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Antimicrobial Activity of the Crude Extracts of *Hamelia patens* on Some Selected Clinical Samples

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Authors' contributions

This work was carried out in collaboration between both authors. Author ELO conceptualized and designed the work. She also revised the manuscript critically for important intellectual content. Author JIE performed the experiment, data acquisition, interpretation of result and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the antimicrobial activity of the crude extracts of *Hamelia patens* on selected Clinical isolates and verify its use in Complementary and alternative medicine for the treatment of Microbial infections.
Study Design: This is an experimental study involving the extraction of crude substances from the leaves of *Hamelia patens* using Ethanol, Methanol, Petroleum ether and water; Preliminary Phytochemical screening, Susceptibility testing and determination of the Minimum Inhibitory/bactericidal Concentrations.
Place and Duration of Study: Study was carried out in the Department of Applied Microbiology and Brewing of Nnamdi Azikiwe University, Awka between January and June 2014.
Methodology: The leaves of the plant were pulverized and active principles extracted. Preliminary Phytochemical analysis was done using standard methods. The antimicrobial activities against *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Aspergillus niger and Candida albicans* were evaluated using agar diffusion and broth dilution techniques in accordance with standard methods.
Results: The result of the preliminary phytochemical analysis revealed the presence of Alkaloids, Tannins, Glycosides, Saponins, Steroids, Phlobatannins, Terpenoids, Flavonoids and Phytosterols.

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The extracts showed varying degrees of antimicrobial activity ranging from 7 mm to 44 mm against the isolates used. *Escherichia coli and Staphylococcus aureus* were the most susceptible bacterial isolates to all the extracts used while *Candida albicans* was more susceptible to the extracts than the *Aspergillus niger* among the fungal isolates. The Minimum Inhibitory Concentration (MIC) ranged from 12.5 mg/ml to 100 mg/ml among the test organisms, while the Minimum Bactericidal Concentration and Minimum Fungicidal Concentration (MBC/MFC) ranged from 25 mg/ml to >100 mg/ml. Ethanolic extract was the most effective antimicrobial agent when compared to the other three extracts.

Conclusion: *Hamelia patens* has shown potent antimicrobial activity *in vitro*, thus could possibly serve as a source of antimicrobial for the treatment of infections caused by the organisms used in the study.

Keywords: Antimicrobial activity; clinical isolates; ethanolic extract; Hamelia patens; phytochemical analysis.

1. INTRODUCTION

The use of substances with antimicrobial properties is known to have been a practice common for at least 2000 years ago [1]. Antimicrobials of plant origin have been shown to have enormous therapeutic potentials in Complementary and Alternative Medicine (CAM) [2,3]. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and Pharmaceutical drugs. Plant products still remain the principal source of Pharmaceutical agents used in traditional medicine [4,5]. Herbal medicines are in great demand in the developed as well as in developing countries for primary health care because of their wide biological and medicinal activities, higher safety margin and lower costs [6]. Plant based antimicrobials represent a vast untapped source of medicine especially in this age of drug resistance. Thus, herbal medicine would promise a greater viable solution for effective treatment of diseases.

In this study, the antimicrobial activity of the crude extracts of *Hamelia patens* on some clinical isolates was assayed. The plant *Hamelia patens*, commonly known as "*redhead*", "*Scarlet*" or "*firebush*" is a perennial bush shrub which grows in full sun and in shade. It grows about 6 feet tall. *Hamelia patens* has a tap and lateral root systems with abundant fine roots. The roots are red brown, Stem bark is grey and smooth and the inner bark is light green. It may have single or multiple stems and the twigs are orange to purple in colour [7]. Firebush is used in herbal medicine to treat athlete's foot, skin lesions and rash, insect bites, nervous shock, inflammation, rheumatism, headache, asthma and dysentery [8]. These claims led to the screening of this plant for antimicrobial activity.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Hamelia patens leaf and stem with flowers were collected from a local area in Ugwulangwu, Ohaozara L.G.A. of Ebonyi State, Nigeria in January, 2014. Identification of the plant was done at the Federal College of Agriculture, Ishiagu, Ebonyi State and voucher specimen of the plant deposited at the College laboratory. The collected materials were washed, Oven dried at 40°C and pulverised with sterile mortar and pestle.

2.2 Extraction of Plant Material

Approximately 30 g portions of the pulverized plant material were each extracted with 100 ml of ethanol, methanol and petroleum ether for 12 hours using soxhlex extractor.

The aqueous extract was obtained by macerating a 30 g portion of the pulverized plant material in distilled water at room temperature for 24 hours. The extracts were filtered with Whatman No 1 filter paper, concentrated to dryness in an Oven at 40°C and stored at 4°C.

2.3 Test Organisms

The test organisms used were clinical isolates obtained from the Microbiology Laboratory of University of Nigeria Teaching Hospital (U.N.T.H), Ituku Ozalla, Enugu State. The isolates include:- *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi*, *Aspergillus niger and Candida albicans.* The

organisms were subcultured to obtain viable strains used for the research work.

2.4 Determination of Antimicrobial Activity

Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) were used as nutritive media during the investigation. The Bacterial isolates were cultured on Nutrient Agar while the fungal isolates were cultured on Sabouraud Dextrose Agar; both by spread plate techniques using a sterile L – shape spreader. Duplicate plates were used for each of the extracts and the isolates were aseptically inoculated in each of the plates. Wells were made on the agar surface with 6mm cork borer. 0.1 ml of the extracts was poured into the well using sterile syringe. The plates were incubated at 37°C for 24 hours for bacterial activity and 48 hours for fungal activity. The plates were observed and the zone of inhibition measured in millimeters. The readings were taken from the duplicate plates and the average values were recorded.

To serve as a negative control, each of the solvents used for the extraction was used in a similar manner like the extracts to ascertain if any of them has antimicrobial effect on the isolates, a method adopted from Jagtap, et al. [9]. Standard antibiotic- Streptomycin and antifungal- Nystatin were used as positive controls.

2.5 Determination of Minimum Inhibitory Concentration (MIC) of the Plant Extracts

For the Minimum Inhibitory Concentration (MIC) tests, each of the plant extracts was concentrated by evaporation and about 1 g of each of the extracts was dissolved in 10 ml of the nutrient broth, to give a concentration of 100 mg/ml. Thereafter, two fold serial dilutions were made from the original stock (containing 100 mg/ml), according to the method of Egorov [10] using nutrient broth to obtain the concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.12 mg/ml and 1.56 mg/ml.

Then, a 0.1 ml of 0.5 McFarland standards of the test organisms were diluted to100 folds by suspending in a 99.9 ml of physiological saline. A 0.1 ml of the bacteria suspension was added to the tubes containing the different concentrations of the extract. The tubes were labeled appropriately and then incubated at 37°C for 24 hours and examined for activity. The lowest concentration of each of the extracts in each case that inhibited the growth of the test organisms by not showing visible turbidity was recorded as the Minimum Inhibitory Concentration (MIC).

2.6 Determination of Minimum Bactericidal and Fungicidal Concentration of the Extracts

Tubes showing no visible growth from the Minimum Inhibitory Concentration (MIC) test above were sub-cultured on to sterile nutrient agar plates for the bacteria isolates and Sabouraud Dextrose Agar plates for the Fungi isolates and then incubated at 37°C for 24 hours. The lowest concentration of the extracts yielding no growth was recorded as the Minimum Bactericidal Concentration.

2.7 Phytochemical Analysis of the Extracts

The four extracts used for this investigation were subjected to phytochemical analysis to determine their chemical components using standard methods of Harborne [11]. The extracts were evaluated for the presence of Alkaloids, Tannins, Glycosides, Saponins, Steroids, Flavonoids, Terpenoids, Phlobatannins, Phytosterols and Reducing sugar.

3. RESULTS AND DISCUSSION

The result of the phytochemical analysis of the extracts of *Hamelia patens* used in this study is shown in Table 1 below. Table 2 shows the result of the antimicrobial activity of the extracts. The ethanolic extract was active against all the organisms used giving a zone of inhibition that ranged from 20 mm- 44 mm. The methanolic extract and aqueous extract were also active against all the organisms apart from *Aspergillus niger*. The petroleum ether extract showed the least activity being only active against four organisms:- *Staphylococcus aureus, Escherichia coli, Aspergillus niger and Candida albicans.* All the positive controls (Streptomycin and Nystatin) showed activity against all the organisms used. Table 3 is the result of the Minimum Inhibitory Concentration (MIC) of the extracts. The MIC of the various extracts ranged from 12.5 mg/ml- 100 mg/ml. Minimum Bactericidal and Fungicidal Concentration (MBC/MFC) of the extracts are

shown on Table 4. The MBC/MFC of the extracts are from 25- >100.

The result of the preliminary phytochemical analysis revealed the presence of Alkaloids, Tannins, Glycosides, Saponins, Steroids, Phlobatannins and Terpenoids in the Methanolic, Ethanolic and aqueous extracts of *Hamelia patens*. Reducing sugar was absent in all the extracts tested. Flavonoids were present in the Ethanolic and Methanolic extracts, while Phytosterols were present in the Ethanolic and aqueous extracts. None of these chemical constituents was found in the Petroleum ether extract of the plant. From this study, Methanol, Ethanol and Water have shown to be better solvents for the extraction of phytochemicals from the above named plant rather than Petroleum ether. This observation also agrees with the work done by Suruchi et al. [12], who extracted similar phytochemicals from *Hamelia patens* using Methanol, Ethanol, Water and Acetone.

The extracts of *Hamelia patens* showed varying degrees of antimicrobial activity against the organisms used in this study. The antimicrobial activities recorded may be attributed to the various phytochemicals that were found present in the extracts analyzed. Previous studies have reported credible antimicrobial activities of Alkaloids, Tannins and Saponins [13]. Flavonoids were also reported to have anti- inflammatory and antioxidant activities [13]. Tannins found in the phytochemical analysis may be responsible for the antimicrobial effects. Akiyama et al. [14], in their study of the antibacterial action of tannins against S. *aureus*, attributed the antimicrobial mechanisms to their (I) astringent property (II) toxicity, and (III) complexation of metal ions.

Generally, the ethanolic extract proved more effective in comparison with the other extracts. Ethanolic extract had a wide range of antimicrobial activity against the test organisms with none of the test organisms being resistant to it. This is in line with the work of Cowan [15],

Table 1. Phytochemical analysis of the extracts of *Hamelia patens*

Chemical constituent	Ethanol extract	Methanol extract	Petroleum ether extract	Aqueous extract
Alkaloid	+	+	-	+
Tannins	+	+	-	+
Glycosides	+	+	-	+
Saponins	+	+	-	+
Steroids	+	+	-	+
Flavonoids	+	+	-	-
Terpenoids	+	+	-	+
Phloba tannins	+	+	-	+
Phytosterols	+	-	-	+
Reducing Sugar	-	-	-	-

Key: + = Present, – = Absent

Table 2. Antimicrobial activity of the extracts of *Hamelia patens*

Test organism	Diameter of zone of inhibition (mm)					
	Petroleum ether extract	Methanol extract	Ethanol extract	Aqueous extract	Streptomycin	Nystatin
Staphylococcus aureus	20	15	20	15	40	-
Escherichia coli	14	17	44	7	37	-
Proteus mirabilis	-	17	20	12	33	-
Pseudomonas aeruginosa	-	18	24	10	40	-
Salmonella typhi	-	14	26	13	38	-
Aspergillus niger	12	-	24	-	-	40
Candida albicans	13	9	20	10	-	35

Key: Streptomycin - Positive control for bacterial isolates
Nystatin - Positive control for fungal isolates

Table 3. Minimum inhibitory concentration (MIC) of the extracts of *Hamelia patens*

Test organism	Minimum Inhibitory concentration of extracts (Mg/ml)			
	Petroleum ether	Methanol	Ethanol	Aqueous
Staphylococcus aureus	50	100	25	50
Escherichia coli	50	50	12.5	100
Proteus mirabilis	R	50	25	100
Pseudomonas aeruginosa	R	50	25	100
Salmonella typhi	R	50	25	100
Aspergillus niger	100	R	50	R
Candida albicans	50	50	25	100

Key: R = Resistant

Table 4. Minimum bactericidal / fungicidal concentration (MBC/MFC) of the extracts of *Hamelia patens*

Test organism	Minimum MBC/MFC of the extracts of *Hamelia patens* (mg/ml)			
	Petroleum ether	Methanol	Ethanol	Aqueous
Staphylococcus aureus	100	>100	50	100
Escherichia coli	100	50	25	>100
Proteus mirabilis	R	100	25	>100
Pseudomonas aeruginosa	R	100	50	100
Salmonella typhi	R	50	25	>100
Aspergillus niger	>100	R	100	R
Candida albicans	100	100	50	>100

Key: R= Resistance

who recommended ethanol as one of the best solvents for extraction. The activity observed with the water extract may be associated with the common practice in traditional medicine to use the plant extracts prepared in the form of infusions and decoctions.

Escherichia coli and *Staphylococcus aureus* were the most susceptible to the various extracts used. *Escherichia coli* recorded the highest zone of inhibition of 44mm with the ethanolic extract which was even higher than the Streptomycin used as positive control. This is highly plausible since *E. coli* is known as a major cause of infantile diarrhoea in developing Countries and of traveler's diarrhoea in visitors to these Countries [16]. The antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* is noteworthy. *Staphylococcus aureus* is incriminated in most nosocomial infections and has been reported by many workers to have developed resistance to most antibiotics and *P. aeruginosa* is an opportunistic organism which has been reported to really receive resistance carrying plasmid from other bacteria species [17]. The extracts of *Hamelia patens* exhibited broad spectrum antibacterial activity since they inhibited the growth of both gram positive and gram negative bacteria. This confirms the work of Rosita & Michael [18], who outlined the various uses of *Hamelia patens* in the treatment of many bacterial diseases.

In line with this, it is evident therefore that the extracts of *Hamelia patens* can find application in the production of antibacterial agents for the treatment of diseases especially those caused by the bacterial species used in this work.

The extracts also showed antifungal activity against the two fungi used: - *Candida albicans* and *Aspergillus niger*. This finding correlates with the work of Khandelwal et al. [19] who reported the antifungal activity of *Hamelia patens* against *Aspergillus* species. Abubacker et al. [20] also reported antifungal activity of *Hamelia patens* against *Candida* and *Aspergillus* species amongst others. The results of the MIC, MBC and MFC on the isolates confirmed the antimicrobial potency of the plant extracts as previously observed by the agar diffusion assay.

4. CONCLUSION

The outcome of this work has demonstrated the effectiveness of *Hamelia patens* against some pathogenic micro-organisms. It has

also showed to a large extent that *Hamelia patens* has potentials to be used in the treatment of many infectious diseases especially in these days of microbial resistance to conventional drugs. *Hamelia patens* showed considerable broad spectrum antimicrobial activity and could serve as a potent source of antimicrobial agents. The study has reiterated the need to explore the use of medicinal plants to curb the growing increase of drug resistant micro-organisms.

It is therefore recommended that more work be conducted to help optimally extract all the bioactive compounds in the plant and formulate them into doses for the treatment of infectious diseases.

CONSENT

It was not sought for because it is not needed.

ETHICAL APPROVAL

It was not sought for because it is not needed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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