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Antimicrobial, Phytochemical and Nutritional Properties of *Tetrapluera tetraptera* Seed and Fruit Extracts

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ABSTRACT

Tetrapluera teteraptera is a known spice in Nigeria and have been utilized for various medicinal purposes. However, not much scientific data is available on its antimicrobial and nutritional properties. The Antimicrobial, Phytochemical and Nutritional properties of *T. teteraptera* seeds and fruits were therefore investigated using standard analytical and microbial procedures. Yield of the seed extracts were; 22.49 and 14.16% for aqueous and ethanol extracts, respectively while 43.09 and 36.64% were recorded for the aqueous and ethanol fruit extracts, respectively. Phytochemical analysis indicated that both aqueous and ethanol fruit extracts reveal the presence of cardiac glycoside, saponins, phenols, flavonoids, terpenoids, steroids, resin, alkaloids and tannins. Steroids, saponins and cardiac glycoside were absent in the aqueous seed extract. Proximate analysis shows high amount of carbohydrate (38.26%), protein (18.66%), fats and oil (20.86%). Mineral content analysis shows that it is rich in potassium (23.29 mg/kg). The Na, Ca and Mg contents of the fruit were 2.34, 0.26 and 1.98 mg/kg, respectively. Anti-bacterial analysis showed that aqueous extract had a higher anti-bacteria activity than the ethanol extract of both the fruit and the seed of the plant. At different concentrations, the aqueous extracts were more effective against *Klebsiella pneumoniae and Staphylococcus aureus* than *Escherichia coli* and *Bacillus* sp. The MIC values ranged between 2.0 and 3.0 mg/mL while the MBC values ranged between 2.5 and 4.0 mg/mL. Further studies are needed to harness the medicinal and nutritional potentials of the plant.

Keywords: Antibacterial, Phytochemicals, *Tetrapluera tetraptera*, Spices.

Introduction

Plants have been the basis for medical treatments through much of human history, and Spices which are plant materials, are commonly used as condiments and flavouring agents in foods.[1] Plants are known sources of phytochemical compounds and have been exploited for their medicinal potentials.[1,2] In Nigeria, most spices are used for the preparation of certain type of soups which are delicacies. They have also been used in traditional medicine for fast relief of ailments such as malaria, stomach upset and tooth ache. It has been noted that traditional medicine is valued worldwide and its practice is still common place especially among the low income group.[3]

Tetrapleura tetraptera is a medicinal plant of the Mimosaceae family, single-stemmed, robust, perennial tree with dark green leaves and thick woody base with spreading branches.[4] In Nigeria, the fruit of *T.tetraptera* is variously known as Uyayak in Ibibio, Edeminang in Efik, Osakirisa or Oshosho in Igbo, Dawo in Hausa, Aidan in Yoruba[5] and Iyanghanyanghan by the Itsekiris. The plant is known to have a wide natural distribution over a large part of tropical Africa, especially in the rain forest belt of West, Central and East Africa.[4]

The fruit consist of a fleshy pulp with some small, brownish–black seeds and the fruit possesses a fragrance, which has been attributed to its insect repellent property.[6] The fruits are reported to be green when tender and dark red-brown when fully ripe.[4] He also reported that the length of the fruits ranged from 22 cm to 27 cm and a width of about 5 cm and possessing four (4) longitudinal wing-like fleshy ridges about 2 cm each. *T. tetraptera* is a known medicinal plant in Nigeria and several documented biological or pharmacological attributes which include; molluscicidal, cardiovascular, neuromuscular, hypotensive, anti-convulsant, anti-ulcerative, anti-inflammatory and anti-microbial activities have also been documented in respect of the plant.[7] The pods are reported to have an appealing culinary use for mothers from the first day of delivery to post parturition and as a lactation aid.[8] It was also reported that *T. tetraptera* fruits have been used in the management of convulsions, leprosy, inflammation and rheumatism.[9] In some parts of Nigeria, the fruits are used locally in flavoring, and in the production of pomades and soaps. Also an infusion of the whole fruit is reported to be usually taken by convalescents to bathe in order to get relief from feverish conditions, for use as relief for constipation and as an emetic.[4] A report has it that the soft parts of the fruit and the bark contain sugars, tannins, traces of saponins and amino acids.[10] Consequent upon the extensive use of *T. tetraptera* in traditional medicine and as spice in foods, this research work was designed to evaluate the antimicrobial, phytochemical and nutritional properties of the fruit and seed of the plant.

Materials and Methods

Collection and Preparation of Specimen

Samples of *Tetrapleura tetraptera* fruits were purchased from Warri municipal market located in Delta State, Nigeria and transported to the laboratory for analysis. The fruit was identified by Prof. Osondu Akoma a Botanist at the Department of Biological Sciences, Faculty of Science, Benson Idahosa University, Benin City. The seed was

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removed from the fruit and processed separately. Both the seed and the mesocarp of the fruit were air-dried in the laboratory for 5 days before they were powdered with a dry sterile Panasonic blender model MX-J120P. The grounded mesocarp and seeds were then stored in an air-tight sterile container until they were used for extraction.

Preparation of Extracts
Extracts were obtained by adopting earlier method of other authours[11] as outlined below. The powdered sample (100 g) was soaked in 400 mL of solvent (Distilled water and Ethanol) in a sterile conical flask and covered with cotton wool. It was then plugged and wrapped with aluminium foil and shaken vigorously. The mixture was left to stand for 24 h in a shaking water bath maintained at 40^{o}C. The mixture was filtered using a clean muslin cloth and Whatman No. 1 filter paper. Thereafter the filtrate was evaporated to dryness by means of a rotary evaporator attached to a vacuum pump. The evaporation was done at 50^{o}C at a rotary speed of 120 rpm. The percentage yields of the aqueous and ethanol crude extracts were determined and estimated as dry weight (extract)/dry sample weight x 100. The extracts were stored in the refrigerator until needed for further analysis.

Microorganisms
The bacteria species used in the investigation were *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Bacillus* sp. The organisms were clinical isolates obtained from the Microbiology laboratory of the University of Benin Teaching Hospital, Benin City. The cultures of bacteria were maintained on nutrient agar slants at 4^{o}C, re-identified by biochemical tests[12] and sub-cultured on nutrient broth for 24 h prior to testing.

Phytochemical screening of seed extract
Phytochemical screening of the seed extract was carried out using methods previously described.[11]

Antibacterial Activity
Antibacterial activity of the seed extracts were tested using the agar-well diffusion test. About 0.2 mL of a 24 h broth culture containing 1 X 10^6 cells/mL of organism was aseptically introduced and evenly spread using bent sterile glass rod on the surface of gelled sterile Mueller Hinton agar plates. Three wells of about 6.0 mm diameter were aseptically punched on each agar plate using a sterile cork borer, allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the Petri dish. Fixed volumes (0.1 mL) of the various concentrations (50, 100, 150 and 250 mg/mL) of the extracts were then introduced into the wells in the plates. A control well was made at the centre and filled with 0.1 mL of the extracting solvent. A separate plate containing antibiotic tetracycline (30 mg/mL) was used as positive control. The plates were allowed to stand on the bench for 40 min for pre-diffusion of the extract to occur and then incubated at 37^{o}C for 24 h. The resulting zones of inhibition were measured using a ruler calibrated in millimetres. The average of the three readings was taken to be the zone of inhibition of the test bacterial isolate at the test concentrations.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)
The MIC was determined using the tube dilution method. Standardized suspensions of the test bacterium was inoculated into a series of sterile tubes of nutrient broth containing different concentrations (1.0 to 10 mg/mL) of the extracts and incubated at 37^{o}C for 24 h. The MICs were read as the least concentration that inhibited the growth of the test organisms.
The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loopful from each tube was sub-cultured onto extract free agar plates, incubated for another 24 h at 37^{o}C. The minimum bactericidal concentration was considered as the lowest concentration that could not produce a single bacterial colony.[13]

Nutritional and Mineral content Estimation
The recommended methods of the Association of Official Analytical Chemists (AOAC)[14] were used for the determination of moisture, protein, lipid, fibre, and carbohydrate contents.

Moisture
Ten grams (10 g) of powdered seeds were dried in a thermostatically controlled ventilated oven at 105^{o}C until constant weight was obtained. The loss in weight was recorded as moisture content.

Protein
Crude protein was determined by the Kjeldahl method**.** Powdered seed (0.2 g) was digested in 2 mL concentrated H_2SO_4 in the presence of selenium catalyst, until a clear digest was obtained. The nitrogen content of digest was determined colorimetrically at 630nm. Protein was calculated as: Nitrogen content x 6.25.

Lipid
Two grams (2 g) of each of the dried samples were weighed into the porous thimble of the Soxhlet extractor with the mouth plugged with cotton wool. The thimble placed in the extraction chamber was suspended above the weighed receiving flask containing petroleum ether (b.p. 40, 60^{o}C) below the condenser. The flask was heated for eight hours to extract the crude lipid. The flask containing the crude lipid was disconnected from the Soxhlet, and then oven dried at 100^{o}C for 30 min, cooled in desiccators and weighed. The difference in weight is expressed as percentage crude lipid content.

Fibre
The crude fibre was determined as the organic residue left after treating the sample under standard conditions with petroleum ether , then boiled in 1.25% H_2SO_4 (w/v) and 1.25% NaOH (w/v) solutions. The residue after crude lipid extraction was used for the assay. Crude fibre content was expressed as percent loss in weight on ignition.

Carbohydrate
Available carbohydrate was calculated using the difference method, by subtracting total or sum of crude protein, crude lipid, crude fibre and ash from 100% dry weight sample.

Minerals
Mineral contents were obtained by ashing 2.0 g dried and powdered seed sample in a muffled furnace at 550^{o}C. The ash was dissolved in10 mL of 20% nitric acid and filtered through acid washed Whatman No.541 filter paper into a 100 mL volumetric flask. The filtrate was made up to the mark with de-ionized water and the resulting solution used for the analysis of Calcium, sodium, potassium, magnesium, zinc and Iron. The analyses were determined by atomic absorption spectrophotometry at 630 nm.[15] All analysis was done in triplicate.

Results and Discussion

Plants resources have been known to possess immense benefits to mankind. The plant *Tetrapleura tetraptera* is basically known for its use as a spice in some of Nigerian diet, however, the medicinal values cannot be overlooked. The percentage yield of the seed extracts were 22.49% and 14.16% for the aqueous and ethanol extracts, respectively while values of 43.09% and 36.64% was recorded for the aqueous and ethanol fruit extracts, respectively (Table 1). Factor like age, polarity of the solvent used and type of plant material could affect extract yield.[16] Phytochemical analysis is very useful in the evaluation of bioactive and biological components of seed and fruit parts of plants. The result of the phytochemical screening of the mesocarp (Table 2) shows the presence of phytochemical compounds such as flavonoids, phenolics, tannins, saponins, and resin. That of the aqueous extract revealed the presence of flavonoids, phenolics and cardiac glycosides. Phytochemical analysis of both the ethanol and aqueous extracts of the seed showed that compounds such as terpenoids, saponins and steroids were absent. This result is significant as compounds like flavonoids, saponins, alkaloids and tannins have been reported to possess several

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biological functions of medicinal importance such as protection against allergies, inflammation, platelet aggregation, hepatotoxins and microbes.[17,18] It has also been reported that plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects often associated with synthetic antimicrobials.[19]

The concentration of nutrients, which included carbohydrates, proteins, fats and oils, moisture, fiber and ash were; 38.26%, 18.66%, 20.86%, 15.38%, 6.84 % and 4.06%, respectively (Table 4). The Fe, Mg, P and K contents recorded for the whole fruit were; 3.60 mg/kg, 1.98 mg/kg, 0.43 mg/kg and 23.29 mg/kg, respectively (Table 5). The Na, Ca and Mg content of the fruit were; 2.34 mg/kg, 0.26 mg/kg and 1.98 mg/kg, respectively (Table 5). Nutritional analysis of the whole fruit (Table 4) indicate that it contain appreciable amount of carbohydrates (38.26%), lipids (18.66%) and proteins (20.86%). Fiber was also present in significant amount (6.84%). Mineral content analysis (Table 5) revealed that potassium was present in appreciable amount (23.29 mg/kg). Content of other minerals present in the fruit were 3.60 mg/kg, 1.98 mg/kg and 0.43 mg/kg for Fe, Mg and P, respectively. These results probably indicate the potential nutritional benefits associated with the consumption of *T. tetraptera* fruits in our diet. Also, the high macro nutritional status of the spice would justify its inclusion in the preparation of "Pepper Soup" a delicacy widely prepared and consumed in parts of Southern Nigeria.[20] Several authors have reported that regular use of plant foods rich in protein makes a valuable addition to a diet and also that consumption of foods rich in macronutrients is beneficial to the body.[21,22] The protein and fiber values recorded for the fruit contrasted with an earlier report,[23] who reported lower concentration of these parameters in *T. tetraptera* whole fruit sampled from Eke Okigwe market, Imo State. These variations may be attributed to factors such as seasonal and maturity variation, geographical origin, genetic variation, growth stages, part utilized and postharvest drying and storage.[24]

Table 1: Yield of Aqueous and Ethanol Extracts of *T. tetraptera* Seed and Mesocarp.

***T. tetraplera* extracts**	**Plant Powder (g)**	**Yield (%)**
Seed $_{aqu}$	100	22.49
Seed $_{eth}$	100	14.16
Mesocarp $_{aqu}$	100	43.09
Mesocarp $_{eth}$	100	36.64

Key: eth: ethanolic; aqu: aqueous.

Table 2: Phytochemical Constitutions of both the Aqueous and Ethanol Extracts of *T. tetraptera* Mesocarp.

Phytochemical parameter	**Aqueous extract**	**Ethanol extract**
Flavonoids	+	+
Phenolics	+	+
Steroids	+	+
Tannins	+	+
Saponins	+	+
Resin	+	+
Terpenoid	+	+
Cardiac glycosides	+	+
Alkaloids	+	+

Key: + = Present; - = Absent

Table 3: Phytochemical Constitutions of both the Aqueous and Ethanol Extracts of *T. tetraptera* Seed.

Phytochemical parameter	**Aqueous extract**	**Ethanol extract**
Flavonoids	+	+
Phenolics	+	+
Steroids	-	+
Tannins	+	-
Saponins	-	+
Resin	+	+
Terpenoid	-	-
Cardiac glycosides	-	+
Alkaloids	+	+

Key: + = Present; - = Absent

Table 4: Nutritional qualities of the whole *T. tetraptera* Fruit.

Parameter	**Content (%)**
Carbohydrate	38.26
Protein	18.66
Fat and oil (lipid)	20.86
Moisture	15.38
Fiber	6.84
Ash	4.06

Table 5: Mineral Content of the Whole *T. tetraptera* Fruit.

Parameter	**Content (mg/kg)**
Fe	3.60
Mg	1.98
P	0.43
K	23.29
Na	2.34
Ca	0.26
Mg	1.98

Results of the antibacterial capability of the extracts as indicated by the inhibitory zone of the test organisms showed that for aqueous extract from the seed, *E. coli* recorded the highest zone of inhibition (11 and 17 mm) at both the least concentration of 50 mg/mL and the highest concentration of 250 mg/mL, respectively. *S. aureus* recorded the least (8 mm) at 50 mg/mL, while at 250 mg/mL, *K. pneumoniae* recorded the least (14 mm) zone of inhibition. The ethanol extract had *S. aureus* showing the highest (7 mm) zone of inhibition at 50 mg/mL while *Bacillus* sp. had the highest (12 mm) at 250 mg/mL (Table 6).

The zone of inhibition for both *E. coli* and *S. aureus* exposed to varying doses of both the aqueous and ethanol *T. tetraptera* mesocarp extracts ranged from 20 to 30 mm, 19 to 26 mm, 24 to 30 mm and 21 to 30 mm. The inhibitory zones elaborated by both *K. pneumoniae* and *Bacillus* sp. exposed to aqueous and ethanol *T. Tetraptera* mesocarp extracts ranged from 22 to 31 mm, 18 to 23 mm, 17 to 23 mm and 16 to 22 mm (Table 7).

The observed differences in the bioactivity of both the seed and mesocarp extracts could be attributed to the presence of greater amounts of phytochemicals in the fruits especially the ethanol extract (Table 2). Previous report[25] had stated that the antimicrobial properties of spices such as *T. tetraptera* are desirable tools in the control of

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food borne infections and in food spoilage. The activity of plants extracts against various bacteria species has been a subject of intense investigation in the last three decades.[2] This is with the hope that important bioactive constituents can be found to help in combating the constantly emerging infectious diseases plaguing the populace. The trend in the degrees of activities against the organisms used is corroborated by the observation that both the seed and mesocarp extracts contained varying amounts of different phytochemicals such as flavonoids, phenolics and alkaloids which have variously been shown[26] to be the key bioactive constituents responsible for the activities against these organisms. However, the seed extracts displayed a comparative lesser bioactivity against the test isolates in comparison with the fleshy mesocarp extracts. The expressed antibacterial activity of *T. tetraptera* seed and mesocarp extracts had earlier been reported.[7] The report recorded significant antibacterial activity of both aqueous and ethanol extracts of *T. tetraptera* against *E. coli*, *S. aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*.

The Analysis of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values in this study shows that for the fruit aqueous extracts, MIC values ranged between 2.5 and 3.0 mg/mL while that of the ethanol extract ranged between 2.0 and 3.0 mg/mL and that of the seed between 2.5 and 3.5 mg/mL for the aqueous while that for the ethanol is between 3.0 and 4.0 mg/mL. The MBC values (Tables 8 and 9) also had varying interesting results. These results are significant because it means they could be of pharmacological relevance. Earlier research[25] has indicated that the active components of plant extracts can interfere with the growth and metabolism of microorganisms in a negative manner and are quantified through the determination of the minimum inhibitory concentration and the minimum bactericidal concentration and that these values can be used as guide for the treatment of most infections.

Table 6: Antibacterial Activity (zone of inhibition in mm) of the Aqueous and Ethanol extracts of *T. tetraptera* Seed.

Test bacterial isolates	Aqueous Extract				Ethanol Extract			
	50 mg/mL	100 mg/mL	150 mg/mL	250 mg/mL	50 mg/mL	100 mg/mL	150 mg/mL	250 mg/mL
E. coli	11	14	15	17	5	7	7	10
S. aureus	8	11	14	16	7	6	8	9
K. pneumoniae	9	11	12	14	5	7	8	9
Bacillus sp.	10	12	14	15	6	8	11	12

Table 7: Antibacterial Activity (Zone of inhibition in mm) of the Aqueous and Ethanol extracts of *T. tetraptera* Mesocarp.

Test bacterial isolates	Aqueous extract				Ethanol extract			
	50 mg/mL	100 mg/mL	150 mg/mL	250 mg/mL	50 mg/mL	100 mg/mL	150 mg/mL	250 mg/mL
E. coli	20	22	24	30	19	23	25	26
S. aureus	24	27	30	30	21	25	27	30
K. pneumoniae	22	25	27	31	18	20	21	23
Bacillus sp.	17	18	21	22	16	19	20	22

Table 8: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Mesocarp Extract in (mg/mL).

Isolates	Solvents			
	Aqueous		Ethanol	
	MIC	MBC	MIC	MBC
S. aureus	2.5	3.5	2.0	3.0
E. coli	3.0	3.0	2.5	3.0
K. pneumoniae	2.5	2.5	2.5	3.5
***Bacillus* sp.**	2.5	3.0	3.0	4.0

Table 9: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Seed Extract in (mg/mL).

Isolates	Solvents			
	Aqueous		Ethanol	
	MIC	MBC	MIC	MBC
S. aureus	3.0	4.5	3.0	3.5
E. coli	3.0	4.0	4.0	4.0
K. pneumoniae	3.5	4.0	3.0	3.5
***Bacillus* sp.**	2.5	3.0	3.0	4.0

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Conclusion

Conclusively, it is pertinent to state that this study reveals and confirm the therapeutic properties and justify the use of the fruits of *Tetrapleura tetraptera* in some of Nigerian diet both as spice and as a major ingredient. The resultant effect of this discovery is that considerable new drugs that could be effective against both present and emerging infections can be derived from the plant fruits and seeds. However, further studies are needed to isolate, quantify and purify the active constituents so as to harness the medicinal and nutritional potentials of the plant especially in assisting to combat antimicrobial resistance.

Conflict of Interest

The authors declare no conflict of interest

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this work will be borne by them.

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