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Antimicrobial Activity of the Methanolic and Crude Alkaloid Extracts of *Acalypha wilkesiana* cv. *macafeeana* Copper Leaf

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Abstract: The antimicrobial activity of methanolic leaf extracts and crude alkaloid extracts of *A. wilkesiana* cv. *macafeeana* was evaluated after a preliminary phytochemical screening of the leaf extracts. The standard agar well diffusion method was used in the bioassay involving test bacteria and yeast isolates, while percentage inhibition of extracts on radial growths of the molds was determined. The Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MBC) were also determined by the broth microdilution assay technique. The microorganisms used were clinical strains of *Escherichia coli*, *Salmonella typhi*, *Streptococcus pyogenes*, *Strept. pneumonia*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), non-methicillin resistant *Staph. aureus*, *Candida albicans*, *Aspergillus fumigatus* and *A. flavus*. Alkaloids, tannins, terpenoids and cardiac glycosides were extracted by the methanol solvent. The crude alkaloid extracts inhibited only the Gram-negative bacteria with mean inhibition zones of 10.0±0.00 to 12.3±0.03 mm while the methanol extracts inhibited all other test organisms, a broad spectrum activity. The water extracts had no activity against the non-MRSA strains. The MIC was 0.4 mg mL^{-1} for all unicells except strains of *C. albicans* which both had MICs of 0.8 mg mL^{-1}. The MBC was 0.4 mg mL^{-1} for tested isolates except the non-MRSA and *C. albicans* which had MBCs of >12.0 mg mL^{-1} and 1.0 mg mL^{-1}, respectively. The methanolic extract totally inhibited all tested aspergilli while the water extract had a varying inhibitory effect (63.0±2.50 to 81.0±2.90%) on the tested fungi strains. The alkaloid had no effect on the molds.

Key words: Antimicrobial, alkaloid, *Acalypha wilkesiana* cv. *macafeeana*, phytochemical

INTRODUCTION

The use of plant, plant extract or plant-derived chemicals to treat diseases; topical, subcutaneous and systemic, has stood the test of time (Oladunmoye, 2006). In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs (Iniaghe *et al*., 2009). Also, there has been little or no report of any form of microbial resistance during the use and administration

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of herbal medicines (Stephen *et al*., 2009). More importantly in Africa, particularly West Africa, new drugs are often beyond the reach of the poor such that up to 80% of the population use medicinal plants as remedy against infections and diseases (Kirby, 1996; Hostettmann and Maston, 2002).

Acalypha wilkesiana belongs to the family euphorbiaceae and grows as an annual bedding plant (Oladunmoye, 2006). This large, fast-growing, evergreen shrub provides a continuous splash of colour in the landscape with the bronze red to muted red, 4 to 8 inch long, heart-shaped leaves available in varying mottled combinations of green, purple, yellow, orange, pink, or white, depending upon cultivar (Gilman, 1999). Although, there are quite a reasonable number of cultivars worldwide, the macrophylla, hoffamanii, godseffiana, macafeeana, hispida, marginata and racemosa are peculiar cultivars within Nigeria (Oladunmoye, 2006; Yusha'u *et al*., 2008; Iniaghe *et al*., 2009).

Investigations have been carried out on almost all the available cultivars within Nigeria with respect to their phytochemicals and antimicrobial action against medically inclined and agriculturally related pathogens (Akinde and Odeyemi, 1987; Alade and Irobi, 1993; Adesina *et al*., 2000; Oladunmoye, 2006; Ogbo and Oyibo, 2008). Consequently, this plant has been reported to specifically have antibacterial and antifungal properties (Alade and Irobi, 1993) as the expressed juice or boiled decoction is locally used within Nigeria and some other parts of West Africa for the treatment of malaria, dermatological and gastrointestinal infections (Akinde and Odeyemi, 1987). There was however no available report to us on the phytochemical screening of the macafeeana cultivar using methanol as the extraction solvent, extraction of alkaloid from this cultivar and antimicrobial activity of its methanol extract and alkaloid, therefore the need for this study.

MATERIALS AND METHODS

Plant Material, Phytochemical Extraction and Screening

Fresh leaves of *A. wilkesiana* cv. *macafeeana* were obtained from the Horticulture Garden in Babcock University, Nigeria where this study was carried out between May, 2008 and April, 2009. The identity of the plant was authenticated by a Plant Taxonomist within the Plant Sciences unit of the Department of Chemical and Environmental Sciences, Babcock University.

The leaf samples were air dried for 3 weeks, pulverized into uniform powder using a blender and extracted using methanol. Fifty grams of the pulverized leaves was soaked in 500 mL absolute methanol (Sigma, USA) for 24 h in a 1 L conical flask after which filtration of the mixture followed using two folds of Whatman No. 1 filter paper. The extract was then concentrated at 40°C in a vacuum using rotary evaporator (Eyela Rotavap N-1001, Rikakikai Co. Ltd., Tokyo). The same procedure was used in the distilled water extraction of phytochemicals.

The phytochemicals screened for were alkaloids, tannins, phlobatannins, saponins, flavonoids, steroids and cardiac glycosides using standard procedures as described by Harborne (1973) and Sofowora (1993).

Crude Alkaloid Extraction

Crude alkaloid was extracted following the method reported by Adeniyi *et al*. (2009). Briefly, 20 g of the ground leaf was weighed and dispensed into 200 mL of 10% (v/v) acetic acid solution in ethanol. The mixture was well shaken and allowed to stand for 4 h before filtering using two folds of Whatman No. 1 filter paper. The filtrate was then evaporated to

one quarter of its original volume on a heating mantle. Concentrated ammonium hydroxide was added drop wise in order to precipitate the alkaloids. A pre-weighed Whatman No. 1 filter paper was used to filter off the precipitate and it was then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was oven-dried to constant weight at 60°C for 30 min and cooled in a desiccator. About 0.4 g of the dried precipitate was dissolved in 5 mL distilled water and tested for the presence of alkaloid using few drops of the Dragendoff's reagent (solution of potassium bismuth iodide). An orange colored precipitate confirmed the presence of alkaloid. The percentage weight of the alkaloid was determined using the formula:

$$\text{Sample weight (\%)} = \frac{W_2 - W_1}{W_0} \times 100$$

where, W_2 is weight of crude alkaloid on filter paper, W_1 is weight of filter paper and W_0 is weight of powdered sample used for extraction (20 g).

Source of Microorganism

Microorganisms used were fresh clinical isolates from Babcock University Medical Centre and Olabisi Onabanjo University Teaching Hospital, Nigeria. Isolate selection was dependent upon their availability and confirmed taxonomy, thus the isolates represented pathogens of both topical and systemic diseases. The bacteria were identified as: *Streptococcus pyogenes* (4 isolates), *Strept. pneumonia* (2 isolates), Methicillin-Resistant *Staphylococcus aureus* (MRSA) (2 isolates), *Staph. aureus* (2 isolates), *Escherichia coli* (2 isolates) and *Salmonella typhi* (2 isolates), using the conventional biochemical and physiological tests described by MacFaddin (2000), Fobres *et al.* (2002) and Leboffe and Pierce (2002). The Bergey's Manual of Systematic Bacteriology (Sneath *et al*., 1986) was used for species authentication. The *Staph. aureus* isolates were confirmed as either MRSA or non-MRSA by serological methods using the Staphytect Plus Reagent kit (DR0850, Oxoid, UK). The fungi used were: *Candida albicans* (2 isolates), *Aspergillus fumigatus* (2 isolates) and *A. flavus* (1 isolates), as identified via macroscopic and microscopic observations as well as germ tube test and chlamydospore production on cornmeal agar fortified with Tween 80 polysorbate for the yeast (Bulmer, 1978; Domsch *et al*., 1980; Brown, 2005).

Bioassay

The standard agar well diffusion method as described by Perez *et al.* (1990) was used in the bioassay involving the bacteria and yeast isolates while the Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MBC) were determined following the broth microdilution assay technique recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2000). Dimethyl sulfoxide (DMSO) was used as dissolving solvent while the dilutions ranged from 0.1 to 12.0 mg mL^{-1}. Two commercial antibiotics, Ciprofloxacin and Penicillin, served as positive controls for the well diffusion assay. The methanolic extract was used for the MIC and MBC. Agar wells were punched using 5 mm cork borer.

The effect of the extracts on the radial growth of the filamentous fungal isolates was determined by slightly modifying the method described by Amadioha (2002). Briefly, 1 mL of each extract (12 mg mL^{-1}) was spread separately on the surface of sterile solidified Potato Dextrose Agar (PDA) to give the extract-PDA medium. One milliliter each of sterile distilled water and methanol served separately as the controls. Five millimeter disc of each isolate was

cut from a 3 days active culture on PDA and placed separately at the center of the Petri dish containing the corresponding extract-PDA medium. All control plates containing the inocula and methanol were wrapped carefully in aluminum foil to prevent volatilization of the solvent. The plates were allowed to stand for 5 h before incubation at 30°C for 5 days. At the end of incubation, radial growths of isolates were measured and the percentage inhibition of various concentrations determined as described by Ogbo and Oyibo (2008). All assays were carried out in triplicates to calculate the mean results.

RESULTS

The inherent bioactive phytochemicals in the *A. wilkesiana* cv. *macafeeana* leaves are shown in Table 1. Methanol as extraction solvent showed a better capacity for extracting total phytochemicals than distilled water and this is evident in the number of phytochemicals extracted and the overall yield of the extracts. Alkaloids and Tannins were present in extracts of both solvents while Terpenoids and Cardiac glycosides were only extracted by methanol. The total extracts yield by methanol was 7.72 g while the water extracts yield was 6.81 g. The percentage crude alkaloid extract was 3.70%.

The *in vitro* inhibitory activity of the extracts revealed that the crude alkaloid extracts were active only against the Gram-negative bacteria (strains of *E. coli* and *S. typhi*) with mean inhibition zones ranging from 10.0±0.00 to 12.3±0.03 mm, while the methanol and water extracts of total phytochemicals were active against all other test organisms, a broad spectrum of activity. The water extracts had no activity against the non-MRSA strains (Table 2). The streptococci were the most susceptible unicells to the methanol and water extracts of the total phytochemicals with mean inhibition zones of 19.7±0.04 to 24.0±0.00 mm and 15.7±0.04 to 21.3±0.03 mm, respectively. Figure 1 shows the zones of inhibition of some

Table 1: Phytochemical analysis of the methanol and water extracts of *A. wilkesiana* cv. *macafeeana*

Phytocompounds	Methanolic extract	Water extract
Alkaloids	+	+
Cardiac glycosides	+	+
Steroids and terpenoids	+	+
Tannins	+	+

+: Present

Table 2: Zones of inhibition (mm) of leaf extracts of *A. wilkesiana* cv. *macafeeana* and standard antibiotics on unicellular microorganisms

	Test treatments (mm)			Control treatments (mm)	
Organisms	ME	WE	AK	PEN	CP
Escherichia coli (OS_2)	18.0±0.01	11.3±0.03	10.0±0.00	nz	28.3±0.03
E. coli (OS_3)	18.3±0.03	10.7±0.04	12.3±0.03	nz	26.0±0.01
Salmonella typhi (BM_1)	18.0±0.00	10.0±0.00	10.7±0.04	nz	33.0±0.01
S. typhi (BM_4)	16.3±0.03	10.3±0.02	11.0±0.00	nz	29.0±0.00
Streptococcus pyogenes (OS_1)	20.7±0.04	16.0±0.00	nz	25.0±0.00	27.3±0.03
Strept. pyogenes (OS_2)	19.7±0.04	16.0±0.00	nz	23.3±0.03	25.0±0.00
Strept. pyogenes (OS_3)	20.0±0.01	15.7±0.04	nz	18.3±0.03	22.0±0.01
Strept. pyogenes (OS_8)	19.7±0.04	17.0±0.00	nz	21.0±0.00	26.0±0.00
Strept. pneumoniae (OS_6)	24.0±0.00	20.0±0.00	nz	31.3±0.03	32.3±0.03
Strept. pneumoniae (OS_{11})	21.3±0.03	19.0±0.01	nz	23.7±0.04	26.0±0.01
Staphylococcus aureus (BM_{17})	16.7±0.04	nz	nz	15.7±0.04	27.7±0.04
Staph. aureus (BM_{18})	18.0±0.00	nz	nz	19.7±0.04	26.3±0.03
MRSA (BM_2)	15.0±0.00	10.3±0.03	nz	11.7±0.04	23.0±0.00
MRSA (BM_9)	16.3±0.03	11.0±0.00	nz	nz	25.0±0.01
Candida albicans (OS_{10})	16.0±0.00	10.0±0.00	nz	nz	nz
C. albicans (OS_{12})	19.3±0.03	10.7±0.04	nz	nz	nz

Results are means of triplicate treatments with standard deviations. nz: No zone of inhibition, ME: Methanolic extract, WE: Water extract, AK: Crude alkaloid extract, PEN: Penicillin; CP: Ciprofloxacin, MRSA: Methicillin resistant Staph. aureus

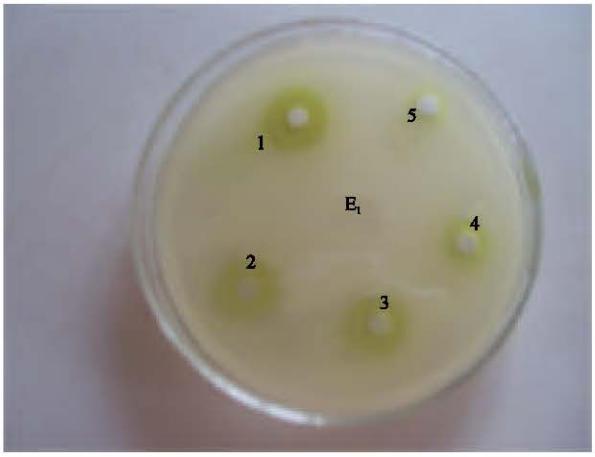

Fig. 1: Zones of inhibition of some concentrations of the crude alkaloid extract against *E. coli* (OS_{12}). 1: 9.6 mg mL^{-1}, 2: 3.2 mg mL^{-1}, 3: 3.2 mg mL^{-1}, 4: 1.6 mg mL^{-1}, 5: 0.8 mg mL^{-1}

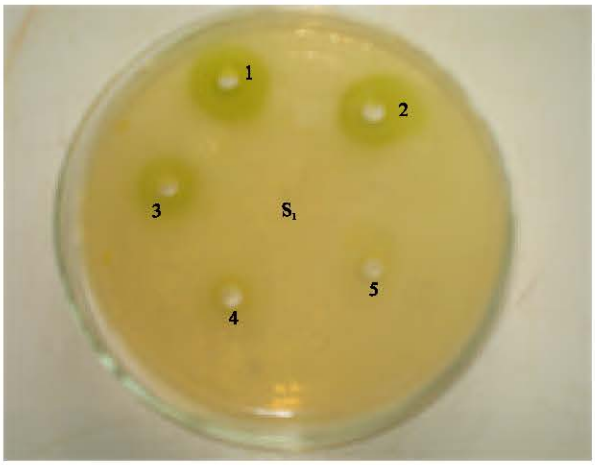

Fig. 2: Zones of clearance of the methanol extract against *Staph. aureus* (BM_{18}). 1: 9.6 mg mL^{-1}, 2: 3.2 mg mL^{-1}, 3: 3.2 mg mL^{-1}, 4: 1.6 mg mL^{-1}, 5: 0.8 mg mL^{-1}

of the concentrations of the crude alkaloid extract against *E. coli* (OS_{12}) while Fig. 2 and 3 show the zones of inhibition of the methanol extract against *Staph. aureus* (BM_{18}) and *C. albicans* (OS_{12}).

The MIC, expressed as the lowest extract concentration at which no visible growth in broth was observed, was 0.4 mg mL^{-1} for all unicellular isolates except strains of *C. albicans* which both had MICs of 0.8 mg mL^{-1} (Table 3). The MBC, recorded as the lowest extract concentration that exhibited capacity to kill 99.9% of unicell inocula, was 0.4 mg mL^{-1} for tested isolates except the non-MRSA and *C. albicans* which had MBCs of >12.0 and 1.0 mg mL^{-1}, respectively.

The results of the effect of the extracts on the radial growth of the filamentous fungal isolates are shown in Table 4. The methanolic extract totally inhibited all tested aspergilli

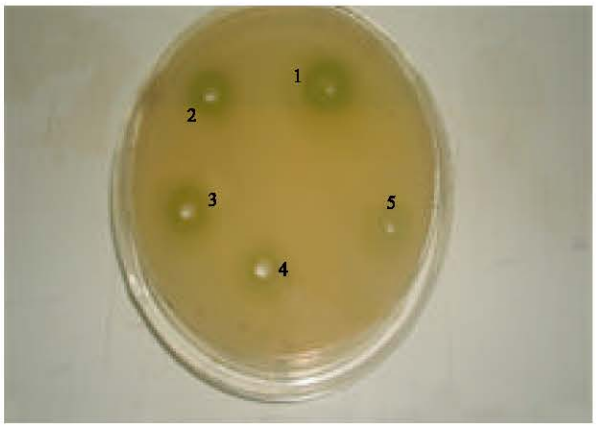

Fig. 3: Zones of inhibition of some concentrations of the methanol extract against *C. albicans* (OS_{12}). 1 = 9

Table 3: The MIC and MBC of leaf extracts of *A. wilkesiana* cv. *macafeeana* on unicellular microorganisms

Organisms	MIC (mg mL^{-1})	MBC (mg mL^{-1})
Escherichia coli (OS_2)	0.4	0.4
E. coli (OS_3)	0.4	0.4
Salmonella typhi (BM_1)	0.4	0.4
S. typhi (BM_4)	0.4	0.4
Streptococcus pyogenes (OS_1)	0.4	0.4
Strept. pyogenes (OS_2)	0.4	0.4
Strept. pyogenes (OS_3)	0.4	0.4
Strept. pyogenes (OS_8)	0.4	0.4
Strept. pneumoniae (OS_6)	0.4	0.4
Strept. pneumoniae (OS_{11})	0.4	0.4
Staphylococcus aureus (BM_{17})	0.4	>12.0
Staph. aureus (BM_{18})	0.4	>12.0
MR *Staph. aureus* (BM_2)	0.4	0.4
MR *Staph. aureus* (BM_9)	0.4	0.4
Candida albicans (OS_{10})	0.8	1.0
C. albicans (OS_{12})	0.8	1.0

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

Table 4: Inhibition (%) of strains of Aspergillus by leaf extracts of *A. wilkesiana* cv. macafeeana

Fungal strains	Test treatments (%) ME	WE	AK
Aspergillus fumigatus (OS_{14})	100±0.00	76.0±2.51	nz
A. fumigatus (BM_3)	100±0.00	81.0±2.90	nz
A. flavus (BM_{11})	100±0.00	63.0±2.50	nz

Results are means of triplicate treatments with standard error. nz: No zone of inhibition, ME: Methanolic extract, WE: Water extract AK: Crude alkaloid extract

(100% inhibition), while the water extract had a varying inhibitory effect on the strains tested. The alkaloid had no effect on the molds.

DISCUSSION

The difference in the yield weights of the methanol and water extracts may be attributed to the number and quantity of absent bioactive molecules when water was used as extracting

solvent. The presence of alkaloids, tannins, terpenoids and cardiac glycosides in the methanol extracts show its capacity to serve as an extraction solvent for phytochemicals from *A. wilkesiana* cv. *macafeeana* leaves.

The results of the *in vitro* inhibitory activity of the crude alkaloids, the methanol and water extracts are quite appreciable when compared to the mean inhibition zones produced by the control antibiotics and the fact that the standard antibiotics are in the purified and concentrated form whereas the extracts are crude and harbor both pharmacologically and non-pharmacologically active compounds. The susceptibility of the tested MRSA strains show that the total leaf extracts could be used as an alternative in treating staphylococcal infections resulting from MRSA pending when the active ingredients of this plant would be isolated, chemically identified and purified for commercial use. This is in line with the findings of Stephen *et al*. (2009), who reported that MRSA are highly susceptible to crude extracts of *Khaya grandifoliola* stem bark as compared to commercial antibiotics.

Alkaloids generally have been noted for their antimalarial and antibacterial activities although it seems their mechanism of action on microbes remains unclear (Karou *et al*., 2005; Raghavendra *et al*., 2008). The susceptibility of only the Gram-negative bacteria to the alkaloid extract is interesting as we may suggest that there could have been a possible interaction between these alkaloids and some constituents of the Gram-negative cell wall thereby causing cytotoxic damage to this group of bacteria. This suggestion is on the basis that structurally, Gram-positive and Gram-negative bacteria differ only in their cell wall composition. Therefore the alkaloid extract could be explored as a narrow spectrum herbal drug especially in cases of gastrointestinal infections since the tested bacteria strains are clinical isolates.

The MIC values are remarkable for a crude extract as well as the MBC values except for the MBC for non-MRSA. The result obtained shows that these staphylococci may be inhibited by a very low concentration of the extract while all studied concentrations had no capacity to trigger lysis of the cells by activation of autolytic enzymes in the cell wall. This situation may be referred to as tolerance, a resistance mechanism, due to the large difference between the MIC and MBC (Brooks *et al*., 1998). This therefore shows that this plant extract may not be suitable as a bactericidal agent in the case of infections arising from non-MRSA unless combined herbal therapies are explored.

The inhibitory mechanism of the total phytochemicals extracted with methanol on the radial growth of the filamentous fungi could be that of blocking the synthesis of the cell wall constituents or a possible interference with the replication of genetic material so as to prevent cell division since it was recorded that the molds did not grow at all.

From present study we can conclude that crude alkaloids can be extracted as well as total phytochemicals from *A. wilkesiana* cv. *macafeeana* and that the alkaloids can significantly inhibit the growth of Gram-negative bacteria only, an indication that if commercially produced could serve as a good first line drug for infections, mainly gastrointestinal, caused by Gram-negative bacteria. Also, that the methanolic leaf extract of this plant of pharmacological importance has a broad spectrum of activity against bacteria (Gram-positive and negative) and fungi (yeasts and molds) of clinical origin. Further studies on this plant are encouraged so as to extract, identify and formulate the active molecules into appropriate dosage forms. This is the first available report on the application of methanol as extraction solvent for phytochemicals from *A. wilkesiana* cv. *macafeeana* leaves, the extraction of crude alkaloids from this cultivar and antimicrobial testing of its methanol extract and alkaloids.

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