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Comparative Evaluation of Antimicrobial Activities and Phytochemical Screening of Two Varieties of *Acalypha wilkesiana*

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Abstract: Comparative studies on the antimicrobial activities and phytochemical screening of two varieties of *Acalypha wilkesiana* were carried to investigate the inhibitory potential on some selected pathogens and determine the phytoconstituents of pharmacologic importance. Ethanol was used as the extraction solvent and the microorganisms used were *Staphylococcus aureus*, *S. epidermidis*, *Clostridium sporogenes*, *Bacillus cereus*, *B. subtilis*, *Klebsiella pneunoniae*, *Trichophyton interdistale*, *Aspergillus niger*, *Penicillium camenberti* and *Fusarium solanni*. Growth inhibition indicates on agar plate was used for the antimicrobial sensitivity. The extract was found to posses' broad spectrum of activity on both fungi and the bacteria; the latter found was to be more susceptible. Generally Macrophylla variety was found to be more effective in inhibiting the growth of the organisms than Hoffmanin. The Gram- negative organisms like *Klebsiella* and *Pseudomonas* was found to be more resistance than the Gram-positive organism as indicated by zone of inhibition. The release of sodium and potassium ions was found to be the mechanism of action of the extract and the amount leaked vary from one organism to the other and is also dependent on the *Acalypha* variety. Minimum Inhibitory Concentration (MIC) vary between 0.2-0.98 comparing the efficacy of the extract with commercial antibiotics showed that the latter was more potent but with narrow spectrum of activity only on the fungi unlike the former with broad spectrum of activity on both fungi and bacteria. The bioactive compounds in the extract include saponin, Tannin, Anthroquinine and glycoside.

Key words: Antimicrobial, phytoconstituent, *Acalypha willkesiana*

Introduction

The use of plant, plant extract or plant derived from chemicals to treat disease is therapeutic modalities which has stood the test of time (Anwannil and Atta, 2005). Early human recognized their dependence on nature in both health and illness. Led by instinct, test and experience, primitive people treated illness by using plant, animal parts and minerals that were not part of their usual diet. About 80% of the population in the developing countries still uses traditional medicine for their health care. Modern pharmacopoeias contain at least 25% of drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants (De Silva, 2005.).

There had been renewed interest in herbal product partly due to high cost involved in the development of patentable chemicals/drugs (Hack, 2005). There is also growing evidence to show that medicinal plants contain synergistic and side effect neutralizing combination (Welzel *et al*., 2003).

Alkalypha wilkesiana belong to the family euphorbiaceae and grow as annual bedding plants. It possesses a spectacular colour and leaves are up to 8 inches long which may be crimson, red or purple (Fauan, 2005). The plant has been shown to possess antimicrobial activity (Adesina *et al*., 2000). However, there has not been any report on comparative evaluation of the antimicrobial activity of the two most important varieties as well the mechanism of action of the extract from the plant during the killing process on the susceptible pathogens which are the research foci of the present study.

Materials and Methods

Plant sample, extraction and fractionation. The two varieties *Acalypha wilkesiana* L. (Euphobiaceae) leaves were obtained from Orchid of the Federal University of Technology, Akure Nigeria. Leaves dried at 40°C were pulverized and extracted with 70% ethanol. The extract was then concentrated in a vacuum using rotary evaporator.

Extract 5 g of the crude extract was adsorbed on silica gel of 60-120 mesh (BDH) and chromatographed on a column of silical gel-60 slurry packed in petroleum ether. The column was gradient eluted with petroleum ether and then with ethyl acetate:ethanol 40:1 and finally with 100% ethanol.

A 100 mL of the fraction was collected and analyzed by Thin Layer Chromatography (TLC) on a pre-coated plated Merck, silica gel 60 254, 0.2 mm thickness. The fractions collected were numbered, fractions showing the same TLC characteristics were bulked together. This was also confirmed by measuring their absorbance with the aid of spectrophotometer. Visualization of the spots on plate were by observing them under ultra violet light and by spraying separately with vanillin-sulphuric acid reagent followed by heating at 1000°C for 5 min.

Antimicrobial Sensitivity Assay, Minimum Inhibitory Concentration Determination and Phytochemical Screening

The antimicrobial bioassay of the extract was measured using the standard method of agar well diffusion method (Sudhakar *et al*., 2006) Minimum Inhibitory Concentration (MIC) was determined by tube dilution method. Phytochemical screening was carried out using the method of Sofowora (1993).

Studies on the Leakages of Potassium and Sodium Lons

The purified extract was mixed with an eighteen-hour old culture of the organism. The cells washed twice in physiological saline by centrifuge before use. The inoculums size was then adjusted to contain approximately 10^6 organism per mL. The supernatant solutions obtained after centrifugation at 7000 rpm were analyzed for potassium and sodium ions using flame photometer.

Results and Discussion

The results of the study indicated that the two varieties of *Acalypha wilkesiana* had antimicrobial activities on most of the tested organisms (Table 1). The efficacy of the two varieties in inhibiting the growth of the organisms also vary with the red macrophylla been more potent than the light green Hoffamanii. The variation may be as a result of the difference in the nature, type and distribution of the phytoconstituents of pharmacologic importance in the two varieties. The susceptibility and resistance also vary among the organisms with the extract inhibiting the growth of the bacteria more than the fungi This might be due to their eukaryotic nature which confers on them a more complex cell wall with rigidity and less permeability that the thin cell wall membrane of bacteria. Another factor that may be responsible for the low sensitivity of the fungi may be due to their ability to produce extracellular enzymes (Tortora *et al*., 2002). These enzymes are known to be degradative in nature and can give them the of degrading and metabolizing their substrate (Brock and Madigan, 1991). Therefore the bioactive molecule in the extracts from the two *Alkalypha wilkesiana* varieties may become food source for the fungi instead of inhibiting their growth after being rendered non-toxic due to degradation. Among the bacteria variation exist from one genus to another and from different species to another in the same genus. The observed variation might be due to variation in their genetic composition. The information for these characteristics may be coded on the chromosome or on the plasmid as R. factor. The distribution of these plasmids had been found to vary among different bacteria species.

Table 1: Zones of Inhibition (mm) of two *Acalypha wilkesiana* varieties on tested microorganisms

Organisms	Macrophylla	Hoffmanin
Bacillus cereus	18.0	12.0
Bacillus subtilis	14.0	-
Clostridium sporogenes	6.0	7.5
Escherichia coli	-	-
Klebsiella pneumoniae	9.0	8.0
Pseudomonas aeuriginosa	10.0	1.0
Staphlococcus aureus	17.0	14.0
Staphylococcus epidermidis	8.0	6.0
Aspergillus niger	6.0	-
Fusarium solani	6.0	2.0
Penicillium camenberi	-	4.0
Trichophyton interdistale	8.0	3.0

Table 2: Minimum Inhibitory Concentration (MIC) in mg mL^{-1} of two varieties of *Acalypha wilkesiana*

Organisms	Macrophylla	Hoffmanin
Bacillus cereus	0.98	0.66
Bacillus subtilis	0.76	0.54
Clostridium sporogenes	0.72	0.80
Escherichia coli	0.44	0.62
Klebsiella pneumoniae	0.66	0.40
Pseudomonas aeuriginosa	0.72	0.96
Staphylococcus aureus	0.22	0.86
Staphylococcus epidermidis	0.40	0.50
Aspergillus niger	0.80	0.50
Fusarium solani	0.66	0.20
Penicillium camenberti	0.78	0.32
Trichophyton interdistale	0.80	0.58

The resulted revealed that the two varieties of *Acalypha willkesiana* has different MIC values for the various organisms (Table 2). The fungi were found to have higher MIC different from that of the bacteria. The reason for the variation in the antimicrobial susceptibility patterns may also be responsible for the observed disparity in the MIC.

From Table 3, the amount of sodium and potassium Ions leaked by the two varieties was shown to be lower in fungi than for bacteria. The lower values for fungi may responsible for lower zones of inhibition and MIC. *Fusarium*, *Pencillium* and *E. coli* do not have any zones of inhibition but still leaked some ions. The reason for this may be that the extract is having microbistatic and not microbicidal activities on these organisms. The mechanism of action of the extract might be due to the leakage of these sodium and potassium ions. Sodium and potassium ions had been shown to affect osmotic balances of the cell and equally activate enzymes. Most cell activities including respiratory and biosynthetic activities are mediated by enzymes (Brock and Madgan, 1991). The observed antimicrobial activities may be as a result of cell damage and inactivation of enzymes due to induced leakage of these ions.

Phytochemical screening of the two varieties of *Alkahypha wilkesiana* showed the presence of bioactive molecules like Saponin, Tannin, Anthraquinone and Cardiac glycoside in both varieties whereas alkaloid and phlobatannin is present in macrophylla and absent in Hoffmanin (Table 4). The presence of these additional factors may be responsible for the observed higher inhibitory potential in Macrophylla.

Comparison of the efficacy of the extract with commercial antibiotics showed that the latter is more effective in inhibiting the growth of the organism than the former. This may be due to the presence of stronger bioactive principles in the antibiotics and their molecular size which permit their penetration into the cells of the organism. However, the extract was found to have a broad spectrum of activities inhibiting the growth of both fungi and bacteria whereas the antibiotics has narrow spectrum affecting the growth of some selected bacteria.

Table 3: Amount of sodium and potassium ions (ppm) released by two varieties of *Acalypha wilkesiana*

	Sodium ions		Potassium ions	
Organisms	Macrophylla	Hoffmanin	Macrophylla	Hoffmanin
Bacillus cereus	26	54	70	6
Bacillus subtilis	18	54	46	12
Clostridium sporogenes	50	56	90	14
Escherichia coli	40	50	84	13
Klebsiella pneumoniae	48	92	28	15
Pseudomonas aeuriginosa	58	82	18	60
Staphilococcus aureus	60	104	62	18
Staphilococcus epidermedis	30	38	54	36
Aspergillus niger	8	2	10	6
Fusarium solani	9	9	2	4
Penicillium camenberi	8	7	6	2
Trichophyton interdistale	6	5	10	2

Table 4: Result of phytochemical screening on the two varieties of *Alkalypha wilkesiana*

Bioactive	Macrophylla	Hoffmanii
Saponin	+	+
Tannins	+	+
Anthraponine	+	+
Candiac glycoside	+	+
Alkloid	+	-
Phlobatannins	+	-

+ = Present, - = Absent

Recommendation and Conclusions

Ethanolic leaf extract of the two varieties of *Acalyphya wilkesiana.* significantly inhibit the growth of the tested microorganisms. The level of inhibition was found to be organism dependent and the particular variety. These finding, may lead support to the traditional use of *Acalypha* wil*kesiana* in the treatment of microbial infections. Current researches and technology can be developed which will help to optimally extract all the bioactive molecules in the plant and formulated into appropriate dosage.

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