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Evaluation of Phytochemical Contents and Antimicrobial Activities of *Pandiaka heudelotii*

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Abstract

The work investigated the secondary metabolites present in different parts of the *Pandiaka heudelotii* plant harvested from Ezira, Orumba south local government area of Anambra state, southeastern Nigeria as well as their antimicrobial activity. The objectives were to extract, determine the phytochemicals present, evaluate the antimicrobial potential and determine the zones of inhibition of the root, stem and leaf extracts of the plant. The emergence of antibiotics has decreased the spread and severity of a wide range of diseases. Plant extracts were tested for antibacterial and antifungal activities against *Staphylococcus aureus*, *Escherichia coli, Proteus vulgaris, Streptococcus pyogenes, Bacillus subtelis, Pseudomonas aerogenes, Salmonella typhi, Aspergillus flavus, Aspergillus niger* and *Candida albicans.* The zone of inhibition of extracts was compared with that of standard drugs like Erythromycin, Ciprofloxacin, Fulcin and Fluconazole. The result revealed the inhibition of bacterial and fungal growth with some test organisms. The microbial activity of the plant parts may be due to the presence of various secondary metabolites. The ethyl acetate extracts of *Pandiaka heudelotii* stood out with a minimum inhibitory concentration (MIC) range of 5 - 10 mg/mL and a minimum bactericidal concentration (MBC)/minimum fungicidal concentration (MFC) of 10 - 20 mg/ml against some test organisms. The plant can thus be worked upon to discover biologically active natural products that may serve as a prelude to the development of new pharmaceutical research undertakings.

Keywords

Pharmaceuticals, Secondary Metabolites, Antibiotics, *Pandiaka heudelotii*, Bacteria

1. Introduction

The discovery of antibiotics and their development for the treatment of infec-

tious diseases is the biggest success story in the history of chemotherapy [1]. However, the efficiency of many antibiotics is being threatened by the emergence of microbes that can resist the existing therapeutic agents due to their uncontrolled use. This was the push that led scientists across the globe to search for natural antimicrobial compounds available from plant sources [2]. It has been estimated from studies carried out on bacterial resistance that about 10 million people will die per year in 2050 with costs of about 100 trillion dollars. As a result, the World Health Organization (WHO) and some other groups around the world agreed on the urgency of developing an action plan that will address the issue globally, especially in the development of new drugs [3] [4].

Plants are the source of several valuable chemical substances referred to as secondary metabolites that bear pharmacognostic and pharmacological implications and have the potential to emerge as super drugs in the future [5]. Secondary metabolites are the various groups of natural products that are not essential for the vegetative growth of the producing organism but are considered differentiating compounds conferring adaptive roles such as functioning as defence compounds or signalling molecules in ecological interactions [6].

Among these plants is the *Pandiaka heudelotii* which is an annual herb belonging to the *Amaranthaceae* family and grows to about 1 m high from a lignified base, of savanna and often a weed of cultivated land of dry sandy areas, occurring from Senegal to Southern Nigeria and across central Africa to Sudan. The leaves are used by the Ezira people of southeastern Nigeria for the treatment of bulging fontanel in infants. The leaves of this plant are often used in southern Nigeria as vegetables and boiled for tea [7]. In Burkina Faso, *Pandiaka heudelotii* has been used as an analgesic, for annexite salpingitis, female genital inflammation and blepharitis. It is claimed by traditional healers that the addition of this plant would improve the efficacy of any drug of plant origin [8]. The current study is aimed at evaluating the phytochemical contents in *Pandiaka heudelotii* plant of which to the best of our knowledge the stem and root have not been studied.

2. Materials and Method

2.1. Chemicals and Reagents

Methanol (BDH UK), N-hexane (Noah USA), Dichloromethane (DCM) (JHD, USA) Ethylacetate (ETOAC) (Qualikems chemicals), Teteraoxosulphate (VI) acid (H_2SO_4), (Loba Chemie PVT LTD India), Ferric chloride. Concentrated hydrochloric acid (Loba Chemie PVT LTD India), Fehlings solution A and B, Olive oil (Andalucia Spain). All chemicals were of analytical grade.

2.2. Preparation of Plant Material

The harvested plant parts were washed under running water to remove sand and other particles. The leaves, stems and roots of the plant were separately dried at room temperature. This was to prevent the formation of fungus which might affect the purity of the sample as well as preserve the thermo labile active compo-

nents. The dried parts were pulverized using an electric-powered blender to obtain a fine powder of the leaves, stem, and root samples and stored in appropriately labelled plastic containers before their use.

2.3. Extraction Method

The dried powdered leaf (500 g) was loaded into a 4000 ml round bottom flask and was successively refluxed using 2500 ml of methanol for three (3) hours. The extract was filtered into a conical flask after extracting for 3 hours. This was repeated four (4) more times to fully extract from the sample. The filtrate was concentrated using a rotary evaporator. The concentrated extract was placed in a water bath to completely remove the solvent followed by a Liquid–liquid separation. The methanol extract was dissolved in 1.5 litres of water and transferred into a 5-litre separating funnel. Equal volumes of N-hexane were added to the aqueous methanol extract, stirred and allowed to stand for two hours after which the organic layer was recovered using a long-neck Pasteur pipette. This was repeated three times. The extract was concentrated in a rotary evaporator and weighed to a constant weight to yield the hexane extract and this procedure was repeated with DCM and ethylacetate. This procedure was repeated for the dried powdered stem (500 g) and root (500 g) samples. The percentage (%) extractive yield was recorded in Table 1.

2.4. Phytochemical Screening

Phytochemical screening was carried out on the extracts and the result is presented in Table 2.

2.4.1. Tests for Glycosides

General Test: A small quantity of each extract was boiled with 2 ml of 2.5 M tetraoxosulphate (VI) acid. This was cooled and neutralized with 20% potassium hydroxide and then boiled again with 5 ml of a mixture of equal volume of Fehling's solution A and B. Formation of a brick red precipitate shows the presence of glycosides [9].

2.4.2. Test for Alkaloids

Dragendorff's, reagent was used to carry out the test. This was done by adding 1 mL of Dragendorff's to 2 mL of the extract. The formation of an orange-red precipitate indicates the presence of alkaloids [9].

2.4.3. Test for Tannins

Put about 2 ml portions of the extract in a test tube gently heat it for 2 min and add 3 drops of Ferric chloride solution. A bluish-black color indicates the presence of Tannin [10].

2.4.4. Test for Flavonoids

Ferric Chloride test: A little portion of the extract was dissolved in ethanol and boiled with a few drops of 10% ferric chloride solution. A violet colouration in-

dicates the presence of a phenolic hydroxyl group [9].

2.4.5. Test for Saponins

Frothing Test: A 0.5 ml portion of the extracts was shaken with water in a test tube and then warmed in a water bath. Persistent frothing indicates the presence of saponin [11].

2.4.6. Test for Carbohydrates

A few drops of Molisch's reagent were added to a clear solution of the extract dissolved in water. This was followed by the addition of 1 ml of concentrated sulphuric acid down the side of the test tube. The mixture was allowed to stand for 2 minutes and then diluted with 5 ml of water. The appearance of red colour at the interphase of the two layers indicates the presence of carbohydrates [12].

2.5. Preliminary Antimicrobial Screening of the Crude Extracts

This was done as a preliminary evaluation of the antibacterial or antifungal activity of the crude extracts of the plant. The pathogens used include; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris, Bascillus subtilis*, *Candida albicans*, *Aspergillus flavus*, *Aspergillus nigre,* and Methicilin resistance *Staphylococcus aureus* (MRSA).

The antimicrobial activities were determined using the punched agar diffusion method [13]. The minimum inhibitory concentration of the extract against the microorganisms was carried out using glucose indicator broth. Punched agar diffusion method was used to determine the minimum inhibition concentration and minimum fungicidal concentrations of the extracts. Standard reference antibiotic Fluconazole, Fulcin, Ciprofloxacin and Erythromycin were used as controls for the tested bacteria. The plates were incubated at 37˚C for 24 hours. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition against the tested bacteria and the results are described in Tables 3 – 5.

3. Results

Table 1. Extractive yield for different solvents (%).

Solvents	Leaf %	Stem %	Root %
N-hexane	1.43	1.50	1.54
DCM	1.31	1.43	1.40
Ethyl acetate	1.52	1.50	1.58

Table 2. Result of preliminary phytochemical screening of *Pandiaka heudelotii* plant extracts.

Test	Leaf			Stem			Root		
	DCM	Hex	ETOAC	DCM	Hex	ETOAC	DCM	Hex	ETOAC
Tannins	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	−	−	−	−	−	−

Continued

Cardiac glycosides	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+
Flavonoids	−	+	+	−	+	+	−	+	+
Phenolic compounds	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+

KEY: + Present, − Absent.

Table 3. Minimum inhibitory concentration (mic) and minimum bactericidal/minimum fungicidal concentrations of leaf extracts of *Pandiaka heudelotii* on test organisms.

Test organisms	N-Hexane		DCM		Ethyl acetate	
	MICs	MBCs/MFCs	MICs	MBCs/MFCs	MICs	MBCs/MFCs
A	10	40	20	40	10	20
B	10	40	20	40	10	20
C	5	40	10	40	5	10
D	10	10	10	10	10	10
E						
F						
G	10	40	20	40	10	20
H	10	40	20	40	10	20
I						
J						
K						

Key: A = *S. aureus.* B = *Streptococus pyogenes*, C = *Bacillus subtilis,* D = *E. coli,* E = *Proteus vulgaris,* F = *Pseudomonas aeruginosa*, G = *Salmonella typhi,* H = *Candida albicans*, I = *Aspergillus flavus,* J = *Aspergillus nigre,* K = Methicilin resistant *S. aureus.*

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal/minimum fungicidal concentrations of root extracts of *Pandiaka heudelotii* on test organisms.

Test organisms	N-Hexane		DCM		Ethyl acetate	
	MICs	MBCs/MFCs	MICs	MBCs/MFCs	MICs	MBCs/MFCs
A	10	20	20	40	5	10
B						
C						
D	10	40	20	40	5	10
E	10	20	20	40	5	10
F						

Continued

G	10	40	10	20	5	20
H						
I	10	40			10	20
J						
K	10	20	10	20	5	10

Key: A = *S. aureus.* B = *Streptococus pyogenes*, C = *Bacillus subtilis,* D = *E. coli*, E = *Proteus vulgaris,* F = *Pseudomonas aeruginosa,* G = *Salmonella typhi,* H = *Candida albicans,* I = *Aspergillus flavus*, J = *Aspergillus nigre*, K = Methicilin resistant *S. aureus.*

Table 5. Minimum inhibitory concentration (mic) and minimum bactericidal/minimum fungicidal concentrations of stem extracts of *Pandiaka heudelotii* on test organisms.

Test organisms	N-Hexane		DCM		Ethyl acetate	
	MICs	MBCs/MFCs	MICs	MBCs/MFCs	MICs	MBCs/MFCs
A						
B	10	20	10	40	5	20
C	5	10	10	10	5	40
D						
E	10	20	10	40	5	10
F	10	20	10	40	5	10
G						
H	10	20	10	20	5	10
I						
J						
K	10	20	20	20	5	10

Key: A = *S. aureus.* B = *Streptococus pyogenes*, C = *Bacillus subtilis*, D = *E. coli,* E = *Proteus vulgaris*, F = *Pseudomonas aeruginosa,* G = *Salmonella typhi,* H = *Candida albicans,* I = *Aspergillus flavus*, J = *Aspergillus nigre,* K Methicilin resistant *S. aureus.*

4. Discussion

Table 1 showed that ethyl acetate extracted the largest amount of the substance from the root and leaf parts of the plant. This was followed by N-hexane and the dichloromethane.

The phytochemical screening of the crude extracts from the leaf, stem and root showed the presence of phytochemicals such as tannins, alkaloids, glycosides, saponins, phenolic compounds and carbohydrates (Table 2) some of which have also been identified in a study by Abubakar *et al.*, 2016 on the leaf of *Pandiaka heudelotii*. Tannins are known for their anti-inflammatory, anti-oxidant as well as anti-microbial properties [14].

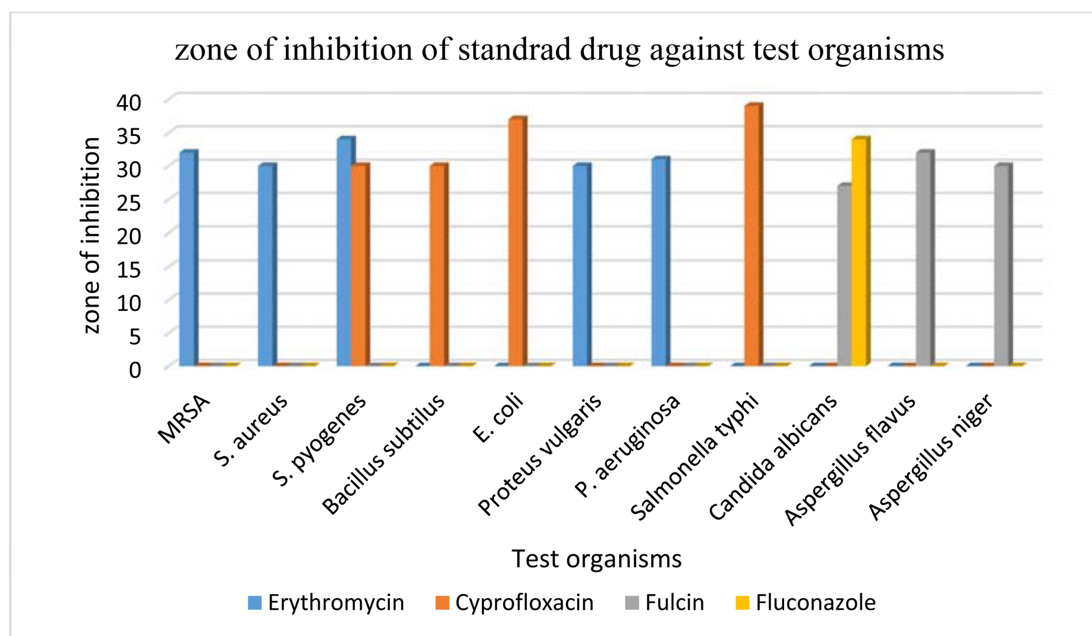

Figure 1. Zone of inhibition (mm) of the standard drug against test organisms (Drug concentration = 40 mg/ml).

The antimicrobial activity of the leaf portions of N-hexane, DCM and ethyl acetate extracts revealed that there was no antibacterial activity on *proteus vulgaris*, *Pseudomonas aerugenosa*, *Aspergillus nigre, Aspergillus flavus* and *MRSA*. However, activity was observed in the rest of the organisms. The MIC and MBC/MFC of the leaf extracts ranged between 5 - 20 mg/ml and 10 - 40 mg/ml respectively (Table 3). Table 4 shows the antimicrobial activity of the root portions of the three solvent extracts. The result revealed no activity on *Streptococcus pyogenes, Bacillus subtilis*, *Pseudomonas aerugenosa, C. albicans,* and *Aspergillus nigre*. However, *Aspergillus flavus* showed fungicidal activity at 40 and 20 mg/ml for DCM and ethyl acetate portions respectively. Table 5 gave the result of the antimicrobial activity for N-Hexane, DCM and ethyl acetate portions of the stem. The result revealed no activity for *Staphylococcus auerus, E. Coli, salmonella typhi, Aspergillus flavus* and *Aspergillus nigre* for all three extract portions of the stem.

The diameter zone of inhibition of standard drugs against test organisms is shown in Figure 1. The zone of inhibition ranged from 27 - 39 mm. Fulcin inhibited the growth of the three fungi strains at 27 mm, 30 mm, and 32 mm for *Candida albicans, Aspergillus nigre*, and *Aspergillus flavus* respectively while Fluconazole inhibited the growth of only *Candida albican* at 34 mm.

Some of the bacteria strains were inhibited by Erythromycin and Ciprofloxacin at a range of 30 - 39 mm. The extracts from the plant compare well with standard drugs as evidenced from **Tables 3-5**. In a previous study of the methanol and aqueous leaf extracts of *Pandiaka heudelotii*, MIC and MBC/MFC showed the inhibition of *S. Aureus* and *E. coli* [8]. This is also in line with the result of the current study using N-hexane, DCM and ethyl acetate.

5. Conclusion

Secondary metabolites are responsible for several actions of pharmacological importance. The ethyl acetate extract portions of the leaf, root and stem had more activity than the N-Hexane and DCM extracts. The MIC of the ethyl acetate portion ranged from 5 - 10 mg/ml and the MBC/MFC ranged from 5 - 40 mg/ml with the most activity observed with MRSA which was inhibited at 5 mg/ml in the root portion. The current study revealed that ethyl acetate is the best-extracting solvent compared to N – Hexane and DCM. Results from the study confirmed that the stem and roots of *Pandiaka heudelotii* are an effective alternative therapy against microbial agents. This is also in line with an earlier work on the leaf as reported by [8].

The possession of antimicrobial activity by *Pandiaka heudelotii* may aid the discovery of new chemical classes of antibiotic substances that could serve as selective agents for the control of certain health conditions.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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