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Assessment of the antimicrobial activity of aqueous and ethanolic extracts of *Piper guineense* leaves

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The antimicrobial activity of aqueous and ethanolic extracts of leaves of *Piper guineense* was determined on some bacteria and fungi, namely, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans* and *Saccharomyces cerevisiae*, using agar well diffusion method and minimum inhibitory concentration (MIC). The ethanolic extract was found to show more activity than the aqueous extract on all the isolates. The diameter of zones of inhibition for the ethanolic extract ranged between 2 and 12 mm, while that of aqueous extract ranged between 5 and 8 mm. The MIC of the ethanolic extract was from 2.5 to 10 mg/ml, while for aqueous extract, the MIC was 10 to 20 mg/ml. *Escherichia coli* was found to show the greatest sensitivity, while *P. aeruginosa* showed the least sensitivity of all the isolates. The phytochemical analysis carried out on *P. guineense* leaves revealed the presence of alkaloids, tannins, saponins, glycosides and flavonoids. The presence of these phytochemicals supports the use of this plant as antimicrobial agent. *P. guineense* can therefore be used as antimicrobial agent.

Key words: *Piper guineense*, antimicrobial, extracts, aqueous, ethanol, phytochemical.

INTRODUCTION

Plants have provided a source of inspiration for novel drug compounds, as plant-derived medicines have made large contributions to human health and well being. Plant extracts have been used for a wide variety of purposes for many thousands of years (Jones, 1996). The antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservatives, pharmaceuticals, alternative medicine and natural therapies (Reynolds, 1996; Lis-Balchin and Deans, 1997).

The use of traditional medicine is wide spread throughout the world. The term, traditional medicine, is interchangeably used with herbal medicine and natural medicine medicine (Hazan and Atta, 2005). Since antiquity, man has used plants to treat common infectious diseases and even long before mankind discovered the existence of microbes; the idea that certain plants had healing potential was well accepted (Rios and Recio, 2005). A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs.

A number of plants have been used in traditional medicine for many years due to their antimicrobial properties (Sofowora, 1993). Specifically, the medicinal value of these plants lies in some chemical substances

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that produce a definite physiological action on the human or animal body (Edeoga et al., 2005). The most important of these bioactive constituents which are mainly secondary metabolites are alkaloids, flavonoids, tannins and phenolic compounds. These phytochemicals are toxic to microbial cells. There is growing interest in exploiting plants for medicinal purposes especially in Africa. This stems from the fact that microorganisms are developing resistance to many drugs and as such created situation where some of the common and less expensive antimicrobial agents are losing effectiveness (Montefore et al., 1989). Medicinal plants generally contain a number of compounds which may be potential natural antibacterial for the treatment of common bacterial infections (Ratnasooriya et al., 2005). Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Kareem *et al.,* 2010). Herbal medicine which uses medicinal plants primarily presents as an alternative to such situation (Sofowora, 1993).

Medicinal plant such as *Piper guineense* has been asserted to provide various culinary and medicinal properties (Scott et al., 2005). These medicinal properties exert bacteriostatic and bactericidal effects on some microorganisms. These effects have been attributed to the peptides, alkaloids, essential oils, phenols and flavonoids which are major components in these plants (Okigbo and Igwe, 2007).

P. guineense, commonly referred to as African black pepper or Ashanti pepper, is very similar to *Piper nigrum* which is the true pepper of commerce from which black and white peppers are processed (Isawumi, 1984). *P. guineense* belongs to the family Piperaceae. It has more than 700 species throughout the tropical and subtropical regions of the world. It is known with different vernacular names in Nigeria: Igbo (Uziza) and Yoruba (Iyere). *P. guineense* has culinary, medicinal, cosmetic and insecticidal uses (Dalziel, 1955; Okwute, 1992). The insecticidal activity of *P. guineense* against *Zonocerus variegatus* is attributable to the piperine-amide component of the plant. The leaves are considered aperitive, carminative and eupeptic. They are also used for the treatment of cough, bronchitis, intestinal diseases and rheumatism (Sumathykutty et al., 1999).

In this study, the antimicrobial activity of crude extracts of the leaves of *P. guineense* has been studied as part of the exploration for new and novel bioactive compounds.

MATERIALS AND METHODS

Collections of samples

The samples of the leaves of *P. guineense* were bought from Ogige market in Nsukka, Enugu State, Nigeria. The variety was chosen because it is widely used in all parts of the country as spice, condiment and in soup making. The plant was identified and authenticated at the herbarium of the Department of Botany, University of Nigeria, Nsukka, Enugu State, Nigeria. The samples were thoroughly washed with tap water and rinsed with distilled water. A voucher specimen was deposited at the herbarium for reference purposes.

Preparation of sample

The washed samples were dried at room temperature for one week after which they were finely ground. The ground samples were put into sterile screw capped container and stored under refrigeration condition in preparation for the extraction process.

Extraction procedure

The extraction was done by the soaking method using two solvents, namely, deionized distilled water and ethanol. Different 200 g portions of the powdered sample were differently extracted with 200 ml distilled water and 200 ml of 70% (v/v) ethanol in different 500 ml Erlenmeyer flasks. The samples were soaked for about 72 h with intermittent shaking after which they were filtered using Whatman No. 1 filter paper. The extracts were then concentrated by evaporating to dryness using rotary evaporator at a temperature of 40°C. A dark-green coloured mass of *P. guineense* was obtained and stored in airtight bottles at 4°C in a refrigerator until it is ready for use.

Reconstitution of extract

The stored extracts were reconstituted using the corresponding solvents to obtain stock solutions which were further diluted serially to obtain concentrations of 1.0, 2.5, 5.0, 10.0 and 20.0 mg/ml prior to determination of the minimum inhibitory concentration (MIC).

Preparation of test organisms

Microorganisms were obtained from the culture collections of the Department of Microbiology, University of Nigeria, Nsukka. Organisms were as follows: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans* and *Saccharomyces cerevisiae*. The organisms were maintained on nutrient agar and Sabouraud dextrose agar (SDA) slants at refrigeration temperature. Overnight cultures were prepared by inoculating approximately 2 ml nutrient broth or Sabouraud dextrose broth with colonies of the appropriate organism taken from the agar slant. Broths were incubated overnight at 37°C for bacteria and 30°C for fungi. Inocula were prepared by diluting overnight cultures in saline to approximately 10^8 cfu/ml for each of the organisms.

Determination of antimicrobial activity of the extracts

The antimicrobial screening of the aqueous and ethanolic extracts was carried out using the agar well diffusion method as described by Lino and Deogracious (2006). Nutrient agar and SDA were, respectively, poured into sterile Petri dishes and allowed to solidify. About 1.0 ml of the test culture was dropped on the appropriate solidified agar and spread over the surface of the medium using a spreader. Wells of approximately 6 mm in diameter were made in the agar medium using sterile cork borer. Each well was filled with 0.2 ml of the appropriate concentration of each extract. The dishes were allowed to stand for 40 min at room temperature to allow for proper diffusion of the extract to occur. Control experiments were set up with 0.2 ml of 70% ethanol and 0.2 ml distilled deionized water in separate wells. The plates were incubated at 37°C for 24 h for the bacteria and at 30°C for 48 h for fungi. All tests were performed in duplicates and antimicrobial activity was expressed as the mean diameter of the clear zone (mm) produced by the plant extracts.

Determination of MIC of extracts

The MIC of the crude extracts was carried out using a modified method of Akinpelu and Kolawole (2004). Serial dilutions of the crude extracts were prepared and 2 ml aliquots of the different concentrations of the solution were added to 18 ml of pre-sterilized molten nutrient agar or SDA for bacteria and fungi, respectively, at 40°C to give final concentration regimes of 1.0, 2.5, 5.0, 10.0 and 20.0 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry before streaking with 18 h old bacterial and fungal cultures. The plates were later incubated at 37°C for 24 h and at 30°C for up to 48 h for bacteria and fungi, respectively, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

Phytochemical analysis

The phytochemical analysis of the ground leaves of *P. guineense* was performed following the methods described by Trease and Evans (1989) and Harbone (1998). The phytochemicals analysed for were plant secondary metabolites which included flavonoids, tannins, saponins, alkaloids and glycosides.

RESULTS

Antimicrobial activity of the extracts

The results of the antimicrobial activity of the extracts against the test organisms, namely, *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis*, *Candida albicans* and *S. cerevisiae* are shown in Table 1. The extracts showed varying degrees of growth inhibition against the isolates. The mean zones of inhibition of growth of the isolates are a function of relative antimicrobial activity of the extracts. The ethanol extract showed higher growth inhibition (2 to 12 mm) than the aqueous extract (5 to 9 mm) against all the isolates. Ethanol and distilled deionized water which served as control showed no activity against the test organisms. The antimicrobial activities of the aqueous and ethanol extracts appeared to be broad spectrum as their activities were independent of Gram reaction. The inhibition zone for *P. aeruginosa* was the least and that for *E. coli* was the highest compared to the other isolates tested.

MIC of the extracts

The results of the MIC of the extracts against the tested isolates are shown in Table 2. The MIC of the ethanol extract for the different organisms ranged between 5.0 and 10.0 mg/ml, while that of the aqueous extract ranged between 10.0 and 20.0 mg/ml. Lower MIC values were obtained for the aqueous extracts. Higher concentrations of the *P. guineense* extracts were needed to inhibit *P. aeruginosa* and the fungi when compared with the other isolates. The MIC of the aqueous extract of *P. guineense* were 20.0 and 10.0 mg/ml for *P. aeruginosa* and *E. coli*, respectively, while that of the ethanol extract were 10.0 and 5.0 mg/ml for *P. aeruginosa* and *E. coli*, respectively.

Phytochemical analysis

The phytochemical analysis of *P. guineense* leaves revealed the presence of flavonoids, tannins, saponins, alkaloids and glycosides.

DISCUSSION

The results of this study showed that the leaf extracts of *P. guineense* inhibited the growth of all the microbial isolates tested. This suggests that the leaf extracts have antimicrobial activity. The extracts are equally broad spectrum in activity as their activities were independent of Gram reaction. Higher antimicrobial activity of the extracts was observed against *E. coli* followed by *S. aureus*. The antimicrobial effect of *P. guineense* extracts is attributable to the phytochemical constituents present in it. The *P. guineense* leaves are rich in phytonutrients such as flavonoids, tannins, saponins, glycosides and alkaloids.

These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with proline-rich proteins (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003) and they hasten the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004).

The biological function of flavonoids includes protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors (Okwu, 2004). These observations therefore support the use of *P. guineense* in herbal cure remedies. The plant, *P. guineense*, also contains alkaloids which are ranked the most efficient therapeutically significant plant substance. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects. They exhibit marked physiological activity when administered to animals.

In the present study, the aqueous extract of *P. guineense*, generally, showed less antimicrobial activity than the ethanolic extract against the isolates. This observed difference may be due to insolubility of the active compounds in water or the presence of inhibitors to the antimicrobial components (Okigbo and Ogbonnanya, 2006). The less activity of the water extract than ethanol extract against most microbial strains investigated in this

Table 1. Antimicrobial activity of leaf extracts of *P. guineense*.

Test organism	Zone of inhibition (mm)									
	Aqueous extract (mg/ml)					Ethanol extract (mg/ml)				
	1.0	2.5	5.0	10.0	20.0	1.0	2.5	5.0	10.0	20.0
S. aureus	-	-	-	5.0	8.0	-	-	2.0	5.0	10.0
E. coli	-	-	-	5.0	9.0	-	-	5.0	8.0	12.0
P. aeruginosa	-	-	-	-	5.0	-	-	-	4.0	8.0
B. subtilis	-	-	-	5.0	8.0	-	-	4.0	6.0	9.0
C. albicans	-	-	-	-	6.0	-	-	-	5.0	8.0
S. cerevisiae	-	-	-	-	6.0	-	-	-	5.0	9.0

Table 2. Minimum inhibitory concentration of *P. guineense* leaf extracts.

Test organism	Aqueous extract (mg/ml)	Ethanol extract (mg/ml)
S. aureus	10.0	5.0
E. coli	10.0	5.0
P. aeruginosa	20.0	10.0
B. subtilis	10.0	5.0
C. albicans	20.0	10.0
S. cerevisiae	20.0	10.0

investigated in this study is in agreement with previous works which showed that aqueous extracts of plants generally showed little or no antibacterial activities (Aliero et al., 2006; Ashafa et al., 2008). It has been reported by Okigbo and Ajale (2005) and Okigbo et al. (2005) that inactivity of plant extracts may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials.

There are variations in the degrees of antimicrobial activities of the extracts on the isolates. The variation is presumed to be due to differences in responses by the isolates to different active compounds present in the plant. Ethanol extracts of *P. guineense* showed more antimicrobial activity against *E. coli* and *Bacillus subtilis* than the other isolates. Moreover, *P. aeruginosa* showed the highest level of resistance to the extracts than all other bacterial isolates.

Conflict of Interests

The author(s) have not declared any conflict of interests.

Conclusion

It can be concluded from this study that the leaf extracts of *P. guineense* showed antimicrobial activity against the tested isolates. Therefore, the plant can be of use in phytomedicine and can be included in health care delivery system particularly in the developing economies. The chance to find antimicrobial activity was more apparent in ethanol than water extracts of the plant. Further studies on more effective methods and other solvents for extracting only the necessary constituents as well as other processing and purification measures would be necessary. The effect of the plant on more pathogenic organisms and toxicological investigations need to be carried out.

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