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Antimicrobial Activity, Phytochemical Screening and Nutrient Analysis of *Tetrapleura tetraptera* and *Piper guineense*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RUBE, UOE, UME, GMI, CAE and APE designed the study, wrote the protocol and interpreted the data. Authors RUBE, APE and UOE anchored the field study, gathered the initial data, performed preliminary data analysis, managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

The study was carried out to examine the antibacterial, antifungal activity, and minimum inhibitory concentrations (MIC) of the petroleum ether, aqueous and ethanolic extracts of *Tetrapleura tetraptera* and *Piper guineense* in additional to their phytochemical screening and proximate composition analyses. All analyses were done using standard techniques. The bacteria isolates used were *Esherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* while the fungi were *Aspergillus, Mucor* and *Rhizopus* species. The highest inhibition of 37.50 mm was seen using aqueous extract of *P. guineense* on *E. coli*. Consistently, the ethanolic and petroleum ether of *P. guineense* was not inhibitory to *P. aeruginosa* and *S. aureus.* The minimum inhibitory concentration (MIC) of the *P. guineense* leaves and *T. tetraptera* fruits were 80 mg/ml for both

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plants. However, the seeds had a higher MIC of 200 mg/ml. The highest sensitivity was shown by *E. coli* while the least by *P. aeruginosa*. None of the extracts showed complete inhibitory activity against all the fungal isolates. The proximate composition of the studied plant parts shows that the leaves of *P. guineense* had the highest amount of moisture (85.15±0.01). *T. tetraptera* had the highest amount of fibre (11.38±0.02) while the seeds of *P. guineense* had the highest amount of protein (11.42±0.03). Phytochemical screening showed the presence of polyphenol, cardiac glycosides, alkaloids, reducing compounds and flavonoids in all the samples with polyphenol being the most abundant in all the samples. Given the antimicrobial potential of these spices, there is a need for more studies aimed at evaluating the bioactive components in these plants.

Keywords: Spices; antibacterial; antifungal; proximate; phytochemicals.

1. INTRODUCTION

Since antiquity, man had discovered the healing power of plants long before microbes and the diseases they cause became known [1]. A number of plants have been used in traditional medicinal practice for many years due to their therapeutic properties and their number is on the increase [2,3,4]. Their use is anchored on the secondary metabolites or bioactive components or phytochemicals that these plants produce. These phytochemicals produce definite physiological action on humans, other animals and even microorganisms. They include alkaloids, flavonoids, tannins, polyphenols, steroids, and so on. As rightly observed, there is a growing interest in the exploitation of medicinal plants especially in Africa due to increasing reports of resistance and high cost of routinely used antibiotics [5,6]. Medicinal plants such as *Piper guineense* and *Tetrapleura tetraptera* although commonly used as spices have been shown to provide various medicinal properties [7,8].

P. guineense commonly referred to as African black or Ashanti pepper is akin to the *Piper nigrum* which is the true commercial pepper [9]. It belongs to the family piperaceae which contains over 700 species spread out throughout the tropical and subtropical regions of the world. The Efik and Ibibio tribes in southern Nigeria call it “Etinghene” and “Oduza”, respectively. The seeds of this plant are consumed by women after child birth to enhance uterine contraction needed for the expulsion of the placenta and other remains from the womb and also in the treatment and management of epilepsy [10,11]. Popularly used as a spices and condiments for making soups, It is also used as an adjuvant in the treatment of rheumatic pains, antiasthmatics and in weight control [2,10,12,13]. Studies have shown the presence of alkaloids, flavonoids, saponins, resins and essential oils [14]. In addition, it is very rich in nutrients [15].

Commonly called “Uyayak” by the Efik and Ibibio tribes in Southern Nigeria, *T. tetraptera* belongs to the family mimosaceae. The pods of the plants have appealing culinary use as spices. In addition, studies have shown that the fruits are used to treat an array of ailments in human such as arthritis, asthma, diabetes mellitus, leprosy, rheumatoid pains and postpartum contraction [16,17]. The dry powdered has been formulated into soaps to increase its antimicrobial activity and foaming properties of the soap. It is also used as fish poison and as an ointment on the skin [18]. A few other studies exist that indicate the antimicrobial potentials of both species on bacteria and fungi [19,20].

Despite the array of culinary, medicinal and non-medicinal benefits of these commonly used spices, there is a dearth of information on their antimicrobial activities. In addition to the proximate composition of these spices, the aim of this study was therefore to determine the phytochemical and antimicrobial activity of *T. tetraptera* and *P. guineense* against some bacteria and fungi.

2. MATERIALS AND METHODS

2.1 Source and Identification of Plants under Study

Dry *T. tetraptera* fruits and fresh leaves and seeds of *P. guineense* were purchased from Itam marketing company, Uyo Akwa Ibom State. These were identified by Mr Frank Okpoyoye of University of Calabar Botanical Garden.

2.2 Collection and Characterisation of Test Bacteria

The microorganisms used in this study were gotten from the University of Uyo Teaching Hospital, Uyo, Akwa Ibom State. The isolates were re-identified using standard microbiological

procedure and they included *Esherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* They were appropriately preserved in slants until required for use.

2.3 Collection and Characterisation of Fungi

The fungi isolates were collected from decaying plants material and were identified using cultural characterisation and microscopic examination. The fungal samples were inoculated sabouraud dextrose agar and the colony morphologies of the isolates were recorded and compared with descriptive features [21]. Slides were then prepared using methylene blue dye. The dye was dropped at the center of the clean glass slides. The hypae was then aseptically removed from the sub-cultured slants with a wire loop and leased apart on the stain. The slides preparation was carefully covered with cover slips with exclusion of air bubbles. Bloating paper was then used to remove the excess stain coming from the edge of the slides. The slides were then observed using x10 and x40 objectives to observe the different type of spore morphologies.

2.4 Plant Sample Preparation

The dry fruits of *T. tetraptera* were prepared as previously described [4,6]. Briefly, the fruits were oven dried using an electric oven maintained at 60℃ for 2 hours. Following drying, the resulting fruits were then pulverized using a mortar and pestle to produce a powder. On the other hand, the seeds of *P. piper* were air dried while the leaves were washed gently in clean tap water without squeezing. After which they were oven dried at 60℃ for 2 hours and made into powders. The powders are then stored in sample bottles until required.

2.5 Proximate Composition Analysis

The proximate components analyzed included carbohydrate, protein, fat, moisture, fibre and ash. These were done as previously described [4,6].

2.6 Preparation of Plants Extracts

This was done as previously described [6]. Ethanolic extract was of the different plants were prepared using 95% ethanol. Exactly 10 g of the powdered plant was weighed out into a sterile beaker container containing 200 ml of 95% ethanol, stirred, wrapped with aluminum foil and allowed to stay for 72 hours at room temperature (25℃). After 72 hours, it was filtered and the solvent was heated in a water bath to evaporate completely. The slurry left behind was then stored in McCartney bottles and kept at 4℃ until required for use. Aqueous extracts was obtained by weighing out 10 g of the ground plant samples separately into 200 ml of sterile distilled water in a beaker. The beaker was wrapped using an aluminum foil and kept for 72 hours. After 72 hours, the filtrate was then was heated in a water bath until a slurry was obtained and stored in McCartney bottles. The petroleum ether extracts were obtained using the Soxhlet extraction method using rotary evaporator and stored appropriately as well.

2.7 Phytochemical Chemical Screening and Quantification

The phytochemical screened for were saponins, tannins, alkaloids, flavonoids, reducing compound, anthraquinones, phlobatannins, glycosides and hydroxymethyl anthraquninones. They were done as previously described [4,6].

2.8 Antimicrobial Sensitivity Testing

Antimicrobial sensitivity testing was done as already reported by Ebana et al. [6] and CLSI [22]. Briefly, a cork borer was used to cut filter papers into tiny disks of 5mm in diameter. The disks were wrapped with aluminum foil and sterilized in the hot air oven at 40℃ for 30 minutes. Colonies of each test isolates were then sub-cultured on nutrient broth and incubated at 37℃ for 6 hours. They were then inoculated on freshly prepared Mueller-Hinton agar plates. The sterilized filter paper disks were soaked in the respective test extracts and then placed on the plates aseptically. The plates were incubated at 37℃ for 24 hours. After incubation, the zones of inhibition were determined in triplicates and the mean values determined and recorded. The antifugal activity was carried out using sabouraud dextrose liquid medium.

2.9 Statistical Analysis

The replicate data obtained in this study were managed and analysed using statistical package for social science (SPSS) version 21. The results were then presented as mean ± standard deviation. Values with p value less than 0.05 were considered significant at 95% level of significance.

3. RESULTS

The results of the phytochemical screening as presented in Table 1 showed that the aqueous and ethanolic extracts of the leaves and seeds of *Piper guineens*e are rich in phytochemicals such as tannins, flavonoids, reducing compounds, polyphenols but not phlobatannins, anthraquninones and hydroxymethyl anthraquinones. On the other hand, the aqueous and ethanolic extracts of *T. tetraptera* lacked saponins and tannins but had other phytochemicals. The most abundant phytochemicals were reducing compounds and polyphenols in both studied plants. On quantification, the most abundant phytochemicals were reducing compounds (4.60±0.02), polyphenol (7.92±0.02) and flavonoids (10.30±0.10) in *T. tetraptera* and seeds and leaves of *P. guineense*, respectively (Table 2). The proximate composition of the studied plant parts shows that both spices are very rich in basic nutrients. As expected the leaves of *P. guineense* was has the highest amount of moisture (85.15±0.01). *T. tetraptera* had the highest amount of fibre (11.38±0.02) while the seeds of *P. guineense* had the highest amount of protein (11.42±0.03) (Table 3).

Antimicrobial sensitivity of the extracts shown in Table 4 reveals that the petroleum ether extracts of the seeds of *P. guineense* did not show any antimicrobial activity on the test isolates. On the other hand, the petroleum ether extract of *T. tetraptera* showed inhibitory activity against the test isolates. The highest inhibition of 37.50mm was seen using aqueous extract of *P. guineense* on *E. coli*. Consistently, the ethanolic and petroleum ether of *P. guineense* was not inhibitory to *P. aeruginosa* and *S. aureus*.

Antifungal activity of the extracts against *Aspergillus, Mucor* and *Rhizopus* species showed varying results. None of the extracts showed complete inhibitory activity against all the fungal isolates. As shown in Table 5, all the aqueous extracts showed inhibitory activity against *Aspergillus* and *Mucor* but not *Rhizopus*. However, the petroleum ether and ethanolic extracts of *T. tetraptera*, and ethanolic extract of *P. guineense* inhibited the growth of *Rhizopus*. The minimum inhibitory concentration (MIC) of the *P. guineense* leaves and *T. tetraptera* fruits were 80 mg/ml for both plants. The seeds however had a higher MIC of 200 mg/ml (Tables 6 and 7).

Table 1. Phytochemical screening of *T. tetraptera* and *P. guineense*

Phytochemicals	*T. tetraptera*		*P. guineense leaves*		*P. guineense seeds*	
	Eth extract	Aq. extract	Eth extract	Aq. extract	Eth extract	Aq. extract
Alkaloids	+	+	++	++	++	++
Glycosides	++	+	++	+	++	++
Saponins	-	-	++	+	+	++
Tannins	-	-	++	+	-	-
Flavonoids	+	+	+	++	+++	++
Reducing compounds	++	+	++	++	+++	++
Polyphenols	++	+	++	+++	++	+++
Phlobatannins	+	+	-	-	-	-
Anthraquinones	+	+	-	-	-	-
Hydroxymethyl anthraquinones	+	+	-	-	-	-

+ = Positive and - = Negative

Table 2. Quantitative estimation of crude phytochemicals

Phytochemicals	*T. tetraptera*	*P. guineense* leaves	*P. guineense* seeds
Alkaloids	1.80±0.10*	2.20±0.10*	2.26±0.01*
Glycosides	1.72±0.02	1.57±0.01	2.53±0.01
Saponins	-	1.40±0.10	1.20±0.10
Tannins	-	0.36±0.01	-
Flavonoids	1.26±0.02	7.19±0.01	10.30±0.10
Polyphenol	4.50±0.10	7.92±0.02	9.83±0.01
Reducing compounds	4.60±0.02	7.20±0.02	9.82±0.02

**Represent significant Mean±SD that were significant across each column ($p < 0.05$) and - = Not detected*

Table 3. Proximate composition of *T. tetraptera and P. guineense* in g/100 g dry matter

Proximate components	*T. tetraptera*	*P. guineense* leaves	*P. guineense* seeds
Moisture	21.13±0.01[a]	85.15±0.01[b]	14.15±0.01[c]
Ash	3.92±0.02	3.23±0.01	1.85±0.01
Protein	4.51±0.01	5.81±0.01	11.42±0.03
Fat	0.52±0.02	2.93±0.01	8.40±0.10
Fiber	11.38±0.02	9.70±0.10	7.75±0.01
Carbohydrate	79.58±0.01	78.27±0.00	70.42±0.00

[a,b,c] Represent significant Mean±SD that were significant across each column (p < 0.05)

Table 4. Antimicrobial sensitivity of the extracts (mm)

Isolates	TT			PGL			PGS		
	PT	AQ	ET	PT	AQ	ET	PT	AQ	ET
E. coli	21.30±1.20	12.00±0.08	21.00±1.40	30.00±2.60	37.50±2 .60	23.00±2.30	-	17.60±1.80	24.00±2.30
P. aeruginosa	20.00±1.40	10.00±0.08	15.00±0.80	-	-	12.50±0.80	-	18.30±1.80	14.60±1.80
S. aureus	20.00±1.14	14.00±0.70	14.30±1.20	-	-	22.00±1.20	-	20.30±2.30	15.60±1.80

Key: *PT= Petroleum ether extract, ET= Ethanol extract and AQ = Aqueous extract. TT= T. tetraptera, PGL = P. guineense leaves and PGS= P. guineense seeds*

Table 5. Antifungal activity of the extracts

Isolates	TT			PGL			PGS	
	PT	AQ	ET	PT	AQ	ET	AQ	ET
Aspergillus sp	-	+	-	-	+	-	+	+
Mucor sp	-	+	-	-	+	-	+	-
Rhizopus sp	+	-	+	-	-	+	-	-

Key: *PT= Petroleum ether extract, ET= Ethanol extract and AQ = Aqueous extract. TT= Tetrapleura tetraptera, PGL = Piper guineense leaves and PGS= Piper guineense seeds. - = Absence of growth and + = Growth*

Table 6. Minimum Inhibitory concentration (MIC) of the petroleum ether extract of *T. tetraptera* and ethanolic extract of *P. guineense* leaves

Isolates	PGL					TT				
	20	40	60	80	100	20	40	60	80	100
Esherichia coli	-	-	±	+	++	-	-	±	+	+++
Pseudomonas aeruginosa	-	±	+	++	+++	-	-	±	+	++
Staphylococcus aureus	-	-	±	+	++	-	-	±	+	++

TT= T. tetraptera, PGL = P. guineense leaves, - = No inhibition, ± = Sparse, ++ = Good and +++ = Very good inhibition

4. DISCUSSION

According to Iwu [23], species are used for culinary purposes as well as medicinal purposes and there is usually no clear line to demarcate whether a plant should be used as food or as medicine. The plants used in this study are common species condiment for special dishes and delicacies in Akwa Ibom state of Nigeria. *T. tetraptera* popularly known as "Uyak uyak" by the Ibibio tribes is principally used for the preparation of the famous white soup of the Efik and Ibibio tribes in Nigeria where it gives a wonderful aroma to this soup. In addition to the unique aroma they impart on the food, they also have a abundance of nutrients such as protein, carbohydrate, fat and fibre [7] In addition, they are equally rich in important micro and macro minerals such as iron, zinc, copper, magnesium, calcium, and potassium [7]. It has been shown that *T. tetraptera* are rich in essential and non-essential amino acids and although they are flavouring agents, they contribute greatly to the health of the individual by supplying all the necessary amino acids [24]. The results of the proximate composition of the whole fruits in our study for fibre and protein are consistent with an earlier study [7].

In another previous study, the proximate composition of *P. guineense* seeds was found to have 12.35% moisture, 6.33% ash, 8.79% crude fibre, 9.89% crude fat, 5.86% crude protein and 57.32% carbohydrate [25]. Compared to our findings, our moisture content, protein, carbohydrate were slightly higher while the rest were lower. In another study, the proximate composition of the leaves were reported as 11.70% moisture, 7.73% ash, 9.26% fibre, 2.24% fat, 16.67% protein and 48.21% carbohydrate [26]. Compared to our findings, our moisture fibre, fat and carbohydrate contents were higher while the rest were lower. Studies have shown that *P. guineense* seeds are also rich in minerals (Calcium, magnesium, iron and phosphorus) and vitamins (A, B, C and E) [25-26].

The results of the phytochemical screening shows the presence of alkaloids, glycosides flavonoids, reducing compounds, polyphenols, tannins, phlobatannins, anthraquninones and hydroxymethyl anthraquinones in the aqueous and ethanolic extract of *T. tetraptera*. On the other hand, the aqueous and ethanolic extracts of the *P. guineense* seeds and leaves all tested negative for phlobatannins, anthraquinones and hydroxymethyl anthraquninones. In addition, the seeds also tested negative for tannins. Our findings is also consistent with earlier studies where the seeds, pulp and woody shells of the *T. tetraptera* fruits were all found to have flavonoids, saponins and tannins [7,27]. The phytochemicals in our studied *P. guineense* is consistent with the report of Nwankwo et al. [26]. On quantification, the most abundant phytochemicals were reducing compounds (4.60±0.02), polyphenol (7.92±0.02) and flavonoids (10.30±0.10) in *T. tetraptera* and seeds and leaves of *P. guineense*, respectively.

The results of the antimicrobial sensitivity of the plants under study showed varying zones of inhibition to the test bacterial and fungal isolates. The highest of inhibition for *T. tetraptera* petroleum ether extract was 21.30±1.20 against *E. coli* while the least inhibition of 10.00±0.08 was observed with *P. aeruginosa* with aqueous extract. Amongst the extracts studies, that of *T. tetraptera* was the most consistent with inhibitory activity. The findings in this study are consistent with the reports of an earlier study where they reported the highest and least zones of 26.40 mm and 11.00 mm with ethanolic and aqueous extracts against *S. aureus.* The zone of inhibition of *E. coli* in their study was 14.00 and 20.00mm using aqueous and ethanolic extracts [27]. Our findings were equally higher than the 5.00 – 18.00 mm reported elsewhere for the leaves, stem, bark and roots of *T. tetraptera* against *E. coli, S. aureus*, *Proteus mirabilis* and so on [28]. A more recent study by Oguoma et al. (2015) on the antimicrobial activity of *T. tetraptera on E. coli, S. aureus, Shigella* spp and *Salmonella typhi* gave zone of inhibition of 21.00 mm for *E. coli* and was similar to our zone of inhibition for *E. coli*.

Table 7. Minimum Inhibitory concentration (MIC) of the aqueous extract of *P. guineense* seeds (mg/ml)

Isolates	PGS				
	120	140	160	180	200
Esherichia coli	-	-	-	±	+
Pseudomonas aeruginosa	-	-	±	++	+++
Staphylococcus aureus	-	-	±	++	++

PGS= P. guineense seeds, - = No inhibition, ± = Sparse, ++ = Good and +++ = Very good inhibition

The antimicrobial of *P. guineense* leaves and seeds also gave varying results. The seed extracts was more consistent than the leaves in our study. However, the highest zones of inhibitions (37.50 and 30.00 mm) were seen with the aqueous and petroleum extracts of the leaves. Our findings were higher than those of an earlier study which recorded the highest zone of 12.00 mm at concentration of 20 mg/ml. The minimum inhibitory concentration of ≥80 mg/ml was observed for *P. guineense* seeds and *T. tetraptera* fruits. For the seeds of *P. guineense*, it was ≥180 mg/ml. Antifungal activity of the extracts against *Aspergillus, Mucor* and *Rhizopus* species showed that none of the extracts had complete inhibition against all the fungal isolates. The most consistent of all the extracts was aqueous extracts which showed inhibitory activity against *Aspergillus* and *Mucor* but not *Rhizopus*. However, the petroleum ether and ethanolic extracts of *T. tetraptera*, and ethanolic extract of *P. guineense* inhibited the growth of *Rhizopus*. An earlier study recorded no inhibition against *Fusarium oxysporum, Penicillium chrysogenum* and *Mucor* species using aqueous and ethanolic extracts of the leaves, stem, bark and roots of *T. tetraptera* [28].

5. CONCLUSION

The present study has confirmed the nutritional and potential antimicrobial importance of the both *P. guineense* and *T. tetraptera*. The proximate composition of the plants edible parts indicates the abundance of basic nutrients. The phytochemical screening reveals the presence of phytochemicals with excellent antimicrobial properties that needs further exploitation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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