

Research Article

Ethnobotany and the Antibacterial Activity of two Indigenous Spice Plants in Healthcare System of Ogbia-Speaking People of Bayelsa State Nigeria

Ihinmikaiye Samuel Olatokunbo^{1*}, Ochekwu, Edache Bernard², Ikuli, Josiah Muonam³, Akinjagunla, Atinuke¹ Odinika, Sylvia Ogechukwu⁴

¹Department of Plant Science and Biotechnology, Federal University Otuoke, Nigeria

²Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria

³Department of Crop and Soil Science, Niger Delta University Bayelsa State, Nigeria

⁴Department of Microbiology, Federal University Otuoke, Nigeria

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Abstract: Ethnomedicinal survey and antibacterial study of ethanol and aqueous extracts of *Piper guineense* and *Tetrapluera tetraptera* were carried out in Ogbia communities and Microbiology Laboratory Federal University Otuoke respectively. Rapid appraisal method and Use-Value index were applied to fetch information on ethnobotanical use of the two spice plants (commonly used among Ogbia people in Bayelsa State), and their antibacterial activities were tested on *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*. Ethnographic data shows that the principal health-care uses of the spices correspond to gastro-intestinal treatment, and fruits were the most important section utilized for managing health disorders. The fruit extracts yielded strong antibacterial activity against the organisms at the treatment regimens (200-12.5mg/ml), the zones of inhibition in both case decreases down concentration gradients. *Escherichia coli* shows the widest Zone-of-Inhibition in *Piper guineense* at each concentration regimen. The widest Zone-of-inhibition in *Tetrapluera tetraptera* extracts were recorded against *S. aureus*, and the lowest Minimum-Inhibitory-Concentration, 2.5mg/ml occurred at the assay of *Piper guineense* against *E. coli*, and similar trend occurred in *Tetrapleura tetraptera* extracts. Also Minimum-Inhibitory-Concentration of 2.5mg/ml and 5mg/ml was recorded on *Streptococcus pyogene* in ethanol and aqueous extract respectively, justifying the ethnomedicinal uses of the spices among the people.

Keywords: Ethnomedicine, Antibacterial activity, Plant extracts, test organisms, Zone of Inhibition, MIC.

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INTRODUCTION

Spice plants are vital components of most Nigeria dishes; they reflect the people's history, culture and geographical location. Spices are plant materials primarily use for garnishing, seasoning or enhancing the taste of local and exotic cuisines [1]; and also for cosmetic, insecticides and curative needs [2]. They are mostly plant parts such as- seeds, fruits, leaves, twig, roots, barks, buds or whole plant: which could be used fresh or dried; whole or grind. Plant species used for spices are usually aromatic and pungent by nature [3], due to the varying degree of essential oils and rich phytochemicals lock up in them [1]. FAO [4] and, Kayode & Ogunleye [5] stressed that spice plants are the most commonly use plant in West Africa, due to their curative value [6].

The use of plant extracts as potential antibacterial agents have been well reported [7-10]. Their medicinal properties have been asserted to exert bacteriostatic and bactericidal effects [11-13] which when employ particularly in the fight against pathogens resistant to major class of antibiotics could prove effective [14]. *Piper guineenses* and *Tetrapleura tetraptera* are two spice plants commonly use in culinary, primarily for health management among Ogbia people, who resides mostly in the tropical forest of Bayelsa State; among who spice plants are employ as tools to manage health disorder either singularly or in combination of other herbs. The antibacterial effect of these plants on certain bacteria isolates (which seem to be the main causal agents of a number of illnesses in the area) has not yet been ascertained, hence this study aimed at documenting ethnobotanical information on the spices, as well as evaluating their antibacterial activities.

METHODOLOGY

Description of the Study Area

Ogbia is one of the three Local Government Areas (LGAs) that constitute Bayelsa East senatorial district. The LGA occupies 1,670km², and its human populations is about 189,926. Ogbia LGA is located on the Cartesian coordinate of Lat 04^o 45 and 6^o 19¹ N and Long 4^o76 and 6^o32¹ E, and is constituted of about 40 communities, some are situated onshore and others are offshore. In fact, Ogbia encompasses Oloibiri, a community where oil struck in quantities sufficient for commercial production in Nigeria was first found in 1956. The oil rich LGA lies within the heaviest rainfall area of Nigeria, and its mean monthly temperature is in the range of 26^oc and 31^oc. The indigenous people are predominantly subsistent farmers, yet a small proportion engages in wild fruit gathering, fishing, hunting, lumbering, palm-oil processing, weaving and trading.

Ethnobotanical Survey

A rapid appraisal method was used to gather ethnobotanical information on *P. guineense* and *T. tetraptera*, commonly used spices among indigenous people of Ogbia LGA. The information was fetched by an open-ended conversation and semi-structured questionnaire matrix following Martin [15]. This allows individual informant to express personal viewpoints freely without being interrupted or contradicted by others. Ten communities (Kolo2, Elebele, Otuabula, Opume, Ewoi, Emeyai, Oloibiri, Otuasega, Oruma and Imringi) were selected for the study. And five elderly people (in each of the community) who have maintained continuous domicile for a minimum period of twenty-five years were identified and interviewed in respect to their knowledge of the spices. Acceptability and suitability of each species were determined in respect to their reported use, using Use-Value index for one species (UV_{is}).

$$\text{Use Value (UV}_{is}) = \frac{\sum U}{N_s} :$$

Where U is the sum of the total number of use citations by all informants for a given species; N_s is the total number of informants. This method quantifies the relative importance of each species based on its relative use among the informants [16]. Also data obtained were subjected to descriptive statistics such as frequency and analysis of variance using SPSS 22 version.

Preparation of the Plants Extracts and Antimicrobial Analysis

The two spice plants assessed were subjected to antimicrobial test on the basis of the information provided by the respondents. The spices (fruits of *Piper guineense* and seeds of *Tetrapleura tetraptera*) were air dried under hot air oven at 55°C for 3 hours and pulverized. Subsequently, thirty gram (30g) of the pulverized samples were extracted successively with 300 ml of ethanol (56 - 60°C) and de-ionized water (90

- 100°C) in Soxhlet extractor until the extracts were clear. The extracts were evaporated to dryness using rotary evaporator and the resulting (pasty form) extracts were stored in labeled sterile plastic containers at 4°C in a refrigerator.

Test Microorganisms and Standardization

Clinical isolates of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the Microbiology Laboratory Federal Medical Centre, Yenagoa, Bayelsa State. The microbes were maintained on nutrient agar slants in a refrigerator at 4°C prior to use.

Determination of Antibacterial Activity

The antibacterial activity was determined by agar well diffusion method of Perez *et al.*, [17]. Bacterial inoculum was prepared by inoculating a single colony of test organisms in 5 ml of Nutrient broth at 37°C for 3 - 5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standard solutions. Nutrient agar plates of Mueller Hillton were prepared and inoculated with the test organisms by spreading the bacterial inoculums on the surface of the media aseptically (streak method) with the help of sterile wire loop. Subsequently, prepared filter disc (of 6mm in diameter) with different concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of both ethanol and aqueous extracts were made and placed on the plates inoculated with the test organisms. The plates were incubated at 37°C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of the zone of inhibition and recorded in mm.

Determination of the Minimum Inhibitory Concentration (MIC)

MIC extracts of *P. guineense* and *T. tetraptera* on the isolate were determined using tube dilution method of Cheesbrough [18]. Various concentrations of the extracts (20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml) were prepared by making tenfold dilution of the extracts, and introduced into different test tubes containing nutrient broth inoculated with 18 hours' cultures of the test organisms. The test tubes were incubated for 24 hours at 37°C. The lowest concentration of the extracts with no visible growth of the isolates was considered as the minimum inhibitory concentration for the isolate.

RESULTS AND DISCUSSION

The Use value (UV) quantified the relative importance, the frequency in which the spice plants were mentioned, in conjunction with the number of uses (report use) mentioned per plant species (Table-1). The Table reveals four uses of *T. tetraptera*, five uses of *P. guineense*, as well as their UV indices. Fruits were reported as the section mostly used for managing health

disorders in the two spectrums, whereas seasoning scored the highest UV index (1.00) of the individual spice, followed closely by their use for managing gastro-intestinal disorder 0.80 and 0.88 for *T. tetraptera* and *P. guineense* respectively. This infers that seasoning is the primary use of the species; however basic acquaintance led credence to their use in health management, and this constitute the secondary use. Moreover, ethnographic data revealed that the main secondary function correspond to gastro-intestinal use, this suggests that the plants yield antibacterial activity against gastro-intestinal tract bacteria. Ethnobotanical researches executed among different ethnic groups indicated the existence of vast knowledge on medicinal plants among indigenous people [19, 20], however the wide spread use of the species could be attributed to cultural acceptability, efficiency against specific diseases, physical accessibility and economic affordability [21].

The results of antibacterial activity of ethanol and aqueous extracts of *Piper guineense* against the test organisms (*S. aureus*, *S. pyogenes*, *E. coli* and *P. aeruginosa*) are presented in Figure 1 and 2. The test organisms were associated with gastric disorder related ailments. The Figures reveal that the fruit extracts yielded strong antibacterial activity against the test organisms at the treatment regimens (200-12.5mg/ml). The zones of inhibition in both case decrease down concentration gradients; and the extracts were significant ($p < 0.05$) in effect against the test organisms. *E. coli* shows the widest zone of inhibition at each concentration regimen compares to others ($p < 0.05$), followed closely by *P. aeruginosa*; while *S. aureus*, yielded the least inhibitory zones in the extracts regimens (Fig 1 and 2).

The trends observed in Fig 3 and 4; favoured the ethanol extract. The widest zone of inhibition was recorded against *S. aureus* at 14.25mg/ml, and the

aqueous extract of the same isolate at 10.5mg/ml; followed by *E. coli* at 11.75mg/ml and 9.75mg/ml respectively. The least zone of inhibition was recorded against *P. aeruginosa* at 1.0 mg/ml and 0.5mg/ml in ethanol and aqueous extracts respectively. The extracts were effective against the test organisms ($p < 0.05$), and the inhibitory zones are directly proportional to concentration. This corroborated Adeniyi *et al.*, [22] and Bansa & Ngbede [23], who noted that Inhibitions expressed by extracts are dose dependent and large size inhibitory zones indicated the potency of the active principles of the plant.

The MIC of ethanol and aqueous extracts of *P. guineense* and *T. tetraptera* assayed against the test organisms are shown in Table 2 and 3 respectively. The Minimum Inhibitory Concentration (MIC) of ethanol and the aqueous extracts of *P. guineense* were comparable. The lowest MIC, 2.5mg/ml occurred at the assay of *P. guineense* against *E. coli*. Similar trend occurred in the MIC of ethanol and aqueous extracts of *T. tetraptera* assayed against the test organisms Table-3. However, the least MIC (2.5mg/ml) was recorded on *S. pyogene* in ethanol extract; the value doubled (5mg/ml) in aqueous extract of the same organism when compared. Variations in the susceptibility of the test organisms to the extracts reveal the degrees of antibacterial activities of the extracts on the isolates. The variation is attributable to physiological differences of the test organisms to the different actives compounds present in the plants [24, 8].

The spice plants, *P. guineense* and *T. tetraptera* used in this study exhibited antibacterial activities: these findings justify their ethnomedicinal uses among the indigenous people, in the management of gastrointestinal disorders. Further study is required to determine the active ingredients responsible for the antibacterial effect of the spice plants.

Table-1: Status and Use-value of the Spice Plants

IBotanical name /Family	Common name	Habit/ Part use	Report Use	*UV _{is} index
1. <i>Tetrapleura tetraptera</i> (Schumach.) Taub. Caesalpiniaceae	Aidan (Asieyesiyie)	Tree/Fruit	for: Seasoning	1.00
			Flu treatment	0.72
			Gastro-intestinal disorder	0.80
			Convulsion	0.64
2. <i>Piper guineense</i> (Schumach. & Thonn.) Piperaceae	Black pepper (Eziza)	Vine/Fruit	for: Seasoning	1.00
			Cough	0.72
			Gastro-intestinal disorder	0.88
			Post partum care: to stabilize womb	0.84
			Pain relieve	0.64
*Use Value index for one Species				

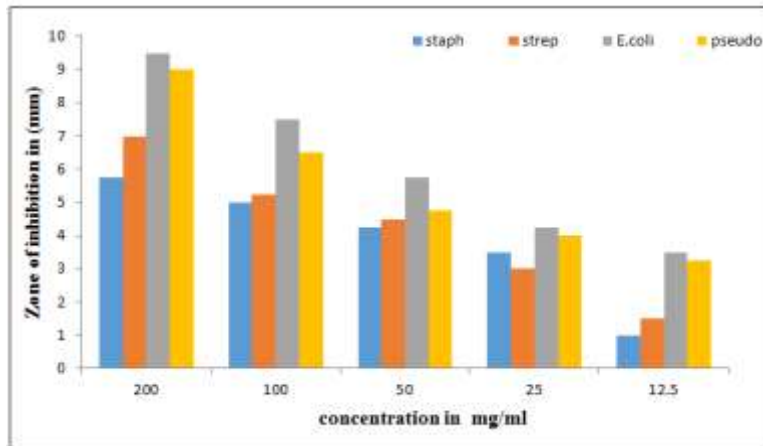


Fig-1: Antibacterial activity of ethanol extracts of *P. guineense* fruits against test organisms

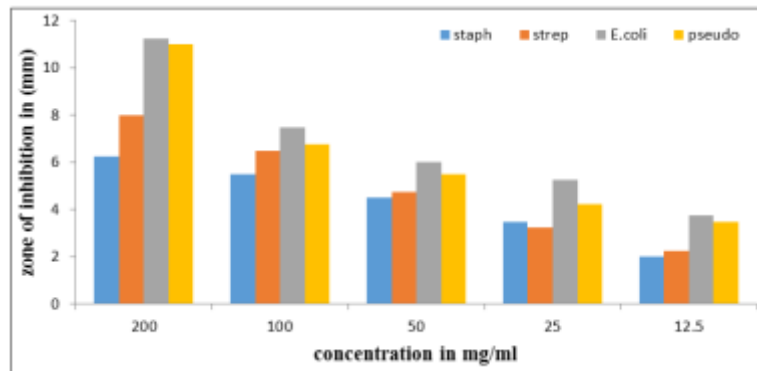


Fig-2: Antibacterial activity of aqueous extracts of *p. guineense* seeds against test organisms

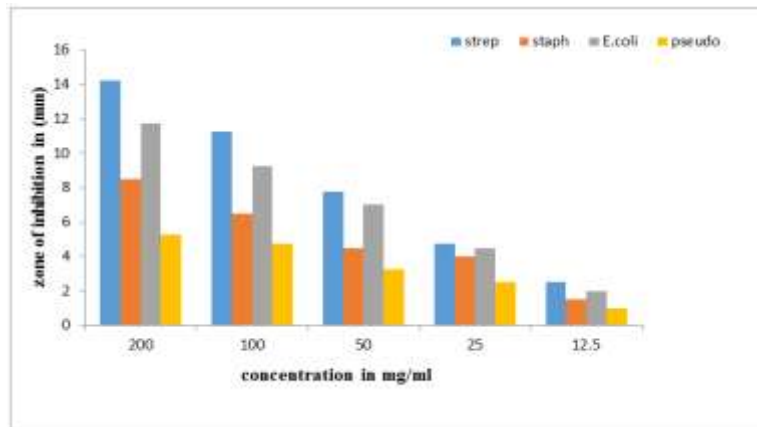


Fig-3: Antibacterial activity of ethanol extracts of *T. tetraptera* fruit against test organisms

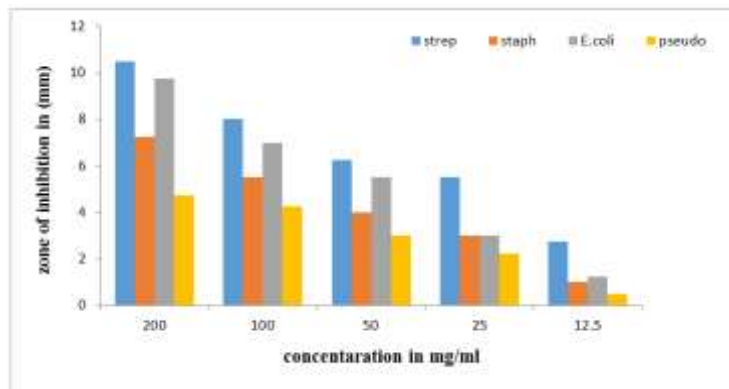


Fig-4: Antibacterial activity of aqueous extract of *T. tetraptera* fruit against test organisms

Table-2: Minimum Inhibitory Concentration (MIC) of Ethanol and Aqueous Extracts (of *P. guineense*) on Different Test Organisms

Test Organisms	MIC of Ethanol extract					MIC of Aqueous extract				
	Concentration of the Extract (mg/ml)					Concentration of the Extract (mg/ml)				
	20	10	5	2.5	1.25	20	10	5	2.5	1.25
<i>Staphylococcus aureus</i>	-	-	+	+	+	-	-	+	+	+
<i>Streptococcus pyogene</i>	-	-	-	-	+	-	-	-	+	+
<i>Esherichia coli</i>	-	-	-	-	+	-	-	-	-	+
<i>Pseudomonas aeruginosa</i>	-	-	-	+	+	-	-	-	+	+
Key + = Growth - = No growth										

Table-3: MIC of Ethanol and Aqueous Extracts (of *T. tetraptera*) on Different Test Organisms

Test Organisms	MIC of Ethanol extract					MIC of Aqueous extract				
	Concentration of the Extract (mg/ml)					Concentration of the Extract (mg/ml)				
	20	10	5	2.5	1.25	20	10	5	2.5	1.25
<i>Staphylococcus aureus</i>	-	-	+	+	+	-	+	+	+	+
<i>Streptococcus pyogene</i>	-	-	-	-	+	-	-	-	+	+
<i>Esherichia coli</i>	-	-	-	+	+	-	-	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	+	+	+	-	+	+	+	+
Key + = growth - = No growth										

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AUTHORS' CONTRIBUTIONS

Ihinmikaiye, S. O.; Ochekwu, E. B. and Odinika, S. O carried out the field ethnobotany research and draft the manuscript. Odinika, S. O., Ihinmikaiye, S. O. and Akinjagunla, A. carried out the microbial laboratory study. Ikuli, J. M. and Akinjagunla, A. participated in the field research and help confirm the species determination. All authors read and approved the final manuscript.

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