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Phytochemical screening and Antimicrobial analysis of *Moringa oleifera* and *Acalypha wilkesiana* plants

Juliana Chineze Obi[1*], **Augustine Enajite Usiakpebru**[1]

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Author Affiliation:
[1]Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria

***Corresponding author**
Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State,
Nigeria
Email: iemmanuel385@gmail.com

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ABSTRACT

Two varieties of medicinal plants (*Moringa oleifera* and *Acalypha wilkesiana*) which were obtained from different locations in Asaba, Oshimili South Local Government Area of Delta State, Nigeria were studied. The extractions were carried out using methanol solvent and the extracts obtained were washed and oven dried at temperature of 35^{0}C. These extracts were further subjected to qualitative and quantitative analysis and the pytochemical screening revealed the different chemical constituents present in them. Amazingly, the phytochemical analysis showed that *Moringa oleifera* had the highest percentage yields of alkaloids (8.56%). The antimicrobial susceptibility and microbial inhibition tests are further proof of their high level of potency.

Keywords: medicinal plants; antimicrobial susceptibility; pytochemical screening

1. INTRODUCTION

The use of traditional medicine has expanded globally and also gaining popularity in the field of medicinal chemistry. It has continued to be used not only for primary health care of the poor in developing countries but also in countries where conventional medicine is predominant in health care system (Balick and Cox, 1996). A medicinal plant is any plant which one or more of its organs contain substances that can be used for therapeutic purposes or serve as precursor for the synthesis of useful drugs. Medicinal plants contain biologically active chemical substances (phytochemicals) such as saponins, tannins, flavonoids, alkaloids and other compounds which have preventive or curative properties. Drugs of natural origin are considered to be less toxic and free from adverse effects than those of the synthesized drugs. The active compounds of many herbal drugs are unknown, and have been widely prescribed by practitioners of traditional medicine due to their minimal adverse effects and low cost (Valiatham, 1998).

Moringa oleifera belongs to the family of morigaceae and it is commonly known as "drumstick or horse radish tree". It is used in India folk medicine for the treatment of various illnesses and also consumed as vegetable food. *Moringa oleifera* is a small graceful plant with thin vegetation often planted locally or used for wall fencing in Nigerian homes (Nikkon et al., 2003). It resembles a

leguminous species at a distance especially when flowering. *Acalypha wilkesiena* is a plant from the family of Euphorbiaceae. The genus comprises about 570 species, a large proportion of which are weeds while the others are ornamental plants (Riley, 1963). The Wilkesiena plants are found all over the world especially in the tropical Africa, America and Asia. *Acalypha wilkesiena* is a fast growing evergreen shrub which provides a splash of colour in the landscape with bronze red to muted red. The leaves appear as heart shaped with combination of colours like green, purple, yellow, orange, pink or white depending on cultivation. The leaves of *Acalypha wilkiesena a*re popularly used in the treatment of skin infections (Akinde, 1986).

Phytochemicals are mostly divided into two main groups; primary and secondary metabolites according to their functions in plant metabolism. Primary metabolites include common sugar, amino acids, protein and chlorophyll while secondary metabolites comprise alkaloids, tannins, etc. Examples of phytochemicals include alkaloids, flavanoids and saponins. The aim of this study is to determine the phytochemical screening *of Moringa oleifera* and *Acalypha wilkisena* and to quantify them as active ingredients in alternative medicine, for production of drugs or as precursors for pharmaceutical industries based on their ethno botanical potentials. Lastly, to determine the antimicrobial activities of these medicinal plants.

2. MATERIALS AND METHODS

All reagents were obtained from commercial sources. Fresh leaves of *Moringa oleifera* and *Acalypha wilkesiana* were collected, washed and air dried. After drying, the leaves were ground in to powdered form and extraction was carried out using methanol solvent. The phytochemical screening analysis was studied using 0.1 g of the dry extracts while the quantitative and antimicrobial analysis were also investigated to determine the active ingredients present in them using the general procedure according to (Debela, 2002).

Determination of Phytochemical Constituents Present

A weighed amount of 0.1 g of the extract was added into 5 ml of distilled water in a 250 mL beaker. The mixture was stirred for 10 minutes and filtered. After that, 1% ferric chloride solution was added to 2 mL of the filtrate.

Quantitative Analysis of *Moringa oleifera* and *Acalypha wilkesiana*

A weighed amount of 5.0 g of the extract was added to 10 % mixture of ethanoic acid and ethanol in the ratio of (1: 9). Thereafter, 2 drops of NH_4OH solution was added and stirred for 5 minutes. The mixture was filtered and a blue black residue was observed.

Antimicrobial Susceptibility Test

The organisms used (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella enterica*) were obtained from Conig-Simonne Laboratory, Awka, Anambra State, Nigeria. The organisms were maintained on nutrient broth for 24 hours. The antibacterial activities of the extracts against the test bacteria were evaluated by modified disc diffusion methods (Agu et al., 2013; Adindu et al., 2016; Awah et al., 2017). Exactly 25 µl of 0.5 Mc Farland standardized suspension of test bacteria ($1.5x10^8$cfu) were cultured onto the Mueller Hinton plates by pour plate method. Exactly 50 µl of the extracts were used to impregnate the 6 mm filter paper discs and placed on two portions of the agar plate. The inhibition zone diameters of the various plates were measured and recorded in millimeters. All experiments were done in triplicates. Negative controls were set up with sterile physiological saline and positive controls were set up using 50 µg/ml ciprofloxacin.

Determination of Minimum Inhibitory Concentration (MIC)

Modified broth dilution methods were used for this study (Agu et al., 2013; Pallotta et al., 2007; Ubaoji et al., 2020; Madzigga, 2010). Two fold serial dilutions of the extracts in Mueller Hinton broth for bacteria was done as follows; 500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml. 0.1 ml of the 0.5 McFarland standardized cultures were each seeded into the various tubes. The Mc Farland standardized cultures were incubated for 2 hours before use. Controls (negative and positive) were set up by using sterile dimethylsulphoxide (negative control) and ciprofloxacin (positive control) for bacteria. The tubes were incubated at room temperature for 24 hours in a metabolic reciprocal shaker (220 rev/min).

3. RESULTS AND DISCUSSION

The phytochemical screening of *Moringa oleifera* and *Acalypha wilkesiana* revealed the presence of chemical constituents as shown in Table 1. The positive sign indicates the presence of chemical constituents while the negative sign indicates the absence of chemical constituents. *Moringa oleifera* and *Acalypha wilkesiana* phytochemical analyses revealed the presence of tannin, saponins, alkaloids,

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flavonoids and cardiac glycosides. However, chemical constituents such as phenol, resins, sterols and anthroquinones were not found in the phytochemical screening of both plants.

Table 1 Phytochemical Constituents Present in *Moringa oleifera* and *Acalypha wilkesiana* Key: + indicates presence; – indicates absence.

S/N	Constituents	*Moringa oleifera*	*Acalypha wilkesiana*
1	Phenol	-	-
2	Tannin	+	+
3	Saponin	+	+
4	Triterpenes	-	-
5	Flavonoids	+	+
6	Alkaloids	+	+
7	Cardiac Glycosides	+	+
8	Resins	-	-
9	Sterols	-	-
10	Anthroquinones	-	-

Determination of the active ingredients which are responsible for their healing processes were conducted and *Moringa oleifera* had the highest percentage yield of alkaloids (8.56%), while tanins, saponins and flavonoids were found to be 0.45%, 1.45% and 6.62% respectively as shown in the Table 2. Similarly, *Acalypha wilkesiana* recorded a high percentage yield of alkaloids (7.14%) and a low yield of tannins (0.51%) and saponnins (0.35%). The percentage yield of flavonoids was relatively high too (6.01%).

Table 2 Percentage Yields of Alkaliods, Tannins, Saponins and Flavonoids in *Moringa oleifera* and *Acalypha wilkesiana*

Phytochemicals	*Moringa oleifera*	*Acalypha wilkesiana*
Alkaloids	8.56%	7.14%
Tannins	0.45%	0.51%
Saponnins	1.45%	0.35%
Flavonoids	6.62%	6.01%

Antimicrobial susceptibility test against test organisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella enterica* revealed that the antimicrobial activities of *Acalypha wilkesiana* extracts had a higher inhibition zone against *Staphylococcus aureus* compared with that of *Moringa oleifera* as seen in Table 3. The mean inhibition zones of *Acalypha wilkesiana* against the test organisms are 18.33 mm, 30.67 mm and 19.70 mm for *Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella enterica* respectively. The mean inhibition zones of *Moringa oleifera* against test organisms are 21.67 mm, 25.00 mm and 10.67 mm for *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella enterica* respectively, and this also revealed their high level of potency.

Table 3 Preliminary antimicrobial susceptibility screening of extracts of *Moringa oleifera* and *Alkalyphawilkesiana* against test organisms

Test Bacteria	MEAN INHIBITION ZONE DIAMETERS (IZD) IN MILLIMETRES ± STANDARD DEVIATION															
	Acalypha wilkesiana				*Moringa oleifera*				Positive control				Negative control			
	ϰ	y	Z	Mean ± SD	ϰ	Y	z	Mean ± SD	ϰ	Y	z	Mean ± SD	ϰ	y	z	Mean ± SD
Pseudomonas aeruginosa	16	19	20	18.33 ± 2.08	22	20	23	21.67 ± 1.53	50	48	46	48.00 ± 2.00	-	-	-	-
Staphylococcus aureus	33	30	29	30.67 ± 2.1	27	25	23	25.00 ± 2.00	60	64	62	62.00 ± 2.00	-	-	-	-
Salmonella enterica	18	20	21	19.70 ± 1.53	10	12	10	10.67 ± 1.15	43	44	46	44.33 ± 1.53	-	-	-	-

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The minimum inhibitory concentration (MIC) is defined as the lowest concentration of anti-microbial activities that will inhibit the visible growth of a micro-organism after overnight incubation. It is the least concentration of the samples with no visible growth. The MIC therefore, conducted on *Moringa oleifera* and *Acalypha wilkesiana* against test organisms (*Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella enterica*), revealed that 500 $mgml^{-1}$ of both plants extracts had no growth in them as shown in table 4. The positive control (ciprofloxacin) showed no growth for all the different concentrations of both plants, while the negative control (sterile dimethylsulphoxide) showed reasonable level of growth in all concentrations of both plants extracts. There was no growth seen in 250 $mgml^{-1}$ of *Acalypha wilkesiana* against *Staphylococcus aureus*.

Table 4 Minimum Inhibitory Concentration (MIC) of the extracts of *Moringa oleifera* and *Acalypha wilkesiana* against the test organisms Key: T= Turbidity; NT = No Turbidity.

	Acalypha wilkesiana ($mgml^{-1}$)				*Moringa oleifera* ($mgml^{-1}$)				Positive control ($mgml^{-1}$)				Negative control ($mgml^{-1}$)			
Test Bacteria																
	500	250	125	62.5	500	250	125	62.5	500	250	125	62.5	500	250	125	62.5
Pseudomona aeruginosa	NT	T	T	T	NT	T	T	T	NT	NT	NT	NT	T	T	T	T
Staphylococcus aureus	NT	NT	T	T	NT	T	T	T	NT	NT	NT	NT	T	T	T	T
Salmonella enterica	NT	T	T	T	NT	T	T	T	NT	NT	NT	NT	T	T	T	T

4. CONCLUSION

In conclusion, *Moringa oleifera* had the highest percentage yields of alkaloids (8.56%), compared *Acalypha wilkesiana* (7.14%). The antimicrobial analysis done on both plants gave further evidence of their antimicrobial potency. Quality herbal plants can be obtained through good quality control measures, standard laboratory techniques and by trained personnel. This will go a long way in meeting local, national and international standards, hence promoting alternative medicine.

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Authors' Contributions

Juliana Chineze Obi: Conceptualized this study and edited the manuscript. Augustine Enajite Usiakpebru: Carried out the various analyses and wrote the manuscript.

Ethical approval

Not applicable.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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