International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: https://doi.org/10.7439/ijbar
CODEN: IJBABN
Original Research Article

Phytochemical analysis, antimicrobial activity and identification of phytoconstituents in *Gleichenia pectinata* (Willd.) C. Presl.

Abiola G. Femi-Adepoju[*1], Paul O. Fatoba[2], Adeyinka O. Adepoju[3] and Abimbola P. Oluyori[4]

[1]*School of Allied Health and Environmental Sciences, Kwara State University, Malete, Kwara State, Nigeria*
[2]*Department of Plant Biology, University of Ilorin, Ilorin, Nigeria*
[3]*Department of Biological Sciences, Wesley University, Ondo, Nigeria*
[4]*Department of Physical Sciences, Landmark University, Omu-Aran, Kwara State*

QR Code

***Correspondence Info:**
Dr. Abiola G. Femi-Adepoju,
School of Allied Health and Environmental Sciences,
Department of Plant and Environmental Biology,
Kwara State University, PMB 1530,
Malete, Kwara State, Nigeria

***Article History:**
Received: 22/11/2018
Revised: 28/11/2018
Accepted: 01/12/2018
DOI: https://doi.org/10.7439/ijbar.v9i12.4974

Abstract

Probing into the bioactive chemical composition of ferns may lead to a validated incorporation into drug manufacture, though they belong to the unpopular group of plants. This present study aimed at investigating the phytoconstituents and antimicrobial potency of the raw methanolic extract and the active chromatographic fractions of *Gleichenia pectinata*. The phytochemical investigation was done via chemical and instrumental (GC-MS) method. The raw methanolic extract of *G. pectinata* contains important phytochemicals such as phenolics, terpenoids, anthraquinone, tannin, alkaloid, saponin, reducing sugar, protein and flavonoids. The minimum inhibitory concentration of the raw methanolic extract against the clinical microbial strains used was 25mg/ml against (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). At this concentration, some *G.pectinata* chromatographic fractions exhibited significant antibacterial and antifungal activities. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most susceptible of the test microorganisms while *Candida albicans* was the least susceptible.

Keywords: Fern, *Gleichenia pectinata*, Chromatography, Fractionation, Antimicrobial.

1. Introduction

Recently, there has been reasonable progress in the development of antimicrobial agents for the treatment of a wide range of infectious diseases. Although the toxicity of these antibiotics to humans and other animals is generally considered low, prolonged use of certain antibiotics can reduce the number of body microflora, which may have a negative impact on the health status of humans [1]. Therefore, plants with medicinal values serve as preferred indigenous sources of novel therapeutic extracts, fractions and compounds which can be employed in complementary drug development.

A very great percentage of the population of developing countries depend on local medicines for their primary health care needs as estimated by WHO and for that reason, the demand for medicinal plants has been on the increase all over the world [2]. The ability to synthesize compounds (secondary metabolites) with antimicrobial potency make plants invaluable in the combat against recently observed resistance of microorganisms to antibiotics [3].

The isolation, identification, and biological activity of several cryptogams (Bryophytes, Pteridophytes and Mushrooms etc.) has been carried out by Asakawa [4] with the identification of hundreds of novel natural products of pharmacological importance. Although these biologically important natural products have been isolated from cryptograms [5], some have even been reported to be fungistatic [7]; the fern (*Gleichenia pectinata*) has not been investigated as much. The plant is overlooked by many and does not seem to have a local name here in Nigeria.

The results obtained by Pradeep *et al* [8], while screening 12 unexploited pteridophytic plants for their antibacterial potential against bacterial strains

(*Agrobacterium tumefaciens, Escherichia coli, Salmonella arizonae, Salmonella typhi and Staphylococcus aureus)* suggests strong antibacterial potency for leaf extracts of ferns. Hence, this work is aimed at the phytochemical analysis and chromatographic fractionation of *Gleichenia pectinata* extract for improved antimicrobial activity.

2. Materials and Methods

2.1 Plant sample/Test microbes collection and preparation

Gleichenia pectinata (Willd.) C. Presl. was collected from Ifetedo (7^o , 10.848' N 4^o 41.857' E; 933ft), Osun State. *Proteus mirabilis; Staphylococcus aureus; Pseudomonas aeruginosa; Escherichia coli, Salmonella typhi Klebsiella pneumoniae* (bacteria) *and Candida albicans* (fungus) were collected from the University of Ilorin Teaching Hospital as clinical isolates and the pure culture of the organisms were obtained by re-culturing.

2.2 Extraction

The plant samples collected were air-dried at room temperature and afterwards ground lightly into powder. The prepared powder (500 g) were soaked in methanol (plant material to solvent ratio was 1:10, w/v) and the extraction process (maceration) lasted for 72 hrs at room temperature with frequent agitation. After filtration, the filtrate was heated at 40°C to obtain the dry extract (GP) [9].

2.3 Chromatographic Fractionation of the Extract

2.0g of the GP dry extract was fractionated by CC using n-hexane: ethyl acetate (1:0; 1:1; 0:1), followed by methanol to obtain a total of 11 fractions which were confirmed by TLC for their purity and to obtain fraction combinations GP-1 to GP-9. These fractions were then subjected to antimicrobial screening against the clinical isolates in order to establish their antimicrobial potentials.

2.4 Antimicrobial Activity

The GP dry extract and the fractions were investigated for their antibacterial and antifungal activities. The *Petri dishes* were kept at 4 °C for 2 hrs, and 20ml of prepared Mueller Hinton agar solutions were poured onto each of the *Petri dish* aseptically. A sterile inoculating loop was used to introduce each of the microorganism strain on the entire surface of separate Petri-dish containing the solidified agar in a sequential order for each of the microorganisms and in respect of each plant extract to be introduced. Disc diffusion method was used according to National Committee for Clinical Laboratory Standards (NCCLS)[10] and Madigan *et al* [11].

The method that was used for the determination of the antimicrobial activity of the raw extracts was micro-dilution method while for the active fractions; disc diffusion method was used according to National Committee for Clinical Laboratory Standards (NCCLS) [10] and Madigan *et al* [11]. In microdilution method, before the inoculation of the microbial strains, 1ml of each extract at different concentrations (20-250mg/ml) was added to the prepared agar media at the molten stage before solidification aseptically. In disc diffusion method, antimicrobial discs of 5mm in diameter were incubated with 10µg of the fractions at different concentrations. Each disc was then placed at the center of the prepared agar plate after the inoculation of the desired organism. The plates inoculated with bacteria were incubated at 37 °C for 24 hrs and at 28°C for 72 hrs for the fungal strains. The turbidity of bacterial cultures were maintained up to 1×10^5 CFU/ml and the fungal strain up to 1×10^4 CFU/ml. All assays were performed in duplicate.

2.5 Determination of Minimum Inhibitory Concentration (MIC) of GP dry Extract

Microorganisms were tested for their ability to produce visible growth on a series of agar plates by agar micro-dilution method containing concentrations; 20mg/ml, 25mg/ml, 50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml and 250mg/ml of the antimicrobial agents. The MIC end-point for each strain was taken as the lowest concentration of GP extract at which there was no visible growth on the agar [10].

2.6 Qualitative phytochemical screening of the plant sample extracts

The GP extract and chromatographic fractions of the medicinal plant under study were screened for the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, glycosides, phlobatanins, phenolic compounds, phytosterols, proteins and anthraquinones using the standard procedures as described by Sofowora [12] and Okwu [13].

2.7 Quantitative Analysis

The quantitative phytochemical screening of G. pectinata was investigated following the reports of the following researchers; Tannin [14], Total Phenol [15], Flavonoid [16], Alkaloid [17], Phytosterol and Protein.

2.8 Identification of the fractionated bioactive components with GC-MS (Gas Chromatography- Mass Spectrometry)

The gas chromatography-mass spectrometry (GC-MS) analysis of selected chromatographic fractions of the GP extract was done using a GC-MS (Model; 7890A GC System, 5675C Inert MSD with triple axis; The column model is Agilent 19091-433HP-5Ms 5% Phenyl methyl silox) equipped with a fused silica capillary column of 30 m length, 0.25 mm diameter, and 250 µm film thickness treated with phenyl methyl silox.

The ion source temperature (EI) was 250°C while the interface temperature was 300°C, Pressure 16.2 psia, out time 1.8mm. 1µl injector in split mode with split ratio 1:50 with injection temperature of 300°C. The column temperature started at 35°C for 5mins and changed to 150°C at the rate of 4°C/min. The temperature was raised to 250°C at the rate of 20°C/min and held for 5mins. The total elution time was 47.5 mins.

The bioactive compounds of GP-4 and GP-7 fractions were identified by comparing their retention indices and patterns of mass spectra with reference to NIST library.

3. Results and Discussion

The results of the qualitative phytochemical screening and quantitative evaluation of some of the phytochemicals in GP are shown in Tables 1 and 2.

Table 1: The qualitative phytochemical screening of the methanolic extract of *G. pectinata*

Phytochemical	Presence/Absence
Alkaloid	+
Glycoside	+
Tannin	+
Phytosterol	+
Flavonoid	+
Phenol	+
Terpenoid	+
Saponin	+
Reducing sugar	+
Protein	+

Present = +; Absent = -

Table 2: The quantitative phytochemical analysis of the *G. pectinata* methanolic extract (mg/g)

Alkaloid	Tannins	Phytosterol	Flavonoid	Phenolics	Carbohydrates	Protein
7.92±1.07	2.10±0.11	0.29±0.57	7.67±0.14	8.42±0.00	14.44±0.17	29.55±0.00

Mean values presented ± standard deviation

From tables 1 and 2 above, it could be deduced that the extract is obviously a rich source of several secondary metabolites which have been associated with various medicinal properties. Apart from the medicinal usefulness of this plant, suspected presence of proteins in the extracts suggests that the cake which is left after extraction might be useful as a protein source in the development of animal feed. Reports from several researchers have established the presence of good number of bioactive components in ferns which have useful therapeutic values like antioxidant [18], antiviral [19], anti-inflammatory [20] and antimicrobial [21]. Herin *et al* [22] investigated the presence of phytochemical compounds in the extracts of five important ferns using different solvents for extraction and discovered that important secondary metabolites such as alkaloids, phenolic compounds, flavonoids, saponins and tannins were present in all the methanolic fern extracts. This is similar to the present study and suggests also that indeed, the choice of solvent for extraction of active ingredients from plants influences the type of bioactive components that will be obtained.

Table 3: The result of the antimicrobial screening of *GP* methanolic extract and the antibiotic drugs at different concentrations

Plant extracts	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Proteus mirabilis*	*Klebsiella pneumoniae*	*Candida albicans*
250mg/ml	+	+	+	+	+	+
200mg/ml	+	+	+	+	+	+
150mg/ml	+	+	+	-	+	+
100mg/ml	+	+	+	-	+	-
50mg/ml	-	+	-	-	+	+
25mg/ml	-	+	-	-	+	-
20mg/ml	-	-	-	-	-	-
Streptomycin (10µg/ml)	+	+	-	+	+	-
Gentamycin (10µg/ml)	-	-	+	-	-	-
Nystacin (10µg/ml)	-	-	-	-	-	+
MIC of GP extracts	100mg/ml	25mg/ml	100mg/ml	200mg/ml	25mg/ml	50mg/ml

Note: + = Inhibition - = No inhibiton

The antimicrobial potency of *G. pectinata* extract was investigated and tested on five clinical strains of bacteria and one fungus. Table 3 shows that the extract of *G. pectinata* exhibited antimicrobial activity against all the test microbes at higher concentrations (250 and 200mg/ml). The activity was retained for four microbes at 150mg/ml and preceded to showing potency against only three at 100mg/ml. Only *K. pneumoniae and P. aeruginosa* were susceptible to the extract at 50 and 25mg/ml while the extract showed no potency against any of the microorganism at 20mg/ml. The MIC was discovered to be 25mg/ml against *K. pneumoniae* and *P. aeruginosa* which were the most susceptible while *S. typhi* and *S. aureus* were the least susceptible microbes. The low MIC obtained is a further confirmation that the extract is a potential antimicrobial resource. The quality of activity and concentration of the positive control drugs used is within the Clinical and Laboratory Standards Institute standard (CLSI) [23]. The degree of inhibition obtained from the activity of Streptomycin against *K. pneumoniae* and *P. aeruginosa* was lower than that of G. *pectinata* extract at 25mg/ml against the same organisms. However, the degree of inhibition obtained for Gentamycin and Nystacin against the susceptible organisms were higher. This further proves the potential of the extract as a possible antibiotic with proper refinement of the active principle. Our results agree with the results earlier obtained by Ojo *et al* [24] and Samir [25] on the antimicrobial activity of ferns against similar organisms employed in this work.

Table 4: The qualitative phytochemical screening of the chromatogram fractions of *G. pectinata*

GP fractions	GP-1	GP-2	GP-3	GP-4	GP-5	GP-6	GP-7	GP-8	GP-9
Tannins	-	-	-	-	-	-	+	+	-
Anthraquinones	-	-	+	-	+	-	-	+	-
Steroids	-	-	+	-	-	-	-	-	-
Flavonoids	-	-	-	-	+	-	-	-	-
Terpenoids	-	-	-	-	-	-	+	+	+
Alkaloids	-	-	+	+	+	+	+	+	+
Phenol	-	-	+	+	-	-	+	-	-
Saponins	-	-	-	-	-	-	+	+	+
Phlobatannins	-	-	-	-	-	-	-	-	-

Present = +; Absent = -

Fractionation sometimes lead to improved biological activity and column chromatography has been projected as one of the means of fractionating crude extracts for improved biologic activity [26]. The column chromatographic fractionation of GP extract was followed by the qualitative phytochemical study of the fractions so as to reveal the distribution of the various secondary metabolites which have been earlier identified in the GP crude extract and to observe the effect of the fractionation of antimicrobial activity. Table 4 shows the absence of all the investigated secondary metabolites in n-hexane fractions (GP-1 and GP-2). That explains the inability of the 2 fractions to inhibit microbial growth. In contrast, the terpenoids and the saponins which have been implicated in antimibacterial activity [27] were observed in the methanolic fractions (GP-7 to GP-9) and this explains the observed wider zone of inhibition by GP-7 to GP-9 against the test organisms. Furthermore, the presence of alkaloids in all the fractions but GP-1 and GP-2 (n-hexane fractions) is worthy of note. This observation coupled with the biological importance of alkaloids suggests that *G. pectinata* should be intentionally explored for novel alkaloids which could be of immense medicinal importance.

Table 5: The antimicrobial activity of the isolated chromatogram fractions of *G. pectinata*

Microorganisms	Conc. (mg/ml)	ZOI (mm)							Positive Control (mg/ml)
		Gp-3	Gp-4	GP-5	Gp-6	Gp-7	Gp-8	Gp-9	
P. aeruginosa	500	17^{a}	18^{a}	20^{a}	17^{ab}	20^{a}	21^{a}	9^{b}	Streptomycin (10 µg/ml) ZOI, 12 mm
	250	16^{a}	16^{b}	19^{a}	18^{a}	20^{a}	17^{b}	15^{a}	
	100	14^{b}	10^{c}	18^{a}	16^{b}	17^{b}	14^{c}	9^{b}	
	50	14^{b}	14^{b}	12^{b}	19^{a}	17^{b}	14^{c}	9^{b}	
	25	17^{a}	19^{a}	11^{b}	11^{c}	17^{b}	10^{d}	9^{b}	
		Gp-3	Gp-4	GP-5	Gp-6	Gp-7	Gp-8	Gp-9	
E. coli	500	11^{a}	17^{b}	11^{a}	18^{b}	19^{b}	18^{a}	20^{c}	Streptomycin (10 µg/ml) ZOI,12 mm
	250	11^{a}	14^{c}	11^{a}	21^{a}	21^{a}	17^{a}	27^{a}	
	100	11^{a}	22^{a}	11^{a}	20^{a}	19^{b}	16^{ab}	24^{b}	
	50	11^{a}	18^{b}	11^{a}	20^{a}	21^{a}	15^{b}	22^{b}	
	25	11^{a}	16^{bc}	11^{a}	15^{c}	21^{a}	13^{c}	17^{d}	
		Gp-3	Gp-4	GP-5	Gp-6	Gp-7	Gp-8	Gp-9	
S. aureus	500	9^{b}	13^{b}	17^{a}	11^{c}	17^{a}	13^{b}	17^{b}	Gentamycin (10 µg/ml) ZOI, 16 mm
	250	9^{b}	11^{c}	16^{a}	17^{a}	16^{a}	11^{c}	21^{a}	
	100	17^{a}	19^{a}	15^{ab}	13^{b}	17^{a}	17^{a}	17^{b}	
	50	9^{b}	18^{a}	13^{c}	17^{a}	15^{b}	17^{a}	16^{bc}	
	25	19^{a}	12^{bc}	12^{c}	10^{c}	13^{bc}	11^{c}	14^{c}	
		Gp-3	Gp-4	GP-5	Gp-6	Gp-7	Gp-8	Gp-9	
P. mirabilis	500	21^{a}	17^{b}	23^{a}	19^{a}	22^{a}	12^{bc}	10^{a}	Streptomycin (10 µg/ml) ZOI, 12 mm
	250	18^{b}	13^{c}	17^{b}	16^{b}	18^{b}	13^{b}	10^{a}	
	100	20^{a}	17^{b}	17^{b}	15^{bc}	18^{b}	16^{a}	10^{a}	
	50	17^{b}	17^{b}	17^{b}	15^{bc}	18^{b}	15^{ab}	10^{a}	
	25	17^{b}	25^{a}	13^{c}	13^{c}	16^{bc}	14^{b}	10^{a}	
		Gp-3	Gp-4	GP-5	Gp -6	Gp-7	Gp -8	Gp-9	
K. pneumoniae	500	20^{a}	18^{a}	21^{a}	21^{a}	22^{a}	11^{c}	10^{a}	Streptomycin (10 µg/ml) ZOI, 12 mm
	250	19^{a}	16^{b}	12^{bc}	13^{b}	18^{b}	17^{a}	10^{a}	
	100	20^{a}	15^{bc}	18^{a}	12^{bc}	18^{b}	15^{b}	10^{a}	
	50	11^{c}	14^{bc}	14^{b}	11^{cd}	17^{b}	15^{b}	10^{a}	
	25	14^{b}	14^{bc}	13^{b}	11^{cd}	17^{b}	13^{bc}	10^{a}	
		Gp-3	Gp-4	GP-5	Gp -6	Gp-7	Gp -8	Gp-9	
C. albicans	500	19^{a}	19^{a}	18^{a}	11^{a}	15^{a}	11^{a}	17^{a}	Nystatin (10 µg/ml) ZOI, 14 mm
	250	10^{c}	17^{ab}	12^{bc}	10^{ab}	13^{ab}	10^{bc}	16^{a}	
	100	16^{b}	15^{bc}	14^{b}	10^{ab}	13^{ab}	10^{bc}	18^{a}	
	50	14^{b}	13^{c}	14^{b}	10^{ab}	12^{bc}	10^{bc}	14^{b}	
	25	18^{a}	11^{d}	13^{b}	10^{ab}	11^{bc}	10^{bc}	11^{c}	

Mean values with different letters are significantly different at 95% confidence level (P≤ 0.05)

Table 5 summarizes the antimicrobial activities of the chromatographic fractions from the *G. pectinata* extract. The values were subjected to one way ANOVA statistical analysis and the level of significance between the mean values were documented at P≤ 0.05 (95% confidence level). The letters at the top of the bars indicates that mean values with different letters are significantly different. It was observed that two isolated chromatogram fractions which are GP-4 and GP-7 which represented the sixth and ninth fractions showed highest inhibition activity against many of the test organisms and performed better than the antibiotic drugs in their activity against *K. pneumonia, P. mirabilis, E. coli* and *P. aeruginosa* at their lowest concentration used. GP-4 also had a good inhibition potential against the activity of *S. aureus*. GP-3 and GP-5 exhibited the most promising antifungal activity. The report of Oluyori *et al* [28], and a close observation of Table 4 suggest that the presence of steroids which was not detected in GP-5 or any other fraction could have contributed to this antifungal activity. Specifically, GP-3 showed a remarkable activity against *Candida albicans*, it therefore suggests that further purification of this fraction may lead to the discovery of a new anticandidal bioactive compound. The concentrations of the standard drugs were within the limit set by Clinical and Laboratory Standards Institute standard (CLSI). The general performance of the two fractions (GP-4 and GP-7) resulted in their chemical analysis and identification of the bioactive components contained in these two fractions.

Table 5: Chemical Analysis of fraction GP-4 by GC-MS Studies

Retention Time	% Relative Area	Name of Compound
7.679	1.24	1-propoxypropanyl-2-pentanoate
8.3	0.93	2-propenoic acid
10.036	2.35	Bromo-cyclohexane
17.341	0.88	1,6,6-trimethylcyclohexene
18.417	0.97	2-Dodecene
25.173	2.61	3-tetradecene
25.424	1.13	Dodecane
27.066	4.19	Precocene l (Quinoline-6-methoxy-1-oxide)
28.771	0.92	2-Fluoro-5-trifluoromethylbenzoic acid, 4-tetradecyl ester
28.959	11.03	2,6-bis (1,1-dimethylethyl)phenol
31.166	6.16	Cetene
33.892	1.78	1-ethyl-1-methylcyclohexane
34.686	1.46	Terephthalic acid, ethyl 4-heptyl ester
36.767	6.96	E-15-Heptadecenal
37.686	0.8	Dodecylcyclohexane
37.859	1.94	9-Heptadecanone
38.095	4.7	Methyl cis-3-(p-anisido) acrylate
38.173	1.19	Succinic acid, 2-methylphenyl 2,3-dichlorophenyl ester
38.393	2.61	Methyl palmitate
38.668	2.89	Dibutyl phthalate
38.794	6.79	2-Butanone,4-[2-Isopropyl-5-methyl-5-(-methyl-5-oxocyclopentyl)] cyclopentyl
38.951	4.15	l-Nonadecene
39.485	1.47	p-Pentylacetophenone
39.548	1.42	Isophthalic acid, ethyl 2-methylprop-2-en-l-yl ester
40.051	1.18	Octadecanoic acid
40.184	3.47	1-Docosene

Table 6: Chemical Analysis of fraction GP-7 by GC-MS Studies

Retention Time	% Relative Area	Name of Compound
9.368	2.55	5-methyl-2-Furancarboxaldehyde
9.91	2.07	(E)-2-Pentenal,
10.758	9.7	1,2-cyclohexandione
11.269	11.72	Phenol
12.628	1.91	3-methyl-1,2-Cyclopentanedione
14.482	5.24	2,5-Dimethyl-4-hydroxy-3 (2H)-Furanone
20.059	50.65	2,3-dihydrobenzofuran,
22.518	1.92	2-Methoxy-4-vinylphenol
27.074	1.77	Precosene 1
38.393	2.49	Methyl palmitate
38.818	2.91	n-Hexadecanoic acid
40.121	2.79	Bergamotol (z-, alpha.-trans)
40.224	2.71	2-Ethyl-3,5-dimethylpyridine
41.174	2.58	Cyclotetracosane
42.093	1.66	Phthalic acid, di (2-propylpentyl ester)
42.415	1.59	Heptacosyl acetate
43.012	1.56	7-(3,4-Methylenedioxy)-tetrahydrobenzofuranone

Table 5 and 6 reveals the isolated compounds present in the GP-4 and GP-7 active fractions as identified by GC-MS. The compounds and which are present in considerable quantities includes in GP-4; Precocene l (Quinoline, 6-methoxy-,1-oxide), Phenol 2,6-bis (1,,1-dimethylethyl), Cetene, E-15-Heptadecenal, Methyl cis-3-(p-anisido) acrylate (new), 2-Butanone,4-[2-Isopropyl-5-methyl-5-(-methyl-5-oxocyclopentyl) cyclopentyl (novel) and l-Docosene. The bioactive compounds present in the GP-7 active fraction and which are present in considerable quantities includes; 1,2-cyclohexandione, Phenol, 2,5-Dimethyl-4-hydroxy-3 (2H)-Furanone and Benzofuran, 2,3-dihydro. The quantity of 2,3-dihydrobenzofuran (50.65%) is a striking revelation that requires further research. This compound has been established to be an analgesic, antidiabetic, antibacterial and antifungal [29]. Dodecane has also been reported by Togashi *et al* [31] to be an antibacterial compound against the activity of *S. aureus.* Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl commonly known as Butylated hydroxytoluene (BHT), an antioxidant, has been established by Turcotte and Saheb [30] to show marked antimicrobial activity inhibiting or decreasing the growth of gram-positive bacteria at a higher degree than the gram-negative bacteria belonging to the family *Enterobacteriaceae* and this was also reported in this present study to be of considerable quantity in the chromatogram fractions of *G. pectinata*. Many derivatives of esters, amides, ketones and fatty acids were identified in the two fractions and many of these have earlier been reported to have antimicrobial properties. For example, the linoleic acid esters present in the stem, hexadecanoic acid methyl ester, are reported to have anti-inflammatory, cancer preventive, hepatoprotective, antiarthritic, and anticoronary properties [32]. Interestingly, 2-Butanone,4-[2-Isopropyl-5-methyl-5-(-methyl-5-oxocyclopentyl)] cyclopentyl and Methyl cis-3-(p-anisido) acrylate are being reported from a plant source for the first time. Quinoline-6-methoxy-1-oxide (a quinoline derivative) was also identified from GP-4 and this suggests that the plant could have antimalarial properties in addition to the established antimicrobial potential.

4. Conclusion

This study was successful in identifying a candidate fern which could serve as rich reservoir of antimicrobial components and may be extremely useful in the treatment of infectious disease. This becomes more important with the emergence of drug-resistant microbes which calls for the production new and efficient antimicrobial agents.

This study is a preliminary validation of the antimicrobial potential of *G. pectinata and* a call for the proper conservation of this important but unpopular plant. Further purification of the fractions could lead to the isolation of one or more pure and novel compounds which could be of significantly interest to the Pharmaceutical Industry.

Conflict of Interest

We declare that there is no conflict of interest.

Acknowledgement

The authors are grateful to the Nigerian government for the funding made available for this research under the Institution Based Research Funding of the Tertiary Education Trust Fund with grant number KWASUNGR/CSP/040914/VOL2/TETF/0020

References

[1]. Levy, S. B. Drug Resistance: The New Apocalypse (special issue). *Trends Microbiol.* 1994; 2: 341–425.

[2]. Farina, M., Preeti, B. and Neelam, P. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. *BioMed Research International* 2014; Article ID 497606, 11.

[3]. Lis-Balchin, M. and Deans S. Bioactivity of selected plant essential oils against Listeria monocytogenes. *Journal of Applied Microbiology*. 1997; 82 (6): 759–762.

[4]. Asakawa, Y. Agnieszka, L. and Fumihiro, N. Phytochemical and biological studies of bryophytes. *J. phytochem.* 2013; 91: 52-80.

[5]. Banerjee R. D. and Sen S. P. Antibiotic activity of pteridophytes. *Econ. Bot.* 1980; 34: 284–298.

[6]. Chen, E.W., Lim, Y. Y. and Chew, Y. L. Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia. *J Food Chem.* 2007; 102: 1214-1222.

[7]. Femi-Adepoju, A.G., Adepoju, A.O. and Ogunkunle, A.T. Assessment of fungicidal and fungistatic properties of some nigerian moss plants. *Global J. Biol. Agric. Health. Sci.* 2014; 3(1):126-131.

[8]. Pradeep, P., Leena, P. and Achaleshwar B. *In vitro* antibacterial activity of fronds (leaves) of some important pteridophytes. *Journal or Microbiology and Antimicrobials*, 2010; 2(2): 19-22.

[9]. Maher, O., Mohamad, S., Mohammad, A., Enas, A., Hanee, A., Maisa, A., Jafar, E. and Ismael, O. Antimicrobial activity of crude extracts of some plant leaves. *Research Journal of Microbiology* 2012; 7: 59-67.

[10]. NCCLS (National Committee for Clinical Laboratory Standards) Approved Standard. (2002). NCCLS M38-A. Villanova, PA, USA.

[11]. Madigan, M. Brock Biology of Microorganisms. San Francisco: Pearson/Benjamin Cummings. 2009: pp. 1152.

[12]. Sofowora, E.A. Medicinal Plants and Traditional Medicine in West Africa. Second ed, John Willey and Sons Ltd. New York, 1993; pp. 289.

[13]. Okwu, D. E. Evaluation of the chemical composition of indigenous Spices and flavouring agents. *Global J. Appl. Sci*. 2001; 7(3): 455-459.

[14]. Padmaja, G. Evaluation of techniques to reduce assayable tannin and cyanide in cassava leaves. *J Agric. Food Chem.* 1989; 37: 712-716.

[15]. Chan, E. W., Lim, Y. Y., Chew, Y. L. Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia. *J Food Chem*. 2007; 102: 1214-1222.

[16]. Kale, A., Gaikwad, S. and Mundhe, K. Quantification of phenolics and flavonoids by spectrophotometer from *Juglans regia. Int J Pharm Biol Sci.* 2010; 1: 1-4.

[17]. Singh, D., Srivastva, B. and Sahu, A. Spectrophotometric determination of Rauwolfia alkaloids, estimation of reserpine in pharmaceuticals. *Anal Sci*. 2004; 20: 571-573.

[18]. Carcia, F., Pivel, J. P., Guerrero, A., Brieva, A., Martinez-Alvazar, M. P., Caamano-Somoza, M. and Conzlez, S. Phenolic components and antioxidant activity of Fernblock, an aqueous extract of the aerial parts of the fern *Polypodium leucotomos*. *Methods Find Exp. Clin. Pharmacol.* 2006; 28: 157–160.

[19]. McCutcheon, A. R., Roberts T. E., Gibbons E., Ellis S. M., Babiuk L. A., Hancock R. E. and Towers, G. H. Antiviral screening of British Columbian medicinal plants. *J. Ethnopharmacol.* 1995; 49: 101–110.

[20]. Liu, B., Diaz, F., Bohlin, L. and Vasange, M. Quantitative determination of anti-inflammatory principles in some *Polypodium* species as a basis for standardization. *Phytomedicine.* 1998; 5: 1487–194.

[21]. Parihar, P. and Bohra, A. Screening of some ferns for their antimicrobial activity against *Salmonella typhi*. *Adv. Plant Sci.*, 2002; 15: 365–368.

[22]. Herin, D.S., John, A. D. and Benjamin, P. J. Qualitative and quantitative analysis of phyto-chemicals in five *pteris* species. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2013; 5(1).

[23]. CLSI (Clinical and Laboratory Standards Institute). (2014). Performance Standards for Antimicrobial Susceptibility Testing. twenty-fourth informational supplement.

[24]. Ojo, O.O. Ajayi, A.O. and Anibijuwon, I.I. Antibacterial potency of methanol extracts of lower plants. *J Zhejiang Univ Sci B.* 2007. 8(3): 189–191.

[25]. Samir, K.P. Study of antimicrobial activity of different plant parts of ferns. *Ind J Biol Stud Res*. 2013; 2(2): 134-138.

[26]. Oluyori A.P., Shaw A.K., Rastogi P., Sammajay R., Olubunmi A., Olatunji G.A. and Fabiyi O.A. Natural antifungal compounds from the peels of *Ipomoea batatas. Nat. Prod. Res.* 2015; 30(18): 2125-2129.

[27]. Adelani-Akande T.A., Ajiba L.C., Dahunsi S.O., Oluyori A.P. Antibacterial activity of watermelon (*Citrullus lanatus*) seed against selected microorganisms. *Afr. J. Biotechnol*. 2015; 14 (14): 1224-1229.

[28]. Oluyori, A.P., Arun, K.S., Olatunji, G.A, Preeti, R., Sanjeev, M., Dipak, D, Ashish A. Sama A., and Puli S.R. Sweet Potato Peels and Cancer Prevention. *Nutrition and Cancer – An International Journal.* 2016; 68(8): 1330-1337.

[29]. Moorthy, V. and Boominathan, M. The antimicrobial activities of crude extracts and fraction of *Psidium guajava* and *Azadirachta indica* against *Staphylococcus aureus* in chronic disease affected patients. *International Journal of Universal Pharmacy and Life Sciences*. 2011; 1(2): 2249–6793.

[30]. Turcotte, P. and Saheb, S. A. Antimicrobial activity of phenolic antioxidants. *Canadian Journal of Microbiology*. 1978; 24(11): 1306–1320.

[31]. Togashi, N., Shiraishi, A., Nishizaka, M. *et al*. Antibacterial activity of long-chain fatty alcohols against *Staphylococcus aureus*. *Molecules*, 2007; 12(2): 139–148.

[32]. Surender S., Vinod N., Sweety J., Gupta Y. K. Evaluation of anti-inflammatory activity of plant lipids containing *α*- linolenic acid. *Indian Journal of Experimental Biology* 2008; 46(6): 453–456.