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Antimicrobial, Phytochemical and Antioxidant Screening of *Acalypha fimbriata* Leaf Extract for Alternative Antimicrobial Therapy

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ABSTRACT

In folklore, *Acalypha fimbriata* have been associated with 'cure all' properties, but without sufficient empirical ethno-pharmacological scientific backups. This research work is therefore necessitated to determine the antimicrobial, phytochemicals and antioxidant activity of *Acalypha fimbriata.* The leaf of *Acalypha fimbriata* were Soxhlet extracted, reconstituted, and screened for phytochemical constituent of antimicrobial importance. Conventional biochemical characterization was carried out on the isolates of *Staphylococcus aureus, Escherichia coli Pseudomonas aeruginosa and Klebsiella pneumoniae* obtained for the research work and the antimicrobial activity of the plant sample was determined using the agar well diffusion technique. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) of the extract were determined by broth dilution method on the isolates. The antioxidant activity of the extract was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and Graphpad prism were used to interprets the data. Alkaloids, flavonoid, anthraquinones, tannins and saponins was found in the leaf extract. *Staphylococcus aureus* exhibited highest zone of growth inhibition (28mm) at 100mg/ml while *Pseudomonas aeruginosa* had the lowest (14mm) at 100mg/ml from the antimicrobial assay. In the determination of the minimum inhibitory concentration and minimum bactericidal concentration, *Staphylococcus aureus* exhibited MIC and MBC at 0.625µg/ml and 12.5 µg/ml, while *Pseudomonas aeruginosa* elicited MIC and MBC at 10 µg/ml and >10 µg/ml of MIC and MBC respectively.Themethanol extract of the plant acted as hydrogen/electrons donor or scavenger of radicals with fifty percent inhibitory concentration (IC_{50}) of 59.83 µg/ml while that of Ascorbic acid (standard) was found to be 92.70 µg/ml using.The varied MIC's and MBC's obtained coupled with the values recorded for the antioxidant radicals validate the antimicrobial and antioxidant potential of *Acalypha fimbriata* that can be explored for therapeutic option, if further purified and optimally processed.

Keywords: *Acalypha fimbriata*, antimicrobial, phytochemical screening, antioxidant potential.

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Introduction

The use of traditional herbs in the treatment of diseases has always been in practice from time immemorial, and it is becoming more popular globally due to ease of access, affordability, and experimental knowledge of uses and efficacy. According to WHO, it has been estimated that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care, while remaining 20% belonging to advanced countries are also actively involved in the use of plant-derived products for medical purposes (Maryam *etal.,* 2012).

Acalypha fimbriata Schumach&Thonn, belong to the family Euphorbiaceae, commonly called Acalypha is a dicotyledonous plant, with a resemblance of *Acalypha cilita*but consistently differing in the teeth of the female bracts, which are up to 1.5mm long, falcate – lanceolate, curved at the apex, often lying perpendicular to each other and almost contiguous. It is expansive in Tropical Africa and Northern South Africa (Wilson and Boderker, 2004).

The genius is represented by sixteen species in Nigeria and they are abundant in the forest and Savannah ecosystems where they show preferences for distributed terrestrial habitats such as farmland, gutter – walls, roadsides, floor crevices and garden (Akinbuluma *et al.,* 2015). The plant is used traditionally for the treatment of bacteria and fungi infections, such as syphilis and other ailments such as worm infections, asthma, ulcer, rheumatism etc (Dahiya and Purkayasha, 2012).

Antioxidants are man-made or natural substances that protect cells from the damage caused by unstable molecules known as free radicals by interacting with them to achieve stability. Although, oxidation reactions are crucial for life, they can also be damaging as they can produce free radicals which start chain reactions that damage cells. Plants and animals maintain complex systems of multiple types of antioxidants such as glutathione, vitamins A, C and E as well as enzymes such as catalase, superoxide, dismutase, and various peroxides. Traditional herbal products and dietary foods were the source of antioxidant for ancient people that protected them from the damage caused by free radicals. Antioxidants are widely used in dietary supplements and have been investigated for the management of diseases such as cancer, coronary heart disease and even altitude sickness. Although some researchers suggested that antioxidant supplements might promote health, later many clinical trials of antioxidant supplements including beta carotene, vitamins A and vitamin E, singly or in combinations suggest that supplementation has no effect on mortality or possibly increases it. They are also used in the food industry in the form of preservatives in foods and cosmetics and prevent the degradation to rubber and gasoline (Ejikeme, 2014).

With the evolution of more resistant pathogenic microorganisms against synthetic antibiotic, it is pertinent to search for plants with broad spectrum antimicrobial compounds from folklore medicine of which *Acalypha fimbriata* is a typical example. Hence, this study is aimed at determining the antimicrobial, phytochemicals, and antioxidant activities of this plant

Materials and Methods

Collection of Plant Materials

The plant was collected at Cacao Research Institute of Nigeria Onigambari, Idi-Ayunre in Ibadan and was authenticated in the herbarium of the Department of Pharmacognosy, Olabisi Onabanjo University with voucher identity OOUPCG 312[A] and was archived.

Collection of samples

Isolates of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella sp* were obtained from routine laboratory bench in

the Department of pharmaceutical microbiology, Faculty of Pharmacy, Olabisi Onabanjo University. They were confirmed by conventional biochemical characterization and preserved on nutrient agar slant in refrigerator at 4°C

Extraction

The air dried leaf powder samples of pulverized *Acalypha fimbriata* leaves (500g) was extracted with distilled methanol (1000 mL) using Soxhlet extractor until complete siphoning. The extract was filtered twice and concentrated with rotary evaporator (Rotavapor-R, India) to give a solid mass.

Reconstitution of plant extract

The solid extract was reconstituted with 5% v/v of DMSO and water to obtained ranging concentrations of 100mg/ml w/v to 6.25mg/ml.

Antimicrobial Assay

The antimicrobial activity of the plant sample was determined using the agar well diffusion technique. The surface of the agar in a culture was seeded with 0.2mL of 1:100 of overnight culture of each isolate. A sterile cork borer was used to bore 8mm wells into the agar medium. Then varied concentrations of plant extract was introduced to the wells and plates were allowed to stand on the bench for 1 hour for pre-diffusion. The inhibitory zones (mm) were taken as a measure of antimicrobial activity after 24hrs incubation.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using broth dilution method. A volume of 2mL of the extract of concentrations ranging from 20µg/mL to 2.5 µg/mL was dispensed into 2 mL of sterile Mueller Hinton broth in a set of 4 test-tubes to obtain a final concentrations of 10µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL and 0. 625 µg/mL respectively. Each test organism was inoculated into the labeled test-tube by taking a loopful of the standardized bacterial suspension using a flame sterilized wire loop except the control; the test- tubes were incubated at 37°C for 18 hours. The MIC was taken as the lowest concentration that prevented visible growth when compared with negative control (broth and extract only).

Determination of Minimum Bactericidal Concentration

The two concentrations which elicited no growth on nutrient agar medium was taken as minimum bactericidal concentration (Mabhisa *et.al.,* 2016).

Antioxidant assays (Assay of DPPH scavenging activity)

The DPPH radical-scavenging activity of the test extracts was examined using the technique of Liyana *et al*. (2001). A volume of 0.136 ml DPPH solution in methanol was prepared and 1.0ml of this preparation was mixed with 1.0ml of the extract in methanol containing 0.02-0.1 mg of the extract. The reaction mixture was thoroughly vortexed and left in the dark chamber of the fume cupboard at 25±2°C for 30 minutes. The absorbance of the mixture was measured at 518nm run on spectrophotometer. Ascorbic acid was used as standard control. The ability to scavenge DPPH radical was calculated using the following equation.

DPPH radical scavenging activity(%)=[(Abs control –Abs sample)/(Abs control) × 100

Where Abs control = Absorbance of DPPH radical + methanol

Abs sample = Absorbance of DPPH radical + sample extract/standard. IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals. The IC_{50} values were calculated with Graphpad Prism version 8.1.

Statistical Analysis

Microsoft® Graphpad Prism version 8.1 (Window 16) was used to analyze the data.

Phytochemical Analysis

Phytochemical screening was performed to identify phytochemicals in methanol extracts of *Acalypha fimbriata* leaf extract. The phytochemicals were detected by color tests.

Test for Alkaloids: Half a gram of *Acalypha fimbriata* leaf extract was diluted to 10mls withacid alcohol boiled and filtered. Two milliliters of dilute ammonia was added to 5mls of thefiltrate. To the extract, 5mls of chloroform was added and shaken gently to extract the alkaloidal base. The layer of chloroform was extracted with 10ml of acetic acid. This was divided into two portions. Mayer's reagent and Draggendorff's reagent was added to the first and second portion respectively. A creamy/reddish-brown precipitate was taken as positive for alkaloids.

***Test for Flavonoids*:** Five milliliters of 10% dilute ammonia solution was added to a portion of the aqueous filtrate of the Acalypha fimbriata crude extract. Concentrated H_2SO_4 (1ml) was added. A yellow colour which disappeared on standing showed the presence of flavonoids.

Test for Saponins: Half a gram of the extract was added to 5mls of distilled water in a test tube. The solution was vigorously shaken and observed for a stable persistent froth. The frothing was mixed with three drops of olive oil and vigorously shaken. Thereafter, it was observed for the formation of an emulsion.

***Test for Anthraquinone*:** Half a gram of the extract was boiled with 10mls of sulphuric acid and was filtered while it is still hot. The filtrate was shaken with 5mls of chloroform. The resultant layer of chloroform was pipetted into another test tube and 1ml of dilute ammonia was added. The resultant solution was checked for colour changes.

Test for Tannins: A quantity of 0.5 g of the extract was boiled in 10mL of distilled water and then filtered, a few drops of 0.1% ferric chloride was added and blue black color was observed.

Results

The isolates of bacterial used in this study elicited varied reactions to conventional biochemical test as shown in Table1. The zones of growth inhibition of the isolates in methanol extract varied from for each isolates tested as showed in Table 2. *Staphylococcus aureus* isolates were inhibited at a minimum concentration of 0.625µg/ mL and exhibited bactericidal activity at 12.5 µg/ mL. The MIC and MBC values of 1.25 µg/ mL and 50 µg/ mL were recorded for *Klebsiella pneumonia* that seems to be relatively effective from MIC point of view than *Escherichia coli* with MIC 5 µg/m mL and MBC 10 µg/mL. *Pseudomonas aeruginosa* was found to be resistant to both *Acalypha fimbriata* and conventional antibiotic (ciprofloxacin) used. The isolates of *Pseudomonas aeruginosa* exhibited the MIC 10 µg/ mL and MBC of >10 µg/ mL respectively as showed in Table 3.

Table 1: Morphological characteristics and biochemical reactions of the isolates

Isolates	Morphological characteristics and Biochemical reactions							
	Gram reaction	Shape	Coagulase	Methyl red	Indole	Catalase	Oxidase	Voges Proskaeur
Escherichia coli	-	Short rod	-	+	+	+	-	-
Staphylococcus aureus	+	Cocci	+	-	-	+	-	+
Klebsiella pneumoniae	-	Bacilli	-	-	-	+	-	+
Pseudomonas aeruginosa	-	Rod	-	-	-	+	+	-

Table 2: Zones of growth inhibition (mm)

Test organisms	Methanol Extract(mg/mL)						
	100	50	25	12.5	6.25	+C	-C
Staphylococcus aureus	28	22	20	18	12	24	0
Escherichia coli	18	14	12	10	10	18	0
Pseudomonas aeruginosa	14	10	0	0	0	12	0
Klebsiella pneumoniae	20	16	14	10	10	24	0

+C: Positive control (10µg/mL ciprofloxacin), -C: Negative control (1% DMSO)

Table 3: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (Broth dilution method)

Test organisms	MIC (µg/mL)	MBC(µg/mL)	Ciprofloxacin (10µg/mL)	
			MIC	MBC
Staphylococcus aureus	0.625	12.5	0.3125	2.5
Escherichia coli	5	10	2.5	5
Pseudomonas aeruginosa	10	>10	10	>10
Klebsiella pneumoniae	1.25	50	5	5

Table 4: Comparative IC_{50} values of *Acalypha fimbriata* and Ascorbic acid

	Acalypha fimbriata		**Ascorbic Acid**	
Conc.	Scavenging Activity (%DPPH)	Conc.	Scavenging Activity (%DPPH)	
	1.0	57	1.0	44
	0.5	54	0.5	42
	0.25	49	0.25	29
	0.125	45	0.125	23
	0.0625	42	0.0625	21
IC_{50}		**0.005983**		**0.009270**

Table 5: Phytochemical screening of Leaf extract of *Acalypha fimbriata*

Phytochemicals	Present
Alkaloids	+
Flavonoids	+
Saponins	+
Anthraquinones	+
Tannins	+

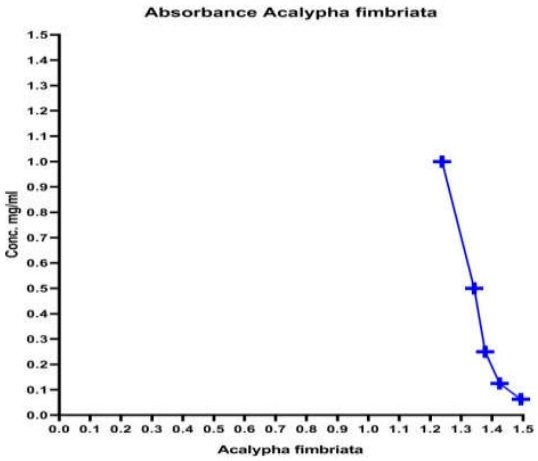

Figure1: Concentrations of the methanol extracts against the absorbance of *Acalypha fimbriata*

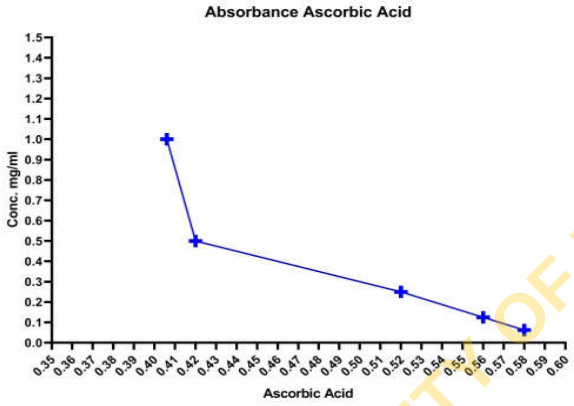

Figure 2: Concentrations of the methanol extracts against the absorbance of Ascorbic acid

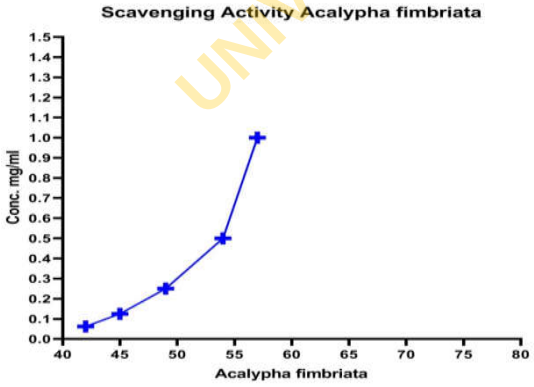

Figure 3: Concentrations of the methanol extracts against the scavenging activity of *Acalypha fimbriata*

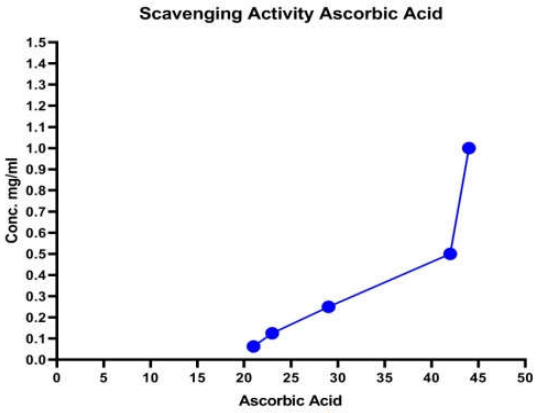

Figure 4: Concentrations of the methanol extracts against the scavenging activity of Ascorbic acid

Discussion

Novel infectious diseases are emerging and bacterial resistance to conventional drugs keeps increasing. The dual factors of antimicrobial resistance and limited scope of orthodox medicine globally have become imperative that global health care be sustained by indigenous herbal medicines (Wagate *etal.,* 2010).

The methanolic leaf extract of the plant studied showed broad spectrum of antimicrobial activity against the isolates tested at varied concentrations. *Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli* were highly susceptible to the extract estimating from pronounced zones of growth inhibition at higher concentrations than *Pseudomonas aeruginosa* that showed resistance to the extract as showed in Table 2 in this study. The antimicrobial activities exhibited by the extract may therefore justify some of the ethno pharmacological claims about this plant in the treatment of the bacterial infections. The MIC and MBC values of the leaf extract of *Acalypha fimbriata* studied showed remarkable antimicrobial activity, The MIC (0.625µg/mL) and MBC(12.5 µg/mL.) of the isolates of *Staphylococcus aureus* elicited the

antimicrobial potency of the plants studied which was similar to the study of Oluwakayode *etal.,*(2016) on phytochemical and antimicrobial screening of Acalypha ciliate plant while . The MIC and MBC values of 1.25 µg/ mL and 50 µg/ mL recorded for *Klebsiella pneumonia* seems to be relatively effective from the MIC point of view in comparison with *Escherichia coli* that had MIC 5 µg/ mL and MBC 10 µg/ mL while *Pseudomonas aeruginosa* was found to be resistant to both *Acalypha fimbriata* and conventional antibiotic (ciprofloxacin) used which corroborates with the findings of Okwute *etal.*(2017) on phytochemical screening and in-vitro antimicrobial activity of *Waltheria indica* leaf extract The MIC and MBC values from both plant could be due of polar constituent of methanolic extract of *Acalypha fimbriata,*, since methanol higher polarity make it an excellent extractant, or genetic components of the isolates of bacterial tested and other inherent factors within the organisms. The MIC and MBC from standard antibiotic tested though effective against the isolates of *Staphylococcus aureus,Klebsiella pneumoniae* and *Escherichia coli* but resistant to *Pseudomonas aeruginosa* could be due to abuse of the antibiotic and underlying genetic factors of the isolates tested which corroborates the study of Rani and Khullar (2004) on antimicrobial evaluation of some medicinal plants for their antienteric activity against multidrug resistance Salmonella (Rani and Khullar,2004)

Plant accumulates an armory of antimicrobial secondary metabolites. Some metabolites represent constitutive chemical barrier to microbial attack (phytoanticipins) and other inducible antimicrobials (phytoalexcins). The presence of the secondary metabolites; alkaloids, saponins, flavonoids, anthraquinone and phenols from the powdered leaf of *A. fimbriata* in this studyas showed in Tables 5 was an indicative of varied pharmacological activities of the extract. This corroborates with the study of Rahua *etal.* (2000) on antimicrobial effect of Finnish plant extracts containing flavonoids and other phenolic compounds (Rahua *etal.,*2000).

The free radical scavenging activity of methanolic extract of *Acalypha fimbriata* showed more activity than ascorbic acid at concentration of 1mg/ml to 0.0625mg/mL as showed in Figure 1-4, an indicative of their antioxidants activity. *Acalypha fimbriata* methanol extract was observed to scavenged DPPH (IC_{50} 0.005983mg/mL) which was observed to be value for the ascorbic acid scavenging DPPH radicals (IC_{50} 0.009270 mg/mL) as shown in Table 4, which was suggestive of *A. fimbriata* potential as a natural source of antioxidant that could be used for the treatment of infectious diseases particularly, that has to do with oxidative consequences.

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, were found in the leaf extract could inhibit hydrolytic enzymes of bacteria and also has anti-inflammatory property (Boakye *etal.,* 2016), which might be responsible for the scavenging of this plant.

Also, the saponins with potential to reduce the body cholesterol by preventing its reabsorption, increasing its excretion thereby reducing blood pressure could be attributed to the additional antioxidant activity of the plant studied (Anita *etal.,* 2019).

The antimicrobial, phytochemical, and antioxidant activities of the leaf extract of *Acalypha fimbriata* against diseases may be responsible for medicinal uses of the plant for therapeutic purposes.

Conclusion

Crude extract of *Acalypha fimbriata* leafin comparison with the standard antibiotic used in this study,exhibited a potent spectrum of antimicrobial activity on the isolates of bacteria

tested (Gram positive and Gram negative). Also, thephytochemicals and antioxidant activity exhibited by the leaf extract of the *Acalypha fimbriata,* is suggestive of the usability of this plant as a safe therapeutic alternative for antimicrobial and antioxidant agent when further purified. Hence, a justification for its ethno-pharmacological uses.

Conflicts of Interest: None

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