while all the extracts exhibited *in vitro* anthelmintic activities against the three helminths used in the assay. The present use of *Acalypha wilkesiana* in the treatment of wounds, swelling and as a worm expellant (Oliver-Bever 1986; Iwu 1993; Burkill 1985) might be explained in the light of these results. This finding is also in accordance with the earlier report of antimicrobial activity of the leaves([Adesina *et al*., 1990).

The observed activity is consistent with the ethno-medicinal uses of the plant. We are looking into isolation of the active principles in these extracts and these will be reported at a later date.

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