

Original Research Article**ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSES OF EXTRACTS OF
Diplazium sammatii and *Pneumatopteris afra* on SELECTED CLINICAL STRAINS OF
BACTERIA****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 distilled 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.*

Results: *The acetone extracts gave the highest zones of inhibition (19.0mm) of the test bacteria at concentrations ranging from 50mg/ml (9.0mm) to 250mg/ml (19.0mm), 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 50.0mg/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 bacteria infectious and other diseases.*

Keywords: Antimicrobials, MIC, Zones of inhibitions, Bacteria, Phytochemicals.

30 1. Introduction

31 In an effort to improve the quality of life, man has always looked up to plants as sources of food, medicine, shelter
32 and for relief from the hardships of life [1]. Since ancient times, varieties of drugs have been obtained from
33 medicinal plants with the search for potent antimicrobial agents shifting to plants [2]. Over 2000 plant species have
34 been found to have medicinal value and these properties have been exploited over the years [3]. Some plants are
35 referred to as medicinal plants because they contain certain bioactive substances, that could be used for therapeutic
36 purposes or which could serve as precursors for the synthesis of useful drugs [4]. The medicinal value of these plants
37 lies in the active phytochemical constituents that produce definite physiological reactions relentless to the cure of
38 diseases of man. The use of medicinal plants in the treatment of human diseases is as old as the disease itself as it
39 predates the introduction of antibiotics [5]. However, the use of antibiotics to treat infections have posed a serious
40 threat to humans and the environment because of the increasing dissemination of antibiotics resistance genes and the
41 acquisition of antibiotics resistance by commensals hence the need for an alternative [6]. So, resistance to drugs
42 especially antibiotics has become a major challenge facing the medical world today coupled with the high cost of
43 production of this drugs and this has brought a renewed interest in plant medicinal drugs [7]. This has necessitated
44 the search for newer drugs which is better and cheaper with plants being the better alternative. The selection of crude
45 plant extracts for screening for antimicrobial effects has the potential of being more successful in the initial stages
46 than the screening of pure compounds isolated from the natural products [8]. Researchers have reported that plant
47 extracts of many higher plants did exhibit antibacterial, antifungal and insecticidal properties during laboratory trials
48 with an observed proliferation of herbal drugs in Nigeria. But very few literature exist on the antimicrobial properties
49 of lower green plants.

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

52 2.0 Materials and Methods

53 2.1 Collection of Plant Materials

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

59 **2.2 Extraction Procedure**

60 **2.2.1 Aqueous Extraction:** Twenty five grammes each of powdered leaves of *Diplazium sammatii* and *Pneumatopteris afra*
61 were separately weighed into a clean sterile Erlenmeyer flask and 100ml of distilled water was added into the Erlenmeyer
62 flask. The mixture was allowed to stand for a period of 120hours. The extract was collected by filter paper.

63 **2.2.2 Solvent extraction:** Twenty five grammes each of dried powdered leaves of *Diplazium sammatii* and *Pneumatopteris*
64 *afra* were each soaked in 100ml of 95% ethanol, methanol and acetone in 250ml Erlenmeyer flasks for a period of
65 120hours. The extracts were then obtained by filtration using filter paper (Whatmann No1 filter paper) into small sterile
66 crucibles. Extracts were evaporated to dryness by the use of rotary evaporator and reconstituted with 50%
67 Dimethylsulphoxide (DMSO, Merck). The stock extracts were kept in the refrigerator at 4°C until used.

68 **2.3 Determination of Antimicrobial Activities**

69 **2.3.1 Source of Microorganisms**

70 Six bacteria strains were used for this study, namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas*
71 *aeruginosa*, *Klebsiella* spp, *Salmonella typhi* and *Shigella dysenteriae*. All the bacteria strains were obtained from the stock
72 culture of the Department of Microbiology, Ekiti State University, Ado-Ekiti. The bacteria isolates was maintained on agar
73 slant at 4 °C.

74 **2.3.2 Standardization of Inocula**

75 The test bacteria were grown at 37°C in Mueller-Hilton broth (Oxoid) (McFarland standard) at optical activity of
76 625nm with Mueller-Hilton Broth and stored at 4°C to prevent further bacteria growth [9].

77 2.3.3 Determination of Antimicrobial Activity

78 Antibacterial activity was measured using agar well diffusion technique [2], whereby the test bacteria were
79 inoculated into the sterile Mueller-Hinton agar plates by aseptically transferring 0.1ml of each of the standardized test
80 bacteria into petri dishes containing solidified Mueller-Hinton agar. A sterile glass spreader was used to evenly spread this
81 over the surface of the Mueller-Hinton agar. A sterile cork borer 6mm in diameter was used to bore wells on the Petri dishes
82 and 0.1ml of each extract was then transferred into the holes. About 0.1ml of DMSO was introduced as control into a well
83 on each plate. The plates were allowed to dry for one hour for diffusion. The plates were then incubated at 37°C for 24hours
84 in an inverted position. The experiment was carried out in triplicates and the mean values were recorded.

85 2.3.4 Determination of Minimum Inhibitory Concentration (MIC) Using Agar Dilution Method

86 The leaves extracts were aseptically introduced into sterile Petri dishes at different concentrations (50mg/ml,
87 100mg/ml, 150mg/ml and 200mg/ml) with the aid of a micropipette at 100µl volume. Fifteen milliliters of sterile Mueller-
88 Hinton agar was added to each of the sterile Petri dishes containing the extracts and was carefully swirled. The agar was
89 then allowed to set. Standardized test bacteria were carefully streaked with the aid of a sterile inoculating loop on the
90 nutrient agar and incubated at 37°C. The plates were observed for growth and the MIC was determined as the lowest
91 concentration that inhibited the growth of the test organisms.

92 2.4 Phytochemical Analysis of the Extracts

93 Qualitative phytochemical analysis was carried out to determine the presence of alkaloids, tannins, saponins,
94 steroids, terpenoids, flavonoids, anthraquinones, cardiacglycosides and cyanoglycosides [4, 26].

95 2.5 RESULTS AND DISCUSSION

96 The result of the antimicrobial activities of the leaf extracts are given in Tables I and II by measuring the diameter of
97 the zones of inhibition compared to standards. The result of the antimicrobial activities of extracts of *Diplazium sammatii*
98 are shown in table I, acetone extracts gave the highest zones of inhibition at 250mg/ml (19.0mm) on *Staphylococcus aureus*,
99 *Salmonella typhi* and *Shigella dysenteriae* with the lowest zone of inhibition occurring at 50mg/ml (9.0mm) on *E. coli*.
100 Methanol extracts of *Diplazium sammatii* also showed considerable level of antibacterial activity with zones of inhibition

101 ranging from 250mg/ml (19.0mm, 18.0mm, and 16.0mm) on *Pseudomonas aeruginosa*, and *Escherichia coli* while it had its
102 lowest zone of inhibition at 50mg/ml (5.0mm) on *Salmonella typhi*. This result obtained is quite higher when compared to
103 results obtained by [5] as the entire results obtained were susceptible to *Diplazium sammatii* to a varying degree.

104 Table II shows the result of the antimicrobial activities of *Pneumatopteris afra*. Acetone extracts of *P. afra* showed
105 the highest antimicrobial activity at 250mg/ml and 200mg/ml (19.0mm) on *S. typhi* with the lowest level at 50mg/ml
106 (4.0mm). *S. typhi* showed reasonable susceptibility to the extracts at 250mg/ml. the aqueous extracts also showed
107 appreciable levels of antimicrobial activity on the tested bacteria except for *E. coli* which showed no susceptibility to the
108 aqueous extracts which agrees with the works of [10, 11].

109 The Minimum inhibitory concentration (MIC) was observed at 50mg/ml for ethanol extract of *Diplazium sammatii*
110 against *Staphylococcus aureus* and *Salmonella typhi* while *Pneumatopteris afra* had an MIC at 50mg/ml for *Pseudomonas*
111 *aeruginosa* and *Salmonella typhi*. When compared with the works of other authors [12, 2, 13, and 3] the solvents used were
112 found to be relatively effective in extracting the polar and non-polar constituents of the plants.

113 Results obtained from the antimicrobial effects of *Diplazium sammatii* and *Pneumatopteris afra* against the bacteria
114 isolates was broad spectrum in activity [14, 11], though with variations in the degree of sensitivity as observed in the Tables
115 presented. The control used in this study showed no inhibitory effect on the microorganisms. From the results obtained
116 acetone, ethanol, methanol and aqueous extracts of both plants inhibited the growth of the test bacteria. Also aqueous
117 extracts of *Diplazium sammatii* and *Pneumatopteris afra* did not have any inhibitory effect on *Klebsiella* spp and
118 *Staphylococcus aureus*.

119 The antimicrobial properties of many medicinal plants have been previously studied [15, 2, 13 and 16]. The acetone
120 extracts of *Diplazium sammatii* and *Pneumatopteris afra* was more effective followed by the methanol extract which
121 correlates with the works of [2] who reported the antimicrobial activities of methanol extracts of lower green plants against
122 *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella* spp. The extracts of *Diplazium sammatii* and
123 *Pneumatopteris afra* had higher inhibitory effects when compared with the works of [17].

124 The effect of the extracts on all the test organisms increased with the increase in concentration of the extracts which
125 is in agreement to the works of other authors. Aqueous extracts of the leaves showed considerable inhibition which also

126 correlates with the work of [13 and 3] who reported higher zones of inhibition for aqueous extracts on both Gram positive
127 and Gram negative bacteria.

128 The phytochemical analysis of the plants showed the presence of certain bioactive (tannins, saponins, alkaloids,
129 flavonoids, cardiacglycosides and anthraquinones except for steroids which was absent in both plants) compounds which
130 have been reported to exhibit various medicinal and physiological activity [18].

131 Differences observed in the antimicrobial activities of the plants could be due to the quantitative and qualitative
132 differences in them [19, 13], the extraction methods employed and the level of concentration of such extracts [20].
133 Alkaloids have also been reported to have antimicrobial potentials [21] as well as antibacterial activities [22]. Flavonoids
134 complex with extra cellular and soluble proteins and with bacterial cell walls [23]. Tannins interfere with protein synthesis
135 by binding to proline rich proteins [24]. Plant glycosides, which are not normally toxic when ingested orally, are known to
136 inhibit chloride transport in the stomach [25, 16].

137 **Conclusion**

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

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148 **Table I: Antimicrobial activities of extracts of *Diplazium sammatii***

149 **Zones of inhibition of the extracts in (mm)**

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	50mg/ml				100mg/ml				150mg/ml				200mg/ml				250mg/ml			
Organisms	ET	AC	ME	AQ	ET	AC	ME	AQ	ET	AC	ME	AQ	ET	AC	ME	AQ	ET	AC	ME	AQ
<i>Staphylococcus aureus</i>	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
<i>Salmonella typhi</i>	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
<i>Pseudomonas aeruginosa</i>	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
<i>Klebsiella spp</i>	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
<i>Escherichia coli</i>	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
<i>Shigella dysenteriae</i>	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

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

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161 **Table II: Antimicrobial activities of extracts of *Pneumatopteris afra***162 **Zones of inhibition of the extracts in (mm)**

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Organisms	50mg/ml				100mg/ml				150mg/ml				200mg/ml				250mg/ml			
	ET	AC	ME	AQ	ET	AC	ME	AQ	ET	AC	ME	AQ	ET	AC	ME	AQ	ET	AC	ME	AQ
<i>Staphylococcus aureus</i>	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
<i>Salmonella typhi</i>	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
<i>Pseudomonas aeruginosa</i>	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
<i>Klebsiella spp</i>	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
<i>Escherichia coli</i>	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
<i>Shigella dysenteriae</i>	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

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

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178 **Table III: Minimum Inhibitory Concentration (MIC) of Extracts of *Diplazium sammatii***

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

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

181 + - Growth of organisms

182 - - No growth of organisms

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190 **Table IV: Minimum Inhibitory Concentration (MIC) of Extracts of *Pneumatopteris afra***

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

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

192 + - Growth of organisms

193 - - No growth of organisms

194 **Table V: Phytochemical analysis of the plants**

Tests	Results	
	<i>Diplazium sammatii</i>	<i>Pneumatopteris afra</i>
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Steroids	-	-
Cardiacglycosides	+	+
Cyanoglycosides	+	+
Anthraquinones	+	+
Terpenoids	+	+
Flavonoids	+	+

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196 Keys: + Present

197 - Absent

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