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Antimicrobial and Phytochemical Analyses of Extracts of *Diplazium sammatii* and *Pneumatopteris afra* on Selected Clinical Strains of Bacteria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OAO and AOA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OOO and FED managed the analyses of the study. Authors OAO and AOA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was carried out to test for the antibacterial effects of *Diplazium sammatii* and *Pneumatopteris afra* plant leaves extracts on some pathogenic bacteria isolates.
Study Design: This study was carried out in triplicates and the results presented are mean values of the recordings.
Place and Duration of Study: This study was carried out in the Microbiology Laboratory of Ekiti State University between January and June, 2011.
Methodology: The plants were collected and air dried at room temperature. The phytochemical constituents were extracted using ethanol, methanol, acetone and cold redistilled water. The agar well diffusion method was used to determine the antimicrobial activity of the plant extracts against *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella species*, *Escherichia coli* and *Shigella dysenteriae*. Minimum inhibitory concentration (MIC) of the extracts against the test bacteria was also determined.

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Results: The acetone extracts gave the highest zones of inhibition (19.0 mm) of the test bacteria at concentrations ranging from 50 mg/ml (9.0 mm) to 250 mg/ml (19.0 mm), while aqueous extracts gave the least zone of inhibition 2.0mm at the same range of concentrations. The MIC was also observed for both plants at 50.0 mg/ml. Phytochemical screening of the plants revealed the presence of tannins, saponins, flavonoids, cardiacglycosides, anthraquinones and alkaloids.
Conclusion: The growth of all bacteria were inhibited at varying degrees thus justifying their use in traditional medicines in treating bacterial infections and other diseases.

Keywords: Antimicrobials; MIC; plant extracts; pathogens; phytochemicals; bioactive.

1. INTRODUCTION

In an effort to improve the quality of life, man has always looked up to plants as sources of food, medicine, shelter and for relief from the hardships of life [1,2]. Since ancient times, varieties of drugs have been obtained from medicinal plants with the search for potent antimicrobial agents shifting to plants [3]. Over 2000 plant species have been found to have medicinal value and these properties have been exploited over the years [4]. Some plants are referred to as medicinal plants because they contain certain bioactive substances, that could be used for therapeutic purposes or which could serve as precursors for the synthesis of useful drugs [5]. The medicinal value of these plants lies in the active phytochemical constituents that produce definite physiological reactions relentless to the cure of diseases of man. The use of medicinal plants in the treatment of human diseases is as old as the disease itself as it predates the introduction of antibiotics [6]. However, the use of antibiotics to treat infections have posed a serious threat to humans and the environment because of the increasing dissemination of antibiotics resistance genes and the acquisition of antibiotics resistance by commensals hence the need for an alternative [7]. So, resistance to drugs especially antibiotics has become a major challenge facing the medical world today coupled with the high cost of production of this drugs and this has brought a renewed interest in plant medicinal drugs [8]. This has necessitated the search for newer drugs which is better and cheaper with plants being the better alternative. The selection of crude plant extracts for screening for antimicrobial effects has the potential of being more successful in the initial stages than the screening of pure compounds isolated from the natural products [9]. Researchers have reported that plant extracts of many higher plants did exhibit antibacterial, antifungal and insecticidal properties during laboratory trials with an observed proliferation of herbal drugs in Nigeria. But very few literatures exists on the antimicrobial properties of lower green plants. *Diplazium sammatii* (*Athyriaceae*) a fern plant belongs to family Athyriaceae in the eupolypods II clade of the order Polypodiales [10] in the class polypodiopsida [11] is known to grow on the banks of streams in the tropics and is increasingly getting endangered due to pollution by industrial effluents. Also *Pneumatopteris afra* (Christ) Holttum is equally a tropical plant.

This study was aimed at determining the antimicrobial activities, and phytochemical constituents of extracts of *Diplazium sammatii* and *Pneumatopteris afra* on some pathogenic bacteria

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh leaves of *Pneumatopteris afra* and *Diplazium sammatii* were collected from the University farm and Ikogosi warm spring, Ekiti State, Nigeria. The plants were identified and authenticated at the herbarium section of Plant Science and Biotechnology Department, Ekiti State University, Ado-Ekiti, Nigeria. The leaves were air-dried at room temperature for twenty-eight days. The dried leaves were then milled into a fine powder using an electronic blender. Plants were stored in an air-tight container at room temperature until required for further use.

2.2 Extraction Procedure

2.2.1 Aqueous extraction

Twenty-five grams each of powdered leaves of *Diplazium sammatii* and *Pneumatopteris afra* were separately weighed into a clean sterile Erlenmeyer flask and 100 ml of distilled water was added into the Erlenmeyer flask. The mixture was allowed to stand for a period of 120 hours. The extract was collected by filtration using Whatmann No1 filter paper.

2.2.2 Solvent extraction

Twenty-five grams each of dried powdered leaves of *Diplazium* s*ammatii* and *Pneumatopteris afra* were each soaked in 100 ml of 95% ethanol, methanol and acetone in 250 ml Erlenmeyer flasks for a period of 120 hours. The extracts were then obtained by filtration using filter paper (Whatmann No 1 filter paper) into small sterile crucibles. Extracts were evaporated to dryness by the use of rotary evaporator and reconstituted with 50% Dimethylsulphoxide (DMSO, Merck). The stock extracts were kept in the refrigerator at 4ºC until used.

2.3 Determination of Antimicrobial Activities

2.3.1 Source of microorganisms

Six bacteria strains were used for this study, namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp, *Salmonella typhi* and *Shigella dysenteriae*. All the bacteria strains were obtained from the stock culture of the Department of Microbiology, Ekiti State University, Ado-Ekiti. The bacteria strains were hitherto authenticated by carrying out biochemical tests and comparing to Bergey's manual. The bacteria isolates was maintained on agar slant at 4ºC.

2.3.2 Standardization of innocula

The test bacteria were grown at 37°C in Mueller-Hilton broth (Oxoid) (McFarland standard) at optical activity of 625 nm with Mueller-Hilton Broth and stored at 4°C to prevent further bacteria growth [12].

2.3.3 Determination of antimicrobial activity

Antibacterial activity was measured using agar well diffusion technique [3], whereby the test bacteria were inoculated into the sterile Mueller-Hinton agar plates by aseptically transferring 0.1ml of each of the standardized test bacteria into Petri dishes containing solidified Mueller-Hinton agar. A sterile glass spreader was used to evenly spread this over the surface of the Mueller-Hinton agar. A sterile cork borer 6mm in diameter was used to bore wells on the Petri dishes and 0.1 ml of each extract was then transferred into the holes. About 0.1ml of DMSO was introduced as a control into a well on each plate. The plates were allowed to dry for one hour for diffusion. The plates were then incubated at 37°C for 24hours in an inverted position. The experiment was carried out in triplicates and the mean values were recorded.

2.3.4 Determination of minimum inhibitory concentration (MIC) using agar dilution method

The leaves extracts were aseptically introduced into sterile Petri dishes at different concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml and 250 mg/ml) with the aid of a micropipette at 100 µl volume [13]. Fifteen milliliters of sterile Mueller-Hinton agar was added to each of the sterile Petri dishes containing the extracts and was carefully swirled. The agar was then allowed to set. Standardized test bacteria were carefully streaked with the aid of a sterile inoculating loop on the nutrient agar and incubated at 37°C. The plates were observed for growth and the MIC was determined as the lowest concentration that inhibited the growth of the test organisms.

2.4 Phytochemical Analysis of the Extracts

The qualitative phytochemical analysis was carried out to determine the presence of alkaloids, tannins, saponins, steroids, terpenoids, flavonoids, anthraquinones, cardiacglycosides and cyanoglycosides [5,14].

3. RESULTS AND DISCUSSION

The extraction process yielded an average of 6.5g for the polar extracts respectively and 8g for the aqueous extracts. The result of the antimicrobial activities of the leaf extracts are given in Tables 1 and 2 by measuring the diameter of the zones of inhibition compared to standards. The result of the antimicrobial activities of extracts of *Diplazium sammatii* are shown in Table 1, acetone extracts gave the highest zones of inhibition at 250 mg/ml (19.0 mm) on *Staphylococcus aureus*, *Salmonellat typhi* and *Shigella dysenteriae* with the lowest zone of inhibition occurring at 50 mg/ml (9.0 mm) on *E. coli*. Methanol extracts of *Diplazium sammatii* also showed considerable level of antibacterial activity with zones of inhibition ranging from 250 mg/ml (19.0 mm, 18.0 mm, and 16.0 mm) on *Pseudomonas aeruginosa*, and *Escherichia coli* while it had its lowest zone of inhibition at 50 mg/ml (5.0mm) on *Salmonella typhi*. This result obtained is quite higher when compared to results obtained by [6] as the entire results obtained were susceptible to *Diplazium sammatii* to a varying degree.

Table 1. Antimicrobial activities of extracts of *Diplazium sammatii* zones of inhibition of the extracts in (mm)

	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml				250 mg/ml			
Organisms	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**
Staphylococcus aureus	8.0	10.0	10.0	4.0	9.0	14.0	13.0	6.0	11.0	16.0	15.0	6.0	12.0	18.0	15.0	6.0	14.0	19.0	17.0	5.0
Salmonella typhi	5.0	11.0	10.0	4.0	8.0	13.0	11.0	5.0	10.0	16.0	8.0	6.0	14.0	18.0	14.0	6.0	15.0	19.0	15.0	6.0
Pseudomonas aeruginosa	6.0	11.0	12.0	4.0	9.0	13.0	13.0	5.0	10.0	15.0	15.0	6.0	14.0	16.0	18.0	6.0	16.0	17.0	18.0	6.0
Klebsiella spp	6.0	11.0	8.0	0.0	7.0	14.0	10.0	4.0	9.0	14.0	12.0	6.0	10.0	16.0	14.0	6.0	12.0	18.0	15.0	7.0
Escherichia coli	6.0	9.0	13.0	2.0	9.0	12.0	15.0	4.0	10.0	15.0	16.0	6.0	14.0	16.0	17.0	6.0	16.0	17.0	19.0	7.0
Shigella dysenteriae	8.0	13.0	5.0	4.0	10.0	14.0	8.0	5.0	10.0	16.0	10.0	6.0	14.0	17.0	12.0	6.0	15.0	19.0	14.0	8.0

Keys: ET – Ethanol AC – Acetone ME – Methanol AQ – Aqueous

Table 2. Antimicrobial activities of extracts of *Pneumatopteris afra* zones of inhibition of the extracts in (mm)

	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml				250 mg/ml			
Organisms	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**
Staphylococcus aureus	4.0	4.0	4.0	0.0	5.0	5.0	5.0	3.0	5.0	6.0	6.0	4.0	6.0	8.0	9.0	6.0	8.0	12.0	11.0	7.0
Salmonella typhi	10.0	13.0	10.0	2.0	12.0	15.0	12.0	4.0	13.0	18.0	12.0	7.0	15.0	19.0	14.0	8.0	16.0	19.0	16.0	8.0
Pseudomonas aeruginosa	11.0	4.0	4.0	2.0	12.0	8.0	7.0	4.0	13.0	6.0	8.0	6.0	14.0	8.0	9.0	9.0	15.0	10.0	10.0	9.0
Klebsiella spp	5.0	4.0	5.0	2.0	5.0	6.0	7.0	3.0	7.0	10.0	10.0	5.0	8.0	9.0	11.0	7.0	10.0	10.0	13.0	8.0
Escherichia coli	5.0	5.0	7.0	0.0	8.0	5.0	9.0	0.0	11.0	10.0	10.0	0.0	11.0	7.0	10.0	0.0	13.0	9.0	11.0	0.0
Shigella dysenteriae	5.0	5.0	5.0	2.0	8.0	6.0	6.0	5.0	11.0	6.0	6.0	7.0	12.0	11.0	8.0	9.0	14.0	14.0	10.0	7.0

Keys: ET – Ethanol AC – Acetone ME – Methanol AQ – Aqueous

Table 3. Minimum inhibitory concentration (MIC) of extracts of *Diplazium sammatii*

	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
Organisms	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**
Staphylococcus aureus	-	+	-	+	-	+	-	+	-	-	-	+	-	-	-	-
Salmonella typhi	-	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	+	+	+	+	-	+	-	+	-	-	-	+	-	-	-	-
Klebsiella spp	+	+	+	+	+	-	+	+	-	-	-	+	-	-	-	-
Escherichia coli	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-
Shigella dysenteriae	+	+	+	+	-	+	-	+	-	-	-	+	-	-	-	-

Keys: ET – Ethanol AC – Acetone ME – Methanol AQ – Aqueous
+: Growth of organisms
-: No growth of organisms

Table 4. Minimum inhibitory concentration (MIC) of extracts of *Pneumatopteris afra*

	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
Organisms	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**	**ET**	**AC**	**ME**	**AQ**
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+
Salmonella typhi	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-
Pseudomonas aeruginosa	-	+	+	+	-	+	+	+	-	-	-	+	-	-	-	-
Klebsiella spp	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	+
Escherichia coli	+	+	+	+	-	+	-	+	-	-	-	+	-	-	-	+
Shigella dysenteriae	+	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-

Keys: ET – Ethanol AC – Acetone ME – Methanol AQ – Aqueous
+: Growth of organisms
-: No growth of organisms

Table 2 shows the result of the antimicrobial activities of *Pneumatopteris afra*. Acetone extracts of *P. afra* showed the highest antimicrobial activity at 250 mg/ml and 200 mg/ml (19.0 mm) on *S. typhi* with the lowest level at 50 mg/ml (4.0 mm). *S. typhi* showed reasonable susceptibility to the extracts at 250 mg/ml. the aqueous extracts also showed appreciable levels of antimicrobial activity on the tested bacteria except for *E. coli* which showed no susceptibility to the aqueous extracts which agrees with the works of [15,16].

The Minimum inhibitory concentration (MIC) was observed at 50 mg/ml for ethanol extract of *Diplazium sammatii* against *Staphylococcus aureus* and *Salmonella typhi* while *Pneumatopteris afra* had an MIC at 50 mg/ml for *Pseudomonas aeruginosa* and *Salmonella typhi*. When compared with the works of other authors [3,4,17, and 18] the solvents used were found to be relatively effective in extracting the polar and non-polar constituents of the plants.

Results obtained from the antimicrobial effects of *Diplazium sammatii* and *Pneumatopteris afra* against the bacteria isolates was broad spectrum in activity [16,19], though with variations in the degree of sensitivity as observed in the Tables presented. The control used in this study showed no inhibitory effect on the microorganisms. From the results obtained acetone, ethanol, methanol and aqueous extracts of both plants inhibited the growth of the test bacteria. Also aqueous extracts of *Diplazium sammatii* and *Pneumatopteris afra* did not have any inhibitory effect on *Klebsiella* spp and *Staphylococcus aureus*.

The antimicrobial properties of many medicinal plants have been previously studied [3,18,20 and 21]. The acetone extracts of *Diplazium sammatii* and *Pneumatopteris afra* was more effective followed by the methanol extract which correlates with the works of [3] who reported the antimicrobial activities of methanol extracts of lower green plants against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella* spp. The extracts of *Diplazium sammatii* and *Pneumatopteris afra* had higher inhibitory effects when compared with the works of [22].

The effect of the extracts on all the test organisms increased with the increase in the concentration of the extracts which is in agreement to the works of other authors. Aqueous extracts of the leaves showed considerable inhibition which also correlates with the work of [4 and 18] who reported higher zones of inhibition for aqueous extracts on both Gram positive and Gram negative bacteria.

The phytochemical analysis of the plants showed the presence of certain bioactive (tannins, saponins, alkaloids, flavonoids, cardiacglycosides and anthraquinones except for steroids which was absent in both plants) compounds which have been reported to exhibit various medicinal and physiological activity [23].

Differences observed in the antimicrobial activities of the plants could be due to the quantitative and qualitative differences in them [17,24], the extraction methods employed and the level of concentration of such extracts [25]. Alkaloids have also been reported to have antimicrobial potentials [26] as well as antibacterial activities [27]. Flavonoids complex with extra cellular and soluble proteins and with bacterial cell walls [28]. Tannins interfere with protein synthesis by binding to proline rich proteins [29]. Plant glycosides, which are not normally toxic when ingested orally, are known to inhibit chloride transport in the stomach [21,30].

Table 5. Phytochemical analysis of the plants

Tests	*Diplazium sammatii*	*Pneumatopteris afra*
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Steroids	-	-
Cardiacglycosides	+	+
Cyanoglycosides	+	+
Anthraquinones	+	+
Terpenoids	+	+
Flavonoids	+	+

Keys:+:Present
-:Absent

4. CONCLUSION

This study shows that lower green plants show much promise in the development of phytomedicines with great antimicrobial properties as observed presently in the traditional context. It can be concluded that this plants showed much antimicrobial potential against the selected test microorganisms and has greater potential in the development of phytomedicines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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