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Determination of the Phytochemical and Antimicrobial properties of *Nauclea latifolia* Root Smith. (Rubiaceae)

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Abstract

Nauclea latifolia is a valuable medicinal plant for treating different ailments. Infectious diseases constitute a world health concern.

This work describes the phytochemical analysis and antimicrobial properties of the root of *N. latifolia*.

Collected root samples were extracted via cold maceration with methanol. The methanol crude extract was fractionated into n-hexane, ethyl acetate, butanol and aqueous fractions. Phytochemical analyses and antimicrobial activity of the methanol extract/ fractions were assessed using agar well diffusion method.

Four different fractions were obtained from the methanol (crude) extract. The phytochemistry carried out showed the presence or absence of certain phytochemicals. The methanol (crude) extract and the different fractions had good antimicrobial properties.

It was concluded that *N. latifolia* root revealed the presence of phytochemicals and has antimicrobial activity.

Keywords: *Nauclea latifolia*; Agar well diffusion assay; Phytochemicals; Antimicrobial

1 Introduction

Plants have formed the foundation of sophisticated traditional medicine systems that have been in existence for thousands of years [1, 2]. Approximately 80 % of the world`s population relies mainly on traditional medicine, predominantly originated from plants, for their primary healthcare, this was estimated by the World Health Organization (WHO) [3]. A plant is considered a medicinal plant only when its biological activity has been ethno-botanically reported or scientifically established [4]. Infectious diseases still represent one of the major health concerns worldwide. According to the National Institute of Health, infectious diseases are the second cause of death and the leading cause of loss of productive life years worldwide [5].

N. latifolia Smith. (Rubiaceae) is a straggling, evergreen, multi-stemmed shrub with sweet scented flowers and well distributed in many parts of Nigeria. It is also known as African peach; it is a medicinal plant that may be used for

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traditional medicinal practices of the East and West African sub-regions of continental Africa where various extracts of the plant are used for the therapeutic management of malaria, hypertension, prolonged menstrual flow, cough, gonorrhoea, stomach disorders, dysentery, ulcers and liver ailment [4]

Amongst the new areas explored to overcome infectious diseases caused by Multi Drug Resistant (MDR) bacteria, medicinal plants seem to offer an ideal alternative since they are readily available source of bioactive agents [6, 7, 8, 9]. Thus, there is need for the phytochemical screening and investigations into the antimicrobial potentials of the extract/fractions of *N. latifolia* roots. This study aimed to evaluate the antimicrobial potential of the extract/fractions of *N. latifolia* root.

2 Material and methods

2.1 Equipment

Grinding machine, Hot air oven (Relitech), Rotary evaporator, Refrigerator, Autoclave, Electrical weighing balance (OHAUS Model 2610), Laboratory Incubator, Laboratory oven (Relitech), Digital water bath (Sanfa-Model No DK420), Bunsen burner.

2.1.1 Apparatus/Glass wares

Beakers, Conical flasks, Round bottom flasks, Filter papers, Aluminum foils, Separating funnel, Petri dishes, Test tubes, Cotton wool, Hand gloves, Facemasks, 10 ml and 0.5 ml Syringe, Marker, Masking tapes, Cork borer, Pasteur pipettes, Swab sticks, Measuring cylinders, Stirrer, Inoculating loop, Micro pipettes.

2.1.2 Reagents

N- Hexane, Ethyl acetate, Butanol, Methanol, Distilled water, Ciprofloxacin, Nutrient Agar, Nutrient Broth, Saubaraud dextrose agar, Dimethyl sulfoxide (DMSO), 70 % alcohol, Mayer's reagent, Wagner's reagent, Conc. H_2SO_4, Dilute ammonia, Glacial acetic acid, Ferric chloride solution, 10 % Lead acetate (Trust Chain Lab.), Neutral 5 % Ferric chloride (Lobachemie) , Chloroform, Miconazole.

2.1.3 Microorganisms

Pseudomonas aeruginosa, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*.

2.2 Plant Materials

Samples of fresh healthy *Nauclea latifolia* root samples were obtained from Nsukka, Enugu State, Nigeria; and authenticated with the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam. The roots were air dried, pulverized into a fine powder, weighed and stored in an air-tight container till use.

2.3 Extraction and Fractionation

The plant materials (500 g) were air-dried, pulverized and cold macerated in methanol for 72 hrs with intermittent shaking and decanting and reconstituted with 500 ml methanol every 24 hrs. The mixture was filtered with Whatman (No. 1) filter paper and evaporated using rotary evaporator at 40 °C and thoroughly dried in a hot air oven at the same temperature. The methanol extract was subjected to liquid-liquid fractionation to obtain n-hexane, ethyl acetate, butanol and aqueous fractions respectively.

2.4 Preliminary phytochemical screening

The test was carried out on the ethanol extract and fractions using standard procedures as described by [10].

2.5 Antimicrobial Screening

2.5.1 Sterilization of Working Materials

Petri dishes, test tubes and pipettes were washed and rinsed with distilled water and wrapped with aluminum foil before they were sterilized in an autoclave at a temperature of about 121 °C for 15 minutes. The laboratory benches were cleaned with 70 % alcohol before and after each experiment.

2.5.2 Preparation of Inoculums

The bacteria used were *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. The fungus used was *Candida albicans*. All isolated from clinical specimens obtained from Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria. The test organisms were separately prepared by sub culturing the pure isolates in Nutrient agar and incubated at 37 °C for 24 hrs for bacteria and in Saboraud dextrose agar for 72 hrs for fungi. Two loopful of the microbial culture were collected using a sterilized (heat fixed) inoculating wire loop into 10 ml nutrient broth contained in sterilized universal bottles and then incubated at 37 °C overnight to be used for the study.

2.5.3 Preparation of extract

A 4 mg dried portion of methanol extract was reconstituted in 4 ml of DMSO, this served as the stock solution (1 mg/ml). Then 2 ml of DMSO was transferred into three different test tubes each, 2-fold serial dilution was prepared by transferring 2 ml of sample from the stock to the first test tube labelled 0.5 mg/ml and the test tube was swirled; 2 ml from the first test tube was transferred to the second test tube labelled 0.25 mg/ml and the test tube was swirled; 2 ml from the second test tube was transferred to the third test tube labelled 0.125 mg/ml and the test tube was swirled, then 2 ml from the third test tube was discarded. The procedure was repeated for n- hexane, ethyl acetate, butanol and aqueous fraction.

2.5.4 Preparation of the culture media

The agar was prepared by suspending 33 g of the nutrient agar in 890 ml of distilled water and 19 g of Saboraud dextrose agar in 290 ml of distilled water. The suspensions were heated to dissolve completely. It was autoclaved at 121 °C for 15 minutes. On cooling, 20 ml of the agar was poured into the different sterile petri dishes and allowed to solidify. *P. aeruginosa*, *S. aureus* and *E. coli* were inoculated in the various nutrient agar petri dishes labeled respectively; *C. albicans* was inoculated in the Saboraud dextrose agar plates using the surface plate inoculation.

2.6 Preparation of standards

A pure drug sample of Ciprofloxacin solution was prepared by weighing 8 mg of the powder and dissolved in 1000 ml to get a concentration of 8 µg/ml. A pure drug sample of Miconazole was prepared by weighing 1 mg of the powder dissolved in 20 ml of 1 % Tween 80 solution. These were kept properly for further process.

2.7 Assay of the antimicrobial activity using Agar well diffusion method

The evaluation of the antimicrobial potentials of the extract and different fractions of the sample was carried out using agar well diffusion assay method as described by [11]. On solidification, five wells were bored on the eight agar plate labelled Methanol extract *P. aeruginosa, S. aureus*, *E. coli* and *C. albicans* (two petri dishes for each organism) using a cork borer of diameter 8 mm. Each well was well labelled according to the serial dilution (1 mg/ml, 0.5 mg/ml, 0.25 mg/ml and 0.125 mg/ml) and the last well was labelled control for each specific organism. 80 µL of the control and different serial dilutions were aseptically poured into the different petri dishes explicitly labeled for it. The petri dishes were covered and incubated for 24 hrs at 37 °C for bacteria and 33 °C for fungi. This procedure was repeated for n-hexane, ethyl acetate, butanol and aqueous fractions respectively.

The present research work does not contain any studies performed on animal / human subjects.

3 Results

The yield from the extraction and fractionation process carried out on the root of *N. latifolia* using various solvent is as presented in Table 1 below

Table 1 Yield from the extraction and fractionation of *N. latifolia* root in various solvents

Extract/ fractions	Weight of powder (g)	Yield (g)	% yield (%)
Methanol extract	500.00	25.70	5.14
N- Hexane fraction	20.70	1.43	6.91
Ethyl acetate fraction	20.70	6.98	33.72
Butanol fraction	20.70	4.65	22.46

3.1 Phytochemical analysis

Table 2 Phytochemical compounds in *N. latifolia* root

Test	Methanol	N-Hexane	Ethyl acetate	Butanol	Aqueous
Anthraquinone	-	+	-	+	+
Alkaloid	+	-	+	+	+
Flavonoid	+	+	+	+	+
Phenol	+	+	+	+	+
Tannins	+	-	-	+	+
Steroids	-	+	+	-	-
Terpenoids	-	+	+	-	-
Glycoside	+	-	-	+	+
Saponin	+	-	-	-	+

Keys: - = Absent, + = Present

3.2 Antimicrobial activity

Table 3 Antimicrobial activity of crude methanol extract of root *N. latifolia*

Concentration mg/ml	Test organisms/ Inhibition Zone Diameter (mm)			
	S. aureus	***E. coli***	***P. aeruginosa***	***C. albicans***
1	6 ± 0.00	4.5 ± 0.70	4 ± 0.00	6 ± 0.00
0.5	4 ± 0.00	3 ± 0.00	3 ± 0.00	5.5 ± 0.70
0.25	4 ± 0.00	0 ± 0.00	0 ± 0.00	5 ± 0.00
0.125	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Positive Ctrl	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

Table 4 Antimicrobial activity of n-hexane fraction of root *N. latifolia*

Concentration mg/ml	Test organisms/ Inhibition Zone Diameter (mm)			
	S. aureus	***E. coli***	***P. aeruginosa***	***C. albicans***
1	4.5 ± 0.70	3.5 ± 0.70	2.5 ± 0.70	4.0 ± 0.00
0.5	3.5 ± 0.70	2 ± 0.00	2 ± 0.00	2.5 ± 0.70
0.25	2 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

0.125	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Positive Ctrl	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

Table 5 Antimicrobial activity of ethyl acetate fraction of root *N. latifolia*

Concentration mg/ml	Test organisms/ Inhibition Zone Diameter (mm)			
	S. aureus	***E. coli***	***P. aeruginosa***	***C. albicans***
1	4.5 ± 0.70	0 ± 0.00	4 ± 0.00	0 ± 0.00
0.5	4 ± 0.00	0 ± 0.00	3.5 ± 0.70	0 ± 0.00
0.25	0 ± 0.00	0 ± 0.00	2.5 ± 0.70	0 ± 0.00
0.125	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Positive Ctrl	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

Table 6 Antimicrobial activity of butanol fraction of root *N. latifolia*

Concentration mg/ml	Test organisms/ Inhibition Zone Diameter (mm)			
	S. aureus	***E. coli***	***P. aeruginosa***	***C. albicans***
1	3 ± 1.40	2 ± 0.00	3.5 ± 0.70	4.5 ± 0.70
0.5	2 ± 0.00	0 ± 0.00	2 ± 0.00	4 ± 1.40
0.25	0 ± 0.00	0 ± 0.00	0 ± 0.00	3.5 ± 0.70
0.125	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Positive Ctrl	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

Table 7 Antimicrobial activity of aqueous fraction of root *N. latifolia*

Concentration mg/ml	Test organisms/ Inhibition Zone Diameter (mm)			
	S. aureus	***E. coli***	***P. aeruginosa***	***C. albicans***
1	3 ± 1.40	2 ± 0.00	2 ± 0.70	3.5 ± 0.70
0.5	1 ± 0.00	0 ± 0.00	1 ± 0.00	3 ± 1.40
0.25	0 ± 0.00	0 ± 0.00	0 ± 0.00	2.5 ± 0.70
0.125	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Positive Ctrl	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

Keys: S. aureus - Staphylococcus aureus; E. coli - Escherichia coli; P. aeruginosa - Pseudomonas aeruginosa; C. albicans - Candida albicans; Positive Control - Ciprofloxacin (8 µg/ml); Miconazole (50 µg/ml).

4 Discussion

From the results, Table 1 showed the yield and percentage yield of the crude methanol extract and its derived fractions. The yield ranged from 1.43 - 25.7 g, while the percentage yield ranged from 5.14 - 33.72 %.

Table 2 gave the phytochemical constituents present in crude methanol extract, n–hexane, ethyl acetate, butanol and aqueous fractions of the root of *N. latifolia*. Anthraquinone, steroids and terpenoids were absent while alkaloids,

flavonoids, phenol, tannins, glycosides and saponins were present in the crude methanol extract; anthraquinone, flavonoids, phenol, steroids and terpenoids were present whereas alkaloid, tannin, glycoside and saponin were absent in n-hexane fraction; anthraquinone, tannins, glycoside and saponin were absent while alkaloid, flavonoids, phenol, steroids and terpenoids were absent in ethyl acetate fraction; anthraquinone, alkaloid, flavonoids, phenol, tannins and glycoside were present whereas steroids, terpenoids and saponin were absent in butanol fraction; anthraquinone, alkaloid, flavonoids, phenol, tannins, glycoside and saponin were present while steroids and terpenoids were absent in aqueous fraction.

Table 3 showed the antimicrobial activity of the crude methanol extract of *N. latifolia* root. It was observed that the different concentrations (1, 0.5 and 0.25 mg/ml) showed Inhibition Zone Diameter (IZD) ranging from 3-6 mm. For the bacteria and fungus, it was observed that 0.125 mg/ml and the positive control had no IZD for both the fungus and the bacteria.

The result of the antimicrobial activity of n-hexane fraction of the root of *N. latifolia* yielded significant data (Table 4). Compared with the control, the concentrations (1, 0.5 mg/ml and 0.25 mg/m) showed antimicrobial activities on *S. aureus* while the control had no antimicrobial activity.

Table 5 showed antimicrobial activity of ethyl acetate fraction of *N. latifolia* root. The different concentrations showed no antimicrobial activity against *E. coli* and *C. albicans* while but showed antimicrobial activity against *P. aeruginosa*.

The antimicrobial activity of butanol fraction as shown in Table 6. The concentrations (1, 0.5 mg/ml and 0.25 mg/ml) showed IZD for both the bacteria and the fungus while the remaining concentrations and positive control had no IZD. All the tested microorganisms (*S. aureus*; *E. coli*, *P. aeruginosa* and *C. albicans*) were most sensitive (with the highest IZD) to the crude methanol extract.

5 Conclusion

It can be concluded that the methanol extract, n- hexane, ethyl acetate, butanol and aqueous fractions of N. latifolia root contain some phytochemicals which possess broad spectrum antimicrobial potentials and can be useful leads in the formulation of antimicrobial agents.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests.

Authors' Contributions

This research idea was conceived by Ezeagha Chigozie Celestina, and the experiments were performed under the close supervision of Ezeagha Chigozie Celestina, who also performed the interpretation and analysis of data. All authors reviewed and approved the final manuscript for publication.

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