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Evaluation of Chemical Compositions and Antimicrobial Activities of *Acalypha ciliata* Leaves

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Abstract

Leaves of *Acalypha ciliata* which serve for both medicinal and nutritive value were evaluated for their chemical and microbial activities. The results revealed that the leaves extracts contain alkaloid, tannins and saponins with alkaloid having the highest percentage composition of 9 ± 0.5%. The proximate analysis results revealed the presence of crude fiber (48.6 ± 0.1%), fat and oil (16.39 ± 0.01%), carbohydrate (17.39 ± 0.016). Ascorbic acid and thiamine occurred as the highest and least percentage vitamin respectively recorded. The minerals which were also present include Ca, Mg, P, Fe, Na, and K. The result of chloroform extract on GC/MS analysis showed nine peaks indicating the presence of nine compounds in the extracts. Compound 8, identified as phthalic acid with m/z 390, had the highest percentage composition with a value of 24.3%. The antimicrobial activity showed that only ethanol extracts exhibited activity against S*almonella*, C*andida* and *Proteus mirabilis* but not against *Bacillus*. The hot water and cold water extracts showed no activity against the bacterial strains tested. The findings from this research justify some of the local applications of leave.

Keywords

Bioactive Compounds, Mineral, Microbial Inhibition, Proximate, Vitamins

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1. Introduction

Acalypha ciliata (Euphorbiacecae) is a plant found widely in tropical Africa. The representative of these large genera and other smaller ones are well distributed in the free area and forest reserves across Nigeria [1, 2]. Its common name or English name is Copper Leaf also known as Indian acalypha and other common names in Senegal, Ghana and Nigeria [3] and *Ege* by Igbo people of South-East Nigeria. *Acalypha ciliata* plant is medium sized often found in the hilly and plain land habitats. The leaves are greenish in colour arranged spirally [4, 5].

The plant serves for both medicinal and nutritive values including antimicrobial activities. They are used as laxative as well as applied in local dressing of wounds and hence have wound healing ability [6]. The foliage of *Acalypha ciliata* provides grazing for donkeys, cattle, sheep and goat but not for horses.

The plant which is indigenous in Nigeria is used for variety of purposes. These include, as foods and fodders, landscape beautification, timber etc. The plant is widely grown for its use as a food wrapper of a local food in South East Nigeria.

This research therefore, evaluated the phytochemical composition, proximate, mineral and vitamin content, GC/MS characterization of chloroform extracts and antimicrobial activity of *Acalypha ciliata* leaves against some selected microorganisms.

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2. Material and Methods

The leave samples used for this research were collected from Umuolakwa in Okoko Item in Bende Local Government Area of Abia State, South East Nigeria while the bacteria strains- S*almonella*, C*andida*, *Bacillus* and P*roteus* were all obtained from the stock culture of the Microbiology Laboratory of the Medical Centre, Abia State University, Nigeria.

The leaves samples were air dried and ground into fine powder with electric blender and stored in airtight container until requires for analysis. All reagents used for this research were of analytical grades.

2.1. Phytochemical Analysis

5 g of sample was soaked with 100 mls of distilled water in a beaker. It was allowed to stand for 24hrs, and then filtered; the filtrate was used for phytochemical screening.

The determination of alkaloids was done according to the method of [7, 8], saponin determination was done according to [9]. Tannin was determined by the spectrophotometer method as described by [10]. Flavonoid was determined by method according to [11].

2.2. Proximate and Vitamin Analysis

The moisture, ash, crude fiber, crude lipid, and protein content determination were done by the recommended methods of [12].

The determinations of vitamins, B_2 (thiamin) and Niacin were done by spectrophotometric method while vitamin C (Ascorbic acid) was determined titrimetrically by the method of [10].

2.3. Mineral Determination

The elements comprising magnesium, sodium, phosphorus, calcium, potassium and iron were determined using Buck 21VGP atomic absorption spectrophotometer (AAS) [13].

2.4. Extraction of Plant Materials for GC/MS Characterization

The extraction of the crude samples of leaves was done by cold percolation using chloroform. 15 g of ground *Acalypha ciliata* was put into a conical flask to which 150 ml of chloroform was added into it and percolated for 24 hours. It was then filtered into a beaker and residue was again re-soaked for another 24 hours with fresh 150 ml of chloroform and filtered into the same beaker for total extraction of the plant materials. The filtrate was then allowed to evaporate to dryness under room temperature. This extract was then subjected to Gas chromatography mass spectrometer analysis.

2.5. Antimicrobial Analysis

2.5.1. Preparation of Plant Extracts

10 g of powdered sample each was soaked in 100 ml of hot water, cold water and ethanol respectively for 24 hours with intermittent shaking. The suspension was filtered through a Whatman No.1 filter paper and the filtrate used for antimicrobial screening.

2.5.2. Preparation of Culture Media

The culture media for antimicrobial activity test was nutrient agar. The milk colour powder has the formulation 28.0 g/L peptone, yeast extract 8 g/L, agar 15 g/L, beef 1.5 g/L and sodium chloride extract 1.55 g/L. The medium used for the antibacterial activity test was prepared by dissolving 28 g of the powder in 100 ml of distilled water, then heated and allowed to cool for some minutes; the medium was dissolved completely and was sterilized by autoclaving for 15 minutes at 121^{o}C. The medium was cooled to 47^{o}C before pouring it into Petri dishes.

2.5.3. Preparation of Inoculators

Stock cultures were maintained at 4^{o}C on slope of nutrient agar. Active culture experiments were prepared by transferring a loopful of cells from the stock culture to test tubes of nutrients agar both (NAB) for bacterial. It was inoculated without agitation for 24 hours at 37^{o}C the culture was diluted to achieve optical corresponding to $2.0x10^{6}$ colony forming units (CFU/ml) for bacterial.

2.5.4. Antimicrobial Screening

The antimicrobial activity screening was performed by filter paper disc method [14]. In vitro anti-bacterial activity was screened by using nutrient agar. The plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. About 5 mg of the extract was loaded on 4 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation for 24 hours at 37^{o}C. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. The experiment was repeated two more times for reaffirmation.

3. Result and Discussion

The results of the phytochemical composition of *Acalypha ciliata* leaves is shown in table I, the vitamin and proximate composition in Table 2 and that of mineral composition in Table 3.

The quantitative analysis of phytochemical result showed that the leaves contained alkaloid, saponin and tannins while phenol and flavonoids were absent. Alkaloid had the highest composition of 9.00 ± 0.5 (Table 1).

Table 1. Phytochemical composition of *Acalypha ciliata* leaves

Phytochemicals	% composition
Alkaloid	9.00 ± 0.5
Tannins	6.00 ± 1.0
Saponins	2.50 ± 0.1
Phenol	-
Flavonoid	-

Alkaloids are among the most efficient therapeutic agents in plant. Tannin has astringent properties, hastens the healing of wound, varicose, ulcer, hemorrhoid, frost-bite and burn in herbal medicine [15].

The results of the proximate and vitamins compositions are shown in Table 2, which shows that this part of the plant is a good source of some food nutrients.

Table 2. Vitamin and proximate composition of *Acalypha ciliata* leaves

Constituents	
Proximate Parameters	% Composition
Crude fiber	48.60 ± 0.10
Protein content	2.40 ± 0.10
Ash content	2.50 ± 0.016
Fat and oil	16.39 ± 0.01
Carbohydrate	17.39 ± 0.016
Moisture content	12.49 ± 0.01
Vitamins	Quantity mg/100g
Ascorbic acid	0.081 ± 0.001
Riboflavin	0.05424 ± 0.00001
Niacin	0.0391 ± 0.0001
Thiamine	0.0117 ± 0.00001

The proximate composition showed that crude fiber had the highest composition by 48.6 ± 0,1%, followed by carbohydrate 17.39 ± 0.016, fat and oil (lipids) 16.39 ± 0.01%, moisture content 12.49 ± 0.01% while protein had the least percentage composition of 2.4 ± 0.1. The presence of crude fiber in the plant leaves indicates that it might act as an index in the intestine by absorbing toxins [16] which are very important in animal diet. Carbohydrate is one of the basic classes of food; it is also the cheapest source of dietary energy [17].

In the vitamin composition mg/100g, ascorbic acid had the highest content of 0.081 ± 0.001 followed by riboflavin 0.05424 ± 0.00001, Niacin 0.0391 ± 0.0001 and the least was thiamine 0.0117 ± 0.00001. The highest content of ascorbic acid makes it act as anti-scurvy and facilitate the transformation of cholesterol into bile acid in the liver. Niacin is active in preventing diseases pellagra while deficiency of thiamine causes beriberi [18].

The result of the mineral compositions is as shown in the Table 3. It shows that sodium had the highest content of 31.167 mg/kg followed by calcium (1.227 mg/kg), while magnesium had the least (0.113 mg/kg). Potassium was not observed in the sample.

Table 3. Mineral composition of *Acalypha ciliata* leaves

Mineral Composition	Quantity mg/kg
Calcium	1.227
Magnesium	0.113
Phosphorus	0.253
Iron	0.393
Sodium	31.167
Potassium	

The result of the mineral composition showed that *Acalypha ciliata* forsk leaves contain rich source of mineral like calcium which is very important for the formation of bones and teeth. Iron is a component of hemoglobin. Sodium reduces the rate of hypertension in human [19].

The result of the chloroform extract of A*calypha ciliate* leaves on GC/MS analysis showed nine peaks indicating the presence of nine compounds in the extracts (Table 4). The compounds present were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literatures.

The first compound was identified as 9-Eicosyne. The compound had the least percentage composition of 3.62% with molecular formula $C_{20}H_{38}O_2$ (m/z 278) which occurred at retention time of 2.71 mins. The second compound is a fatty acid identified as Hexadecanoic acid (palmitic acid), its composition in the extract was 5.80% with molecular formula $C_{16}H_{32}O_2$ (m/z 270) at retention time of 27.7 mins. Compounds 3 is a saturated fatty acid identified as Arachidoic acid. Its percentage composition was 9.06% with molecular formula $C_{20}H_{40}O_2$ (m/z 250) at retention time of 28 mins. It is used in production of detergents, photographic materials and lubricants. Compound 4 is also a fatty acid identified as 9, 12-Octadecenoic acid. It had 7.97% composition with molecular formula $C_{18}H_{34}O_2$ (m/z 294) at retention time of 28.8min. Compound 5 is also a fatty acid identified as 11-octadecenoic acid. Its percentage composition is 13.4% with molecular formula of $C_{18}H_{36}O_2$ (m/z 296) at retention time of 28.9 mins. 11-octadecenoic acid is more lipid soluble form of free acid that can be used as a standard for analysis and could be found as ethyl esters in organs of animals with ethanol. Compound 6 is an unsaturated fatty acid identified as Oleic acid. Its percentage composition was 16.3% with molecular formula $C_{18}H_{34}O_2$ (m/z 282) at retention time of 29.2 mins. Oleic acid occurs

naturally in animals and vegetable fats and oil; it serves as emulsifier or solubilizing agent in the aerosol product. Compound 7 is also a fatty acid identified as stearic acid used to produce dietary supplement. Its percentage composition in the extract was 9.06% with molecular formula $C_{18}H_{36}O_2$ (m/z 284) at retention time of 29.4 mins. Compound 8 had the highest percentage composition of 24.3%. It was identified as phthalic acid an aromatic dicarboxylic acid. Its molecular formula is $C_{24}H_{38}O_4$ (m/z 390) at retention time of 31.5 mins. Compound 9 was identified as squalene with percentage composition of 10.5% and molecular formula $C_{30}H_{50}$ (m/z 410) at retention time of 34.0 mins, it is a chemopreventive substance that protects against cancer.

Table 4. Identified bioactive compounds in *Acalypha ciliata* leaves from GC/MS Analysis

S/No	Peak weight (cm)	% composition	Retention time (Min)	Molecular weight	Molecular formula	Name of compounds
1	1.0	3.62	27.1	278	$C_{20}H_{38}O_2$	9-Eicosyine
2	1.6	5.80	27.7	270	$C_{16}H_{32}O_2$	Hexadecanonic acid
3	2.5	9.06	28.0	250	$C_{20}H_{40}O_2$	Arachidic acid
4	2.2	7.97	28.8	294	$C_{18}H_{34}O_2$	9,12 octadecenoic acid
5	3.7	13.4	28.9	296	$C_{18}H_{36}O_2$	11, octadecenoic acid
6	4.5	16.3	29.2	282	$C_{18}H_{34}O_2$	Oleic acid
7	2.5	9.06	29.4	284	$C_{18}H_{36}O_2$	Stearic acid
8	2.7	24.3	31.5	390	$C_{24}H_{38}O_4$	Phthalic acid
9	2.9	10.5	34.0	410	$C_{30}H_{50}$	Squalene

The result of the growth of the microbial zone of inhibition of hot water, cold water and ethanol extracts of *Acalypha ciliata* leaves is shown in Table 5.

Table 5. Results of inhibition (mm) zone of *Acalypha ciliata* leaves

Microibial strain	Ethanol	Hot water	Cold water
Salmonella	1.2		
Candida	1.2		
Bacillus			
Proteus	1.0		

The result of the in-vitro antimicrobial activity against bacterial strains using hot water, cold water and ethanol extracts showed that only ethanol extracts was most sensitive to *salmonella* (1.2 mm) and *candida* (1.2 mm) and less in *proteus* (1.0 mm) while there was no activity observed in *Bacillus*. Hot water and cold water extracts showed no activity to all the bacterial strains tested. The observed inhibition role of the extracts explains the medicinal use of the plant.

4. Conclusion

The results obtained from this research revealed that the leaves of *Acalypha ciliata* plant samples contain essential phytochemicals, food nutrients, vitamins, minerals, and other bioactive compounds identified from the GC/MS results justifies some of its uses that provides foliage grazing for animal and antimicrobial use of the extracts of the part of the plant.

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