Clinical Phytoscience

ORIGINAL CONTRIBUTION

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Phytochemical screening, antioxidant and antimicrobial activities of *Acalypha ciliata* plant

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Abstract

Background: Phytochemical screening, antioxidant and antimicrobial activities of hexane, ethylacetate and methanol crude extracts of *Acalypha ciliata* plant was studied in this work. This plant according to folk medicine has been reported to be used for treatment of female sterility, dressing of sores and schistosomiasis.

Methods: The preliminary screening of the various extracts was carried out using standard methods and the results revealed the presence of flavonoids, tannins, alkaloids, reducing sugar, anthraquinones, resins and glycosides. The antimicrobial screening was carried out using the following organisms; Staphylococcus aureus, Eshericha coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi, Klebsiellae pneumoniae, Candida albicans, Aspergillus niger, Penicillium notatum and Rhizopus stolonifer. The free radical scavenging capacity using hydrogen peroxide was equally determined to evaluate the antioxidant activities of the extracts.

Results: The result obtained showed that *Acalypha ciliata* extracts have interesting pharmacological active compounds and antimicrobial effects, and as such could be used in ethno medicine for treatment of bacterial infections and ailments.

Conclusion: The extracts of Acalypha ciliata plant contains notable chemical compounds that are responsible for its antioxidant and antimicrobial activity. Further investigations on the chemical compositionsand possible isolation of active ingredients would be carried out.

Keywords: Antioxidant, Antimicrobial, *Acalypha ciliata*, Phytochemicals, Organisms

Background

Herbal and natural products of traditional medicine have been used by men since the advent of human race. Every culture, including western culture has evolved indigenous system of traditional healing [1]. Traditional healing in different cultures has a long history of ancestors creating primitive medicine during their struggle against natural calamities and diseases. Equally while searching for food; the ancient humans discovered that some foods have specific properties of relieving or eliminating certain diseases and maintaining good health [2].

In recent years, secondary plant metabolites have been extensively investigated as a source of medicinal agents. It is anticipated that phytochemicals with good antibacterial activity will be used for the treatment of bacterial, fungal and viral infections [3]. During the last two decades, there has been a considerable increase in the study and use of medicinal plants all over the world, especially in advanced countries. There was also a tremendous increase in the international trade and commercial exploitation of herbal medicines over the counter labeled products. In some countries, herbal medicines are still a central part of the medical system e.g. China, Ethiopia, Argentina and Papua New Guinea [4–7].

Antioxidants have become synonymous with good health; they are a class of compounds thought to prevent certain types of chemical damage caused by an excess of free radicals, charged molecules that are generated by a variety of sources including, smoking, pesticide and fumes from exhaust. Destroying free radicals may help

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fight cancer, heart diseases, stroke and other immune compromising diseases [8, 9].

Interest has increased recently in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their carcinogenicity [10].

Virtually, all medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in absorbing and neutralizing free radicals; many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rate of several human diseases [11].

Acalypha cilita is specie in the botanical family Euphorbiaceae. It occurs widely in Africa where it is eaten as a vegetable, or fed to animals. In West Africa and East Africa it is used as a medicinal plant [12]. In Nigeria *Acalypha ciliata* is known locally as Efiri or Owu in Yoruba land and Agukwu in Igbo land. In Cote d'voire, the leaf decotion is drunk to treat female sterility; In Ghana, mashed leaves are applied as a dressing to sores, whereas in East Africa, a root infusion is taken to treat schistosomiasis and in Senegal, Benin and Nigeria, the leaves are eaten as vegetable [13].

The purpose of this research is to evaluate the phytochemicals, antimicrobial and antioxidant activities of this plant since it is been eaten as vegetables.

Method

Sample preparation

Acalypha ciliata plant was purchased from Ojee market in Ibadan North-East Local Government and Obada market, Tapa in Ibarapa North Local Government, both in Oyo state, Nigeria. The plant was identified and authenticated at Herbarium unit of Botany department, University of Ibadan, Oyo state, Nigeria. The vegetables was picked to remove debris, cut into small pieces and air dried for 14 days inside the laboratory and later milled into mesh sizes.

Preparation of extracts

Two hundred eighty grams of dried and milled plant materials were extracted successively with Soxhlet extractor at temperature of 80 °C. Each of the solvent; hexane, ethyl acetate and methanol were allowed to remain in contact with the plant material for 12 h; the extracts were evaporated to dryness using rotary evaporator and 5 g, 9.7 g and 14 g of the extract respectively were obtained.

Phytochemical analysis

The extracts were analyzed for the presence of alkaloids, resins, tannins, saponins, flavonoids, glycosides, phenols, anthraquinones, cardiac glycosides, steroids, phlobatannins, reducing sugars [14–17].

Test for alkaloids

0.2 g of extracts was shaken with 1 % HCl for two minutes. The mixture was filtered and drops of Dragendorff's reagent added. Formation of a precipitate indicated the presence of alkaloids.

Test for Saponins

0.2 g of extracts was shaken with 5 ml of distilled water in a test tube. Frothing which persists on warming was taken as evidence for the presence of saponins.

Test for tannins

0.2 g of extracts was stirred with distilled water and filtered. Ferric chloride was added to the filtrate. A blue-black, green or blue-green precipitate was taken as an evidence for the presence of tannins.

Test for Steroids (Salkowski's test)

0.2 g of the extracts was dissolved in 2 ml of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interphase indicated the deoxy sugar characteristics of cardenolides.

Test for cardiac-active glycoside (Keller-Killani Test)

0.2 g of the extracts was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution followed by the addition of 1 ml of concentrated sulphuric acid. A brown ring at the interface confirmed the presence of cardiac glycoside.

Test for reducing sugars

0.2 g of the extract was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for two minutes. An orange precipitate on boiling with the Fehling's solution indicated the presence of reducing sugars.

Test for flavonoids

A little amount of magnesium powder and few drops of concentrated hydrochloric acid were added to 3 ml of the extracts. A red or intense red colouration indicated the presence flavonones.

Test for resins

Five milliliters of copper acetate solution was added to 5 ml of the methanolic extract. The resulting solution was shaken vigorously and allowed to separate. A green coloured solution is an evidence of the presence of resin.

Test for anthraquinones

0.2 g of the extracts was shaken with 4 ml of benzene. The mixture was filtered and 2 ml of 10 % ammonia solution was added to the filtrate. The mixture was shaken and the presence of pink, red or violet colour in the

ammonical (Lower) phase indicated the presence of free anthraquinones.

Test for phenols

0.2 g of methanol extract was dissolved in Ferric chloride solution. A green or dirty green precipitate indicated the presence of phenolic compound.

Test for phlobatannins

The extracts (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2 % HCl solution. Red precipitate shows the presence of Phlobatannins.

Test for glycosides

The extracts was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehlings solution A and B were added. Red precipitate indicates the presence of glycosides.

Scavenging of hydrogen peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method by Nabavi et al., 2008a and 2009a [18, 19]. A solution of hydrogen peroxide (2 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 285 nm using a UV/Vis spectrophotometer. The samples at '1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625 mg/ml' were added to H_2O_2. The decrease in absorbance of H_2O_2 at 285 nm was measured spectrophotometrically after ten minutes (10 min) against a blank solution containing the test sample in phosphate buffer saline (PBS) without H_2O_2 and blank solution containing phosphate buffer without hydrogen peroxide (control). All the tests were performed in triplicate. The percentage of hydrogen peroxide scavenged by the extracts was calculated as follows:

$$\% \text{ Scavenged } [H_2O_2] = (A_c - A_s)/A_c] \times 100 \qquad (1)$$

Where A_c is the absorbance of the control and A_s the absorbance in the presence of the sample of extract and standard [18, 19]. The values of % inhibition were obtained from Eq. 1. For the 50 % Inhibitory Concentration (IC_{50}) evaluation of the extract, graphs showing the concentration of the test samples (hexane extract, ethyl acetate extract, methanol extract and the alpha tocopherol) versus % Inhibition (%H_2O_2 reduction) were plotted and shown in Figs. 1a, b, c and d.

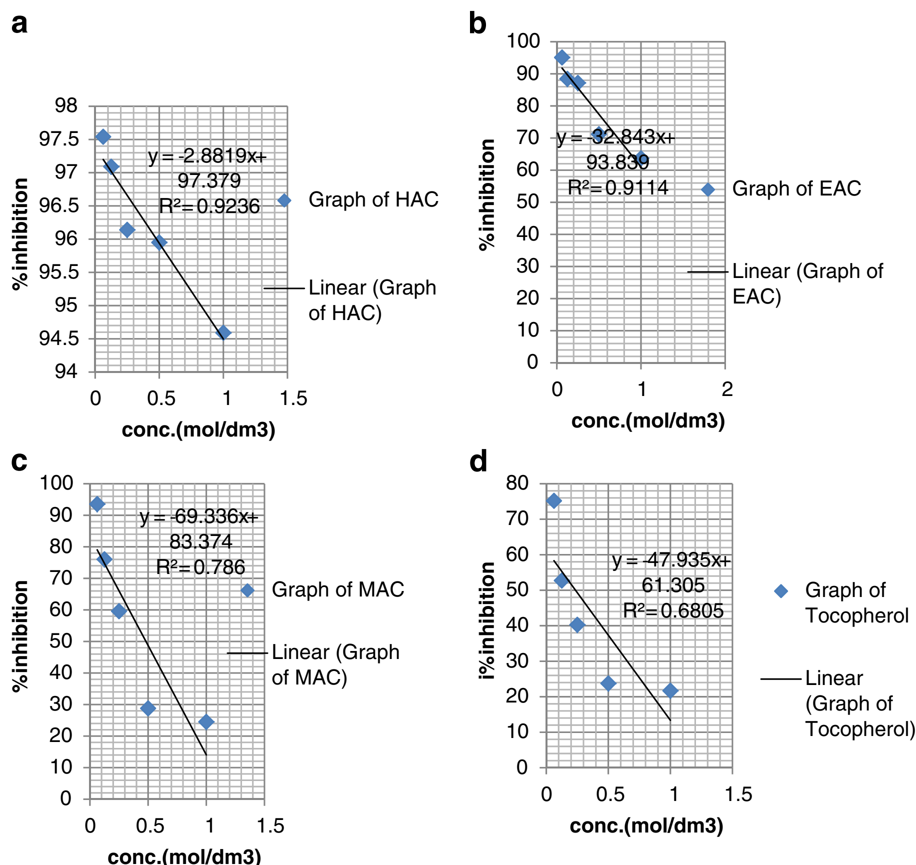

Fig. 1 a,b,c,d Plots of % inhibition against concentration for HAC, EAC, MAC, α-tocopherol (control) respectively showing the IC_{50}

Preparation of graded concentration of the samples

One thousand milligram of each sample was weighed and dissolved into 5 ml of the solvent of extraction in order to obtain proper dissolution. From the 200 mg/ml solution, 2.5 ml was taken into another sample bottle and 2.5 ml of solvent was added to give 100 mg/ml, from this, 2.5 ml is taken into another sample bottle and 2.5 ml of solvent was added to give 50 mg/ml solution. From the 50 mg/ml solution, 2.5 ml was taken into another sample bottle and 2.5 ml of solvent added to give 25 mg/ml solution. Similar procedure was followed to obtain the 12.5 mg/ml and 6.25 mg/ml concentrations, using dry filter paper dispersion method.

Organisms

Bacteria- *S.a : Staphylococcus aureus, E.c : Escherichia coli, B.sab: Bacillus subtilis, Ps. a : Pseudomonas aeruginosa, Sal. t : Salmonella typhi, Klebs : Klebsiella pneumoniae.,*

Fungi- *C. a : Candida albicans, A. n : Aspergillus niger, Pen : Penicillum notatum, Rhi. : Rhizopus stolonifer.*

-ve: Negative control; Methanol for methanolic extract, ethylacetate for ethylacetate extract and hexane for hexane extract.

+ve: Positive control; Gentamicin 10 μg/ml (bacterial) and Tioconazole 70 % (fungi).

Antimicrobial screening

The microbes used were suspended in suitable nutrition media and was poured into a sterile petri-dish and allowed to incubate for 24 h at 37 °C. Suitably cut circular filter paper pieces containing 100, 50, 25, 12.5, 6.25 mg/mL antibiotic solutions test samples and standard were introduced into the media. Then the pieces were placed on the nutritional microbial media all over with suitable gaps in between and incubated again.

After 24 h of incubation, the plates were removed and the diameter of the zone of inhibition of test and standard samples were measured in millimeters.

By comparing the areas of zone of inhibition of test extracts with standard, the concentration and potency of test samples were determined.

Results

The preliminary phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, tannins, reducing sugar, phenols, glycosides and resins in some of the extracts (hexane, ethylacetate, methanol) as shown in the Table 1. The methanol extract shows the presence of saponins, tannins, alkaloids, reducing sugars, phenols, glycosides and resins; the ethyl acetate extract shows only alkaloids while the hexane extract shows the presence of alkaloids and flavonoids.

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method by Nabavi et al.; 2008a and 2009a [18, 19]. In the hydrogen peroxide scavenging radical method, the percentage inhibition of the methanolic extract was in the range of 24.53 % – 93.56 %. The lowest concentration (0.0625 mg/mL) showed the highest percentage inhibition value (93.56 %). There is a characteristic increase in inhibition as the concentration decreases. The ethylacetate extract also showed similar trend as revealed in Table 2 (63.68 % at 1.0 mg/mL, 71.19 %, 87.17 %, 88.43 % at 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL respectively, and 95.09 % at 0.0625 mg/mL). The hexane extract followed similar trend, showing 94.59 % at 1 mg/mL and 95.95 %, 96.14 %, 97.09 % at 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL respectively and 97.94 % at 0.0625 mg/mL. At the lowest concentration of 0.0625 mg/mL the percentage inhibition was the highest for all the extracts.

Discussion

The results of the phytochemical screening shows that the methanol extract contains saponins, tannins, alkaloids, reducing sugar, phenols, glycoside and resins, while the hexane extract contains flavonoids and alkaloids and the ethyl acetate extract only contain the alkaloids.

The IC_{50} (the concentration of the samples required to scavenge 50 % of the peroxide radicals) was actually used to examine the antioxidant effectiveness of the samples. The lower the IC_{50}, the greater the overall effectiveness of the suspected antioxidant sample in question. From the results obtained, it was revealed that the synthetic antioxidant (α-tocopherol) showed the best antioxidant effectiveness with IC_{50} of 0.24 as shown in Fig. 1d. However, the test samples; HAC, *EAC*, and *MAC* had IC_{50} of 16.44, 1.34 and 0.481 respectively and shown in the Figs. 1a, b and c. Therefore, the synthetic antioxidant (α-tocopherol) was more effective than samples, although they are good antioxidants. The trend thus: α- tocopherol > *MAC* > *EAC* > *HAC* (order of decreasing antioxidant effectiveness). Noumedem et al. 2013, reported the

Table 1 Different constituents of the various extracts of *Acalypha ciliata* plant

Solvent	Saponin	flavonoid	Tanin	Phlobatannin	Steroid	Cardiac Glycoside	Akaloid	Reducing sugar	Phenol	Anthraquinone	Glycosides	Resin
HAC.	-	+	-	-	-	-	+	-	-	-	-	-
EAC	-	-	-	-	-	-	+	-	-	-	-	-
MAC	+	-	+	-	-	-	+	+	+	-	+	+

HAC hexane extract of *Acaylpha ciliata*, *EAC* ethylacetate extract of *Acaylpha ciliate*, *MAC* methanol extract of *Acaylpha ciliate*

Table 2 The percentage inhibition (antioxidant levels) of the different concentrations of the three different extracts

Conc. (mol/dm^3)	HAC	EAC	MAC	α-Tocopherol
1.0	94.59	63.68	24.53	21.69868
0.5	95.95	71.19	28.79	23.7504
0.25	96.14	87.17	59.60	40.2791
0.125	97.09	88.43	76.05	52.73975
0.0625	97.54	95.09	93.56	75.19368

antioxidant activities of the methanol extract, hexane, ethyl acetate and residual fractions of *Acalypha manniana* with all the extract especially the residual fractions displaying important radical scavenging activities against 2-diphenyl-1-picryl hydrazyl hydrate (DPPH) (RaS_{50} = 3.34–4.80 μg/mL) when compared with L-ascorbic acid used as reference antioxidant (RaS_{50} = 1.74 μg/mL) [20]. These observations demonstrate that the *Acalypha* species are free radical inhibitors or scavengers acting possibly as primary antioxidants. This is very promising in the perspective of new antioxidant discovery from plant extracts.

According to the antimicrobial results, at a high concentration of 100 mg/mL, it was observed that the methanol, ethyl acetate and hexane extracts of all the tested isolates exhibited good antibacterial and antifungal activities. At a low concentration of 12.5 mg/mL, the hexane extracts could no longer inhibit the growth of the micro organisms but the ethyl acetate and methanol extracts was still very active with the methanol extract showing the greatest inhibition.

All the extracts were no longer susceptible to fungal growth at 12.5 mg/mL except the methanol extract which only inhibited the growth of *Candida albicans* and *Aspergillus niger* at this concentration. This means that the minimum bactericidal concentration for the hexane extract is 25 mg/ml except for *Salmonella typhii* and *Klebsiella pneumoniae* which is 50 mg/mL as shown in Table 3.

This study has shown that the methanol extract of this plant possess high antimicrobial activities even at low concentration of 6.25 mg/mL; compared to the ethyl acetate and hexane extract placing it on a broad spectrum plane.

In a similar study using *Acalypha manniana*, the methanol extract prevented the growth of all tested micro-organisms with minimum inhibitory concentration (MIC) values ranging between 0.12 and 2.04 mg/mL for bacteria with *Staphylococcus aureus* being the most resistant [20]. In the case of *Acalypha indica* as reported by Govindarajan et al., 2008; the specie inhibited the growth of gram + ve bacteria but did not for gram –ve except *Pseudomonas aerugonisa* [21]. This may be due to the composition of gram –ve bacteria which is mainly lipopolysaccharide layer along with proteins and phospholipids. *Acalypha*

Table 3 The zones of inhibition of the isolates at different concentrations compared to the control drugs

Conc.(mg/mL)	Sa	Ec	B.Sab	Ps.a	Sal	Klebs	C.a	A.n	Rhi	Pen
MAC										
100	22	20	22	20	24	26	18	18	16	14
50	18	18	18	18	20	22	16	14	12	12
25	14	16	16	16	18	20	14	12	10	10
12.5	12	12	14	12	14	16	12	10	-	-
6.25	10	10	10	10	10	12	10	-	-	-
HAC										
100	16	18	16	16	14	12	16	12	14	14
50	14	16	14	14	10	10	12	10	10	10
25	12	12	12	10	-	-	10	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
EAC										
100	16	16	20	20	16	16	16	14	16	16
50	14	14	18	18	14	14	14	12	14	14
25	10	12	16	16	12	10	10	10	10	10
12.5	-	10	14	12	10	-	-	-	-	-
6.25	-	-	10	10	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	38	40	38	38	38	28	28	26	28

wilkesiana aqeous extract did not exert any inhibitory effect on *Klebsiella pneumoniae* and *Proteus mirabilis* but the ethanol extract was active with the MIC ranging from 0.25 and 32 mg/mL [22]. From the comparative studies and the results of our research, *Acalypha manniana* specie seems to be the most bioactive since it inhibits the growth of gram –ve and gram + ve bacteria as well as fungi.

Conclusion

The results of this study indicated that the plant contains some major bioactive compounds that inhibit the growth of micro-organisms, thereby showing great potency as effective source of drugs. The phytochemical analysis also reveals that the plant contains similar constituents which are useful for medicinal purpose. The high antioxidant activities of the plant indicates that, it will be potent in fighting diseases associated with oxidative compounds. This work reassures the safety in the consumption of this plant as the general trend in its edibility is diminishing.

Competing interests
Oluwakayode Odeja, Christiana Ene Ogwuche, Elias Emeka Elemike and Grace Obi declare that they have no competing interests.

Authors' contributions
OO carried out the samples collection and took part in the laboratory analysis and write up. OCE was involved in the antioxidant and antimicrobial

analysis as well as results interpretation. EEE was involved in the laboratory work as well as technicalities and made necessary corrections in the write up. OG performed part of the laboratory work, was involved in the plant extraction process, result interpretation and made necessary correction in the write up. All authors read and approved the final manuscript.

Acknowledgment
The authors are grateful to the technologists of Department of Chemistry of the Federal University of Petroleum Resources Effurun for their assistance during the course of this research.

Received: 27 October 2015 Accepted: 2 June 2016
Published online: 21 June 2016

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