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Research Article

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Phytochemical and Antimicrobial Properties of *Piper guineense* (Shumach and Thonn) on Selected Human Pathogens

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ABSTRACT

Background: *Piper guineense commonly known as "West African black pepper", "Ashanti pepper" or "iyere" in Yoruba land (Southwestern Nigeria).*
Objective: *Powdered seed was screened for its phytochemical and antimicrobial properties because for its ethno medicinal claims as being used in the treatment of various microbial and parasitic infections.*
Methodology: *Sun dried seeds of Piper guineense were screened for its phytochemical constituents and antimicrobial activity using ethanol, n-hexane and water extracts. The antimicrobial activities of the extracts were determined based on the presence and absence of zones of inhibition.*
Findings: *The seeds extracts of P. guineense contain secondary metabolites such as carotenoids, alkaloids, tannins, saponins anthraquinones, steroids, phenols, flavonoids and terpenes. The aqueous extracts exhibited highest zones of inhibition that ranged from 3-29 mm, followed by ethanol extracts inhibition with zones from 4-22 mm and least with n-hexane inhibition which ranged from 7-14 mm even with concentrations of 400 and 500 mg only.*
Conclusion: *The results of this research suggest that P. guineense is a promising therapeutic agent that could have potential for the treatment of various pathogenic diseases based on the valuable phytochemicals known for microbial inhibition.*

Keywords: Antimicrobial properties; Aqueous; Ethanol; Hexane; Phytochemical constituents; *Piper guineense*

INTRODUCTION

Plants are valuable antimicrobial agent known for the treatment of various microbial and non-microbial diseases origin from time immemorial. Medicinal plants range from those used in the production of mainstream pharmaceutical products to plants used in herbal medicine preparations in various cultures of the world. Herbal medicine is one of the oldest forms of medical treatment known to man and could be considered one of the forerunners of the modern pharmaceutical trade hence some modern drugs have been synthesized from plants derivatives. Plants of medicinal values are all over the world thus different cultures use different plants in diseases ailments. Most chemists are interested in studying plants that have not been researched on to identify which compounds in the plants are active and how those compounds work. Usually, the goal is to develop a synthetic version of the compound that can be easily produced in a lab and packaged in pharmaceutical preparations [1].
The use of medicinal plants for the treatment and control of diseases around the world has gained popularity and acceptability in different ethnic groups because of their effectiveness and accessibility by the common man that cannot afford the cost of some modern medicines. The medicinal values of plants lie in some chemical substances present in them which possess definite physiological action on the human and animal system. The most important classes of these bioactive constituents of plants are alkaloids, flavonoids, tannins, saponins, terpenoids and phenolic compounds, generally referred to as phytochemicals or metabolites [2] that have been potentially known for their inhibitory actions on microorganisms. With what man have suffered by the various diseases that surround him,

intensive research has made it possible to isolate medicinal compounds from a number of valuable plants and the healing properties of such metabolites determined. Many of these plant derived compounds are now synthesized in laboratories for use in pharmaceutical preparations [3].

Piper guineense (Shumach. and Thonn.) (Ashanti pepper) is an erect herbaceous climbing liana native to tropical Africa, ranging from Guinea to Kenya and South to Zambia. The fruits (berries) of the plant are commonly known in English-speaking countries as "West African black pepper", "iyeree" in Yoruba, and "poivrie" in French. The fruits are usually sold in Nigerian markets as an edible medicinal plant or additive in foods to offer aroma and flavour [4]. Species of Piper are located in all types of vegetation but mostly serve as components of pioneer vegetation [5]. Some of the members of this family include *P. tuberculatum, P. longum, P. nigrum* etc. [6] and belongs to the family Piperaceae or Sapotaceae [7].

MATERIALS AND METHODS

Source of Microbial Isolates

All bacterial isolates which include *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella dysenteriae, Aspergillus flavus and Aspergillus niger* and *Candida albicans* were obtained from the Department of Microbiology, Afe Babalola University, Ado-Ekiti, purified and stored on slants in a refrigerator at 4°C for use.

Collection and Identification of Plant Samples

The whole plant of *P. guineense* was bought in Oyingbo market, Oyingbo, Lagos State in the month of April. The plant was identified and authenticated by Dr. (Mrs.) O.T. Ogunmefun, a botanist in the Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria.

Processing of Plant Samples

The seeds of *P. guineense* was thoroughly cleaned and dried at room temperature for one month. The dried plant material was then ground to fine powder using an electric blender and stored in sterile containers until needed.

Preparation of Samples for Antimicrobial Analysis

Three different solvents were used (ethanol, distilled water and n-hexane). 150 g of seed powder was soaked in 750 ml each of the solvents and mixed by stirring with a glass rod. The mixtures were left at room temperature for 24 h. The extracts were filtered using a sari cloth filter and finally with Whatman No one filter paper. The resulting solution from ethanol and n-Hexane were evaporated in a rotary evaporator while, the aqueous extract was evaporated in a water bath at a temperature of 55°C until semi solid extract was attained. The dried underlying crude extracts were kept in a plastic vials and stored in refrigerator at 4°C until required for use.

Preliminary Qualitative and Quantitative Phytochemical Screening of *P. guineense*

The phytochemical screening was carried out using the methods described [8-10].

Test for phenols and tannins:

Two millilitres of 2% solution of $FeCl_3$ was mixed with the crude extract. Black or blue green colour indicated the presence of tannins and phenols.

Tests for flavonoids:

Shinoda test: pieces of magnesium ribbon and concentrated HCl was mixed with the crude plant extract, after few minutes, the appearance of a pink colour scarlet indicated the presence of flavonoids.

Alkaline reagent test:

Two millitres of 2% NaOH solution was mixed with plant crude extract, intensive yellow colour was formed, which turned into colourless when 2 drops of diluted acid was added to the solution, this result indicated the presence of flavonoids.

Test for anthraquinones:

Five miilitres of chloroform was added to 0.5 g of the extract. The mixture was shaken foe 5 minute after which it was filtered. The filter was then shaken with equal volume of 10% ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of anthraquinones.

Test for cardiac glycosides:
Five millilitres of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristic of cardenolides.

Test for saponins:
Five millilitres of distilled water was added to crude plant extract in a test tube and it was shaken vigorously. The foam formation indicated the presence of saponins.

Tests for glycosides:
Liebermann's test: 2 ml of acetic acid and 2 ml of chloroform mixed with entire plant crude extract. The mixture was then cooled and concentrated H_2SO_4 was added, a green colour indicated the entity of aglycone steroidal part of glycosides.

Salkowski's test:
About 2 ml of concentrated H_2SO_4 was added to the entire plant crude extract. A reddish brown colour produced indicated the entity of steroidal aglycone part of the glycoside.

Test for carotenoids:
Two millilitres of the extract was filtered and 85% sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

Test for steroid:
Two millilitres of chloroform and concentrated H_2SO_4 was mixed with the entire plant crude extract. In the lower chloroform layer, the appearance of a red colour indicated the presence of steroids. A green colour indicated the entity of steroids.

Test for terpenoids:
Two millilitres of chloroform was mixed with the plant extract and evaporated on the water bath, then boiled with 2 ml of concentrated H_2SO_4 A grey colour produced indicated the entity of terpenoids.

Antimicrobial Assay
The antimicrobial assay was done using the agar diffusion method. An overnight culture of each organism was prepared by using small portions of the organism from the stock and inoculating each into 8ml sterile peptone water and incubated for 24 hours at 37°C. The various test bacteria were standardized using the 0.5 McFarland turbidity standards. From the overnight culture, 0.1 ml of each organism was taken and put into 9.9 ml of sterile distilled water to get (1:100) of the dilution of the organism. An aliquot 0.1 ml was taken from the dilution onto the surface of the sterile plates of Muellar Hinton agar (MHA). A cork borer (6 mm) was used to make wells on the inoculated MHA agar. One millilitre of each crude extract was constituted with Dimethyl sulphoxide (DMSO) and introduced into designated wells. These were left on the work bench for the duration of 2 hours after which it was then incubated at 37°C for 24 h. The antimicrobial activities were determined by the width of the zone of growth inhibition.

Antibiotic Susceptibility Test
The antibacterial susceptibility testing was done using the agar-disk diffusion method [11]. Fresh isolates were suspended in peptone broth in comparison to 0.5 McFarland turbidity standards. Each of the isolates was inoculated onto the surface of sterile Mueller Hinton agar plates using a sterile swab in order to ensure even distribution while streaking. The plates were allowed to dry for 15 minutes and the antibiotic discs were placed on the surface of the agar plates using sterile forceps. The plates were then inverted and incubated for 24 hours at 37°C. The antibacterial disc include the Gram negative disc comprising of Septrin (30 µg), Chloramphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Amoxicillin (30 µg), Augmentin (30 µg), Gentamycin (10 µg), Pefloxacin (30 µg), Tarivid

(10 µg), Streptomycin (30 µg) which serves as positive control for Gram negative organisms. The antibacterial activities were determined by the width of the zone of growth of inhibition. The tests were conducted.

Statistical Analysis of Data

All data were analyzed using statistical tools such as distribution table, simple bar chart, analysis of variance (ANOVA) and statistical package for social sciences (SPSS 20). A p value of ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Properties of Seed Extracts of *P. guineese*

The seeds of *P. guineense* were extracted with n-hexane, ethanol and water. This plant has been reported for so many uses including culinary, medicinal, cosmetic and insecticidal uses (Martins) and therefore need the scientific validation for it's widely variety uses. The phytochemical report of the plant shows the presence of flavonoids, alkaloids, tannins, anthraquinones, steroids, phenolics, carotenoids, terpenes and saponins but with the absence of cardiac glycosides. These same phytochemicals were screened from the leaf ethanol and aqueous extracts of *P. guineese* by [12]. However, they obtained higher values in tannins and alkaloids; and lower values in flavonoids, glycosides, saponins, steroids, terpeniods and phenols than our report in this study. Though valuable plant chemicals were screened in various quantities, carotenoid content was more than other screened phytochemical in *P. guineense* seed extract in this study. Nevertheless, Alinnor and Ejele, [13] have reported similar plant chemicals from the leaf extracts of *P. guineense*. The presence of phenolic compounds screened from the seed of *P. guineese* was envisaged hence it's various used in traditional medicine in various cultures of the world. Polyphenolic compounds are primarily responsible for the antioxidant activity of natural extract due to their redox properties and chemical structures [14]. The tannins contents in the cells of plants are potent inhibitors of many hydrolytic enzymes such as pectolytic macerating enzymes used by plant pathogens. This plant chemical have also been found to form irreversible complexes with proline-rich protein as reported in the research work of [15,16], which result in the inhibition of cell protein synthesis. Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorder such as diarrhoea and dysentery [16]. Biological functions of flavonoids, as one of the vital screened phytochemical with high value in this study has protection against allergies and inflammation [17]. The large amounts of beta-carophyllene in flavonoids are valued for its anti-inflammatory agent [18], free radicals, platelet aggregation [19,20] ulcer, hepatoxins and tumor [21]. The high value of alkaloids screened in the seed is also a notable potential for its value in traditional medicine hence alkaloids are ranked the most efficient therapeutically significant plant substance. Alkaloids have being used as CNS stimulant, powerful pain relievers, topical anesthetic in ophthalmology among others as reported by Ashok and Upadhyaya. Saponins which is also a vital plant chemical screened from the seeds of *P. guineese* have been reported to possess anti-carcinogenic properties by Adesokan and Akanji [22]. The presence of these phytochemicals in the seed extracts of *P. guineense* signified its vital role in folklore and other uses for human benefits. *P. guineense* has been confirmed to contain dillapiol, 5-8% of piperine, elemicine, 10% of myristicine and safrole which could further make it potent in addition to other screened chemicals to exhibit the antimicrobial effects on some microorganisms in this report. Phytochemicals are bioactive compound found in plant foods that work with nutrient and dietary fibers to protect against disease [23]. The numerous and rich plant chemicals in the seeds of *P. guineese*, count it most importantly as a valuable medicinal source to be employed in the cure and control of diseases (Table 1).

Table 1: Qualitative and quantitative phytochemical analyses of *Piper guineense*

Quality phytochemicals (mg/100g)		Quantity phytochemicals
Alkaloids	++	935.0 ± 8.660h
Saponins	+	203.3 ± 4.410e
Tannins	+	141.7 ± 4.410d
Anthraquinones	+	61.667 ± 1.667b
Cardiac Glycosides	+	6.667 ± 1.667a
Flavonoids	++	675.0 ± 12.583f
Steroidal Nucleus	+	90.0 ± 2.887c
Carotenoid	+++	1841.7 ± 10.138i
Phenols	+	24.13 ± 0.1667a
Terpenoids	+	778.33 ± 10.138g

Keys: + = present; ++ = Moderate; +++ = Abundant; - = absent

Antibiotic Inhibition Properties on Tested Isolates

The antibiotic susceptibility and resistance pattern of the test organisms is shown in Table 2. Chloramphenicol, ciprofloxacin, Sparfloxacin and streptomycin inhibited all test bacterial species. However, chloramphenicol was the inhibitoriest to the isolates with zones that ranged 18-22 mm, followed by Sparfloxacin with inhibition zones of 10-22 mm on the isolates and ciprofloxacin with zones of between 12-18 mm. Least inhibition has observed with Augmentin that inhibited the isolates with zone range of 0-12 mm. However, Pefloxacin and Tarivid were not inhibitory to any of the tested isolates. Chloramphenicol most inhibited *S. dysenteriae* with zone of 22 mm, Sparfloxacin on *P. aeruginosa* with zone of 22 mm and ciprofloxacin with zone of 18 mm on *S. dysenteriae* and *E. coli* respectively.

Table 2: Antibiotics susceptibility test

	Septrim	**Chloramphenicol**	**Sparfloxacin**	**Ciprofloxacin**	**Amoxicillin**	**Augmentin**	**Gentamycin**	**Pefloxacin**	**Tarivid**	**Streptomycin**
	(30 µg)	(30 mg)	(10 mg)	(10 mg)	(30 mg)	(30 mg)	(10 mg)	(30 mg)	(10 mg)	(30 mg)
Klebsiella pneumonia	0	20	20	16	0	0	0	0	0	16
Pseudomonas aeruginosa	0	24	22	12	0	0	12	0	0	14
Shigella dysenteriae	18	22	19	18	0	0	0	0	0	13
Escherichia coli	0	18	18	18	0	0	0	0	0	15
Salmonella typhi	14	18	16	16	12	10	16	0	0	13

Antimicrobial Properties of Seed Extracts of *P. aeruginosa*

Table 3 represented the antimicrobial activities of the seed extracts. The extracts inhibition on the tested isolates was on concentration dependant. However, the aqueous extract most inhibited the isolates where 500 mg/ml exhibited zone of 29 mm on *P. aeruginosa*, 100-400 mg/ml created high zones of 20, 22, 25 and 26 mm respectively on *S. typhi*. The ethanol extracts (100-500 mg/ml) respectively most inhibited *P. aeruginosa* with zones of 12, 14, 16, 20 and 22 mm. The n-Hexane extracts was only effective at 400-500 mg/ml on *K. pneumonia* with zone of 12 and 14 mm respectively. However, Konning [24,25]; have reported on the antibacterial activity against Gram positive and negative bacteria and also antifungal activity with *P. guineese*. Tekwu et al. [26] have also reported that *P. guineense* could be important sources of bactericidal compounds on several bacteria. The several reports on the inhibitory effect on microorganisms with either the leaf, stem and seed of *P. guineense*, could ascertained its employment in the treatment of diseases by traditional healers in different cultures in Africa and the world as a whole.

All extracts used in this research exhibit antimicrobial property at varying degrees with the n-hexane extract exhibiting the least potency (7-14 mm), ethanol extract (4-22 mm) and the aqueous extract showing a stronger antibacterial activity with zones of inhibition ranging from 5-29 mm. This result is in agreement with Olusimbo who reported the antimicrobial potential of the aqueous extract of *P. guineense*. This is not surprising as the antimicrobial nature of many edible plant extracts such as cranberry, lime and lemon juices have been positively reported on by several authors [27]. There were significant differences in the zones of inhibition of the different concentrations of ethanol, n-hexane and aqueous extracts of *P. guineense* on the tested bacteria isolates, whereas with the fungal isolates, there was no significant difference in the aqueous extract concentrations but significantly different in the ethanol extract concentrations.

Table 3: Antibacterial activity of extract of *P. guineense*

	Ethanol extracts (mg/ml)						Aqueous extracts (mg/ml)						n –Hexane extracts (mg/ml)					
	100	200	300	400	500	DMSO	100	200	300	400	500	DMSO	100	200	300	400	500	DMSO
Klebsiella pneumonia	-	9	11	13	16	–	17	19	21	23	25	-	-	-	-	12	14	-
Pseudomonas aeruginosa	12	14	16	20	22	-	17	19	21	23	29	-	-	-	-	9	11	-
Shigella dysentarie	-	-	7	9	12	-	-	6	8	11	13	-	-	-	7	9	12	-
Salmonella typhi	10	12	14	16	19.5	-	20	22	25	26	31	-	-	-	-	7	10	-
Aspergillus flavus	-	5	8	10	13	-	6	8	10	12	10	-	-	-	-	-	-	-
Aspergillus niger	-	-	4	6	9	-	-	5	7	10	12	-	-	-	-	-	-	-
A. clavatus	-	6	7	8	11	-	8	8	10	12	12	-	-	-	-	-	-	-
Candida albicans	-	10	12	14	14	-	7	9	12	13	13	-	-	-	-	-	-	-

Our findings with the comparative aqueous and ethanol seed extract of *P. guineense* correlates with the studies of Nwinyi et al. [28], where they reported that ethanol extract of *P. guineense* has less antimicrobial activity than the aqueous extract. This may be due to the solubility of the active compounds in water or the presence of inhibitors of antimicrobial components in ethanol. The antimicrobial effect of the seed solvents extracts of *P. guineense* may be attributed to the phytochemicals present in it; hence notable quantities of carotenoids, flavonoids, tannins, saponins and alkaloids were detected. This property in the seeds of *P. guineense* has been evaluated to be desirable as spices on condiments in food preparation. *P. guineense* seeds have been known also to stimulate the production of hydrochloric acid in the stomach and promote the health of the digestive tract. The high level of sensitivity observed in the aqueous extracts towards the microbial pathogens showed that the active components were soluble in water [29,30]. From the results there were variations in the degrees of antimicrobial activities of the extracts on the test microbial isolates. The variation is presumed to be due to different active compounds present in the seed of *P. guineense*. Comparing the sensitivity of the bacterial strains to both the plant extracts and to synthetic antibiotics, the result showed that the plant extracts can be used as an alternative to the antibiotics as the zones of inhibition shown were very comparable and the plant extracts have lesser side effects which are often associated with the use of antibiotics [31].

The seed extracts of *P.guineense* showed antifungal activity on the investigated filamentous fungi and yeasts. Previous antifungal effect with the plant extract of *P. guineese* has been reported by Gabriele [32].

CONCLUSION

The seed extracts of *P. guineense* demonstrated notable antimicrobial potentials and were also screened of vital phytochemical agents of value in medicine known for the treatment of diseases. The aqueous extract exhibited greater antimicrobial activities than ethanol and n-Hexane extracts against the microorganisms. The use of the seeds of *P. guineese* by traditional healers in all culture of the world could be traced to the portrayed plant chemicals that a effective in microbial inhibition, thus its uses as prevention, treatment and cure of diseases of microbial and non-microbial disease origins.

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