Nutrient analysis and antimicrobial activities of the leaves and fruit pulp extracts of *Tetrapluera tetraptera* on clinical bacteria isolates

Abstract

Nutritional, phytochemical and antimicrobial activities of the leaves and the fruit pulp extracts of *Tetrapluera tetraptera* was carried out. Collection and identification of isolates and plants parts, and preparation of extracts and nutrient (proximate, vitamins and minerals and anti-nutrients) evaluations were all done using standard protocols previously reported. Evaluations of phytochemicals were carried out using various crude screening, quantification and gas chromatography coupled to mass spectrophotometer for a robust analysis. Antimicrobial activities were evaluated using the standard disc method and chloramphenicol as control. The results revealed varying amounts of proximate nutrients (ash, fibre, moisture, carbohydrate, protein and fat) in both samples. Vitamin analysis showed the presence of vitamin A, total and soluble C, niacin, riboflavin and thiamin in both samples. Furthermore, both samples had minerals Na, K, CA, P, Mg, Zn, Fe, and Cu, and K, Na, Ca, Mg, P, Fe, Zn and Cu in order of decreasing abundance in the fruit pulp and leaves, respectively. Furthermore, both showed the presence of permissible levels of anti-nutrients compared to edible vegetables. Using all three methods, several phytochemicals such as terpenes, alkaloids, flavonoids, polyphenol, organic and fatty acids, amongst other in various amounts were obtained in the samples. Antimicrobial activities against identified clinical isolates used in this study which were *Escherichia coli*, *Staphylococcus aureus* and *Proteus spp* using both extracts ranged from 12.78±0.03 to 17.34±0.34 mm. Given the rising cases of antimicrobial resistance and absence of newer antibiotics, the antimicrobial activity of our study plant is worth further evaluations.

Keywords: Phytochemical, proximate composition, clinical isolates, medicinal plant, antimicrobial sensitivity

Introduction

Since time immemorial, medicinal plants have been in use by humans to treat certain ailments, and humans in many rural regions of the world especially Africa and Asia, have been shown to rely heavily on medicinal plants for therapeutic purposes (Ebana, 2019). For instance, in Nigeria and as well Ghana, childhood cases of malaria are treated with medicinal plants (Allkin, 2017). Medicinal plants have been defined as plants or plant parts whose organ or organs contain substances called phytochemicals that are used for therapeutic purposes or from which potential synthetic drug precursors can be obtained (WHO, 1977). In many countries, medicinal plants are becoming more and more recognized globally. For instance, China, will hopefully integrate medicinal plants into mainstream health care therapeutics plans while 90% of Germans use medicinal plants(Allkin, 2017). Around the world, there are 28,187 medicinal plants recorded in Kew's Medicinal Plant Names Services (Allkin, 2017).

Tetrapluera tetraptera is a flowering plant species that is common to West Africa countries and grows wells in the rainforest fringes and are also widespread in the tropics. In Nigeria, it is called Uyayak in the south especially in Cross River and Akwa Ibom States, and Aridan (or Aidan) in western Nigeria. *Tetrapleura tetraptera* is deciduous; it reaches 20-25 m in height, with a girth of 1.5-3 m (Orwa *et* al., 2009; Schum. & Thonn, 2009). It fruit pulp is commonly used as spices in cooking several delicacies such as Banga and Pounded yam soups in southern Nigeria. As a medicinal plant, it has several documented pharmacological and therapeutic uses (Enwere, 1998;

Ekwenye and Okorie, 2010). Some of these uses include antimicrobial, molluscicidal, cardiovascular, neuromuscular, hypotensive, anti-conversant, anti-ulcerative, anti-bacterial and anti-inflammatory. Furthermore, the pods and its content are particularly used by mothers from day one of delivery to aid them with lactation (Enwere, 1998).

These properties are due to the presence of phytochemicals in these plants. Using crude screening techniques, some of these phytochemical found in *T. tetraptera* include saponin, tannin, steroids, terpenoids, alkaloids, flavonoids and glycosides amongst others (Godfrey, 2015; Ogugor et al., 2018). In our earlier study, we showed the presence of various alkaloids, saponins, flavonoids, polyphenol, anthraquinones, and hydroxylmethyl anthraquinones (Ebana et al., 2016a). Several studies abound in Eastern Nigeria that has evaluated the nutritional profile and antimicrobial potential of the various parts of the plants (Achi, 2006; Ezeonu et al., 2017; Adadi and Kanwugu, 2020). Constituently, these studies have shown the presence of various nutrients including minerals, vitamins and proximate nutrients such as water, proteins, carbohydrate, fibre and fats/oil in varying amounts as seen with the phytochemicals. The difference in the phytochemical composition and nutrients could be due to the differences in the various climatic or edaphic factors or even dependent on the solvent type (Adusei et al., 2019).

Despite advances in medical sciences especially as regards to infectious diseases therapy, infections of microbial origin still remains a challenge globally due to rising cases and emergence of antibiotic resistant phenotypes, and absence of vaccines amongst others (Ebana et al., 2019a; Njimoh et al., 2015; Ebana et al., 2019b). Several studies have shown that the study plant have abundance of phytochemical with antimicrobial activities that can be optimized and exploited further in the face of dwindling effective antibiotics. In an earlier study, the zones of inhibition reported for the pods of *T. tetraptera* ranged from 16.40 to 28.10 mm for *S. aureus, Bacillus subtilis, P. aeruginosa* and *E. coli* isolates (Achi, 2006). Thus, the aim of this study was to evaluate the nutritional potentials, phytochemicals, and antimicrobial activity of the leaves and fruit pulp of *T. tetraptera* harvested and collected from southern Nigeria state of Cross River State.

Materials and methods

Collection of study plant

The leave and fruit pulp of *T. tetraptera* used for this study was collected together from a single *T. tetraptera* plant from the University of Calabar in 2018, and identified by Prof. Rose Ebana of Obong University, Obong Ntak, Etim Ekpo LGA, Akwa Ibom State, Nigeria. The collected samples were then taken to the laboratory for further analysis.

Morphological and identification of clinical Isolates

Clinical isolates tentatively identified as *Staphylococcus aureus, Proteus species* and *Escherichia coli* were obtained from University of Calabar Teaching Hospital. The isolates were further identified as using standard morphological and biochemical tests previously reported (Holt et al., 1994; Cheesebrough, 1998). Identified isolates were stored in nutrient agar slants until required for use.

Preparation of the plant samples

Processing of the collected plants samples was done as previously described (Ebana et al., 1995; Ebana et al., 2015; Ebana et al., 2016). The freshly collected leaves and dry pods were first sun-dried for 48 hours. After sun drying they were then pulverized mechanically into powder and further dried to get rid of any extra moisture. The powders were then stored for further use in sterile moist free bottle at room temperature until required for further use.

Preparation of extracts

From the powders, ethanolic and aqueous extracts were prepared as previously reported (Ebana et al., 1995; Ebana et al., 2015; Ebana et al., 2016a). For the preparation of the aqueous extract, 20g of the powder was weighed out using an electronic weighing balance and then dissolved in 200ml of sterile distilled water and stirred using a sterile glass stirrer, and allowed to stand for 48 hours. The mixture was then filtered using Whatman filter paper and the filtrate heated at 60^0C to evaporate the water until slurry was left behind. The ethanolic extract was prepared by similarly weighing out 20g of the powder dissolving it in 200ml of freshly prepared 70% ethanol, and the moisture stirred vigorously. The conical flask holding the mixture was wrapped with a foil and stored away from direct sunlight for 48 hours at room temperature. The solution was then filtered and heated in a water bath as done for aqueous extracts until a slurry was obtained. The slurries were then stored in sterile bottles until further use.

Phytochemical screening of extracts

The extracts were screened qualitatively and quantitatively for the following phytochemicals alkaloids, tannins, saponins, flavonoids, glycosides, polyphenol, anthraquinones, hydroxylmethyl anthraquinones and reducing compounds amongst other phytochemicals. The qualitative screening was reported as using either plus signs (+, ++ and ++ depending on the intensity of the colour changes obtained) or minus sign where a negative result was obtained. These were done as previously reported (Guile, 1982; Cullei, 1984; Trease & Evans 1989; Harbone, 1993). Saponin, tannin, alkaloid and flavonoid were all quantified using the method reported previously (Harbone, 1993; Kirk & Sawyer, 1998). Reducing sugar, glycosides and polyphenol were also quantified using the methods of AOAC (2002) and Ekwenye & Okorie (2010).

Proximate composition analysis and anti-nutrient

The freshly collected leaves and the pods of *T. tetraptera* were also subjected to proximate nutrients (carbohydrate, protein, fat, moisture, fibre and ash) analysis as reported previously (AOAC, 1995; AOAC, 2002). Anti-nutrients evaluated in this study included phytic acid, soluble oxalate, total oxalate and hydrocyanic acid and these were carried out as previously (Ebana et al., 2016a and Ebana et al., 2016b).

Determination of vitamins and minerals

Vitamins A, C, riboflavin (B2), thiamine (B1), and nicotinic acid (B3) were all determined using methods previously reported (Bender, 2003; Ball, 1998; Bessy, 1944; Bessy, 1946; Kirk and Sawyer, 1973). Mineral which included potassium, sodium, calcium, magnesium, iron, zinc, copper and phosphorus were all determined using the dry ash methods of AOAC (1995).

Antimicrobial sensitivity test

The isolates were subjected to antimicrobial sensitivity tests as reported previously by CLSI (2014). Sterile discs of diameter 5mm were oven dried at 40^oC for 30 minutes with the discs

wrapped with aluminium foil. The isolates were sub-cultured onto nutrient agar plates overnight and then lawned onto freshly prepared Mueller-Hinton agar (MHA) plates in triplicates. The discs were then impregnated with the slurries and then carefully placed on the MHA plates aspetically. The plates were then incubated at 37°C for 24 hours. The plates were then examined for zones and these were recorded.

Gas chromatography mass spectrophotometer (GC-MS) analysis

The samples were also subjected to GC-MS analysis as previously described (Ebana et al., 2018). Sample preparation was done using standard methodologies. The analysis was carried out using a Clarus 500 Perkin- Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold - Perkin Elmer Turbomass 5.1 spectrometer. All other operating conditions were set according to manufacturer's instructions.

Statistical analysis

Triplicate readings obtained from the analysis were managed in Microsoft excel spreadsheet and then subjected to one way analysis of variance, and the result presented as mean ± standard deviation. Probability values less than 0.05 were considered significant. The mean readings of the leaves and fruit pulps were compared for significant differences using student t-test.

Results

Table 1 shows the result of the proximate composition, vitamins, minerals and anti-nutrients in the leaves and pods of the study plant. The result of the proximate composition revealed the presence of moisture, ash, protein, fat, fibre and carbohydrate in both plant parts but in different concentrations. The leaves as expected had the highest abundance of moisture compared to the dry fruit pulp with abundance of 83.80±4.37 and 18.13±1.34 g/100g, respectively. Carbohydrate content was almost similar in both samples. It was similar with protein and ash but these were slightly higher than those in the leaves. Fat and fibre contents in the fruit pulp were atleast twice higher than those of the leaves. The result of the vitamin analysis shows the presence of vitamins A, total C, soluble C, riboflavin, thiamin and niacin. Vitamins A and C were more in the leaves than in the pods. However, soluble vitamin C, riboflavin, thiamin and niacin contents were more in the pods than the seeds. The most abundant minerals in both samples were sodium and potassium, respectively for the pulp and leaves with concentration of 4.53±0.03 and 4.03±0.01 mg/ 100g. The order of abundance was Na, K, CA, P, Mg, Zn, Fe, and Cu in decreasing order of abundance in the pulp. On the other hand, the order of abundance of the minerals in the leaves was K, Na, Ca, Mg, P, Fe, Zn and Cu. The result of the anti-nutrients shows that the most abundant was phytic acid in both samples but was higher in the leaves compared to the pulp. Total and soluble, oxalate contents and hydrocyanic acid were also more in the leaves than the pulp by a factor of atleast two.

Table 2 shows the phytochemical profile of the study plant parts (leaves and fruit pulp). Consistently, the extract whether aqueous or ethanolic had the same sets of phytochemical but with different intensities. The phytochemicals present in the leaves were alkaloids, glycosides, saponins, flavonoids, reducing compounds, and polyphenol. On the other hand, the fruit pulp did not test positive for saponins but tested positive for phlobatannins. On quantification as presented in Table 3, the most abundant phytochemical was polyphenol with an abundance of 22.45 and 18.38%, respectively in the leaves and pods, respectively. For both plant parts studies, the next most abundant phytochemical was flavonoids with abundance of 7.89 and 15.47%, respectively. In the leaves, the least abundant phytochemical was glycosides, however, for the pods, the least abundant was saponins. The order of abundance of these phytochemicals in the leaves were

polyphenol, flavonoids, reducing compounds, alkaloids, saponins and glycosides in decreasing order of abundance. For the pods, the order was polyphenol, reducing compounds, flavonoids, alkaloids, glycosides, and saponins.

The result of the phytochemical screening using gas chromatography mass spectrophotometer is presented in Tables 4 and 5. Table 4 shows the phytochemicals in the leaves as revealed by gas chromatography mass spectrophotometer and they were 14 in total. They included terpenes, phytosterol, oxalate, steroids, tannin, phenol, saponin, alkaloid, coumarin, anthocyanins, flavonoids, phytate, cardiac glycosides and cyanogenic glycosides. The abundant phytochemical was alkaloids followed by terpenes, phytate and tannin. The least abundant was coumarin. Table 5 shows the phytochemicals in the pulp. It revealed fewer categories than that of the leaves although it gave a total of 20 compounds. These were 8 alkaloids, 6 organic and fatty acids, 5 flavonoids and 1 terpenes. The most abundant was alkaloid followed by flavonoids while terpene was the least.

The results of the antimicrobial sensitivity are presented in Table 6. The zones in mm showed that none of the clinical isolates were resistant to the extracts of the samples. The zones ranged from 14.30 to 15.63 mm and 15.60 to 17.34mm for the ethanolic extracts of the leaves and pulp, respectively. On the other hand, the ranges were 12.78 to 13.50mm and 13.25 to 14.56mm, respectively for the aqueous extracts. Compared to the standard antibiotics, chloramphenicol used in this study, the zones obtained by the extracts were slightly lower.

Table 1: Proximate composition, vitamins and elemental composition of the leaves and pulp

Nutrients	Leaves	Pulp
Proximate composition ((g/100g)		
Moisture	83.80±4.37[a]	18.13±1.34[a]
Ash	2.64±0.14	2.74±0.04
Protein	4.73±0.24	4.61±0.10
Fat	0.97±0.01	2.47±0.01
Fibre	2.98±0.13	5.48±0.34
Carbohydrate	88.65±2.47	88.34±3.08
Vitamins		
Vitamin A (IU/100g)	287.22 ± 10.58[a]	145.89±7.93[a]
Total vitamin C (mg/ 100g)	84.81 ± 4.81	69.78±3.51
Soluble vitamin C (mg/ 100g)	39.43 ± 1.64	46.23±2.01
Riboflavin (mg/100g)	0.78± 0.06	2.12±0.11
Thiamin (mg/100g)	0.28 ± 0.02	1.89±0.04
Niacin (mg/100g)	0.18 ± 0.01	2.02±0.03
Elemental composition (mg/ 100g)		
K	4.03±0.01[a]	3.98±0.07[a]
Na	3.85±0.02	4.53±0.03
Ca	2.12±0.02	3.45±0.04
Mg	1.47±0.03	2.14±0.02
Fe	0.40±0.01	0.86±0.01
Zn	0.07±0.01	1.05±0.01
Cu	0.06±0.01	0.41±0.01
P	0.91±0.03	2.14±0.04
Anti-nutrients		
Hydrocyanic acid	5.45±0.34[a]	2.13±0.20[a]
Total oxalate	68.24±2.34	14.23±1.01
Soluble oxalate	20.84±1.33	7.14±0.97
Phytic acid	251.31±4.69	35.08±3.45

[a]Represents analysis of variance showed significance ($p < 0.05$) for the replicate readings obtained for proximate composition, vitamins, minerals and anti-nutrients. Student t-test comparism only showed significance for minerals but not for the rest.

Table 2**:** Preliminary phytochemical screening of the leave of *T. tetraptera*

Phytochemical	**Leaves**		**Pulp**	
	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract
Alkaloids	++	+	+	+
Glycosides	+	+	++	+
Saponins	+	+	-	-
Flavonoids	++	+	+	+
Reducing compounds	++	+	++	+
Polyphenol	+++	++	++	+
Phlobatannins	-	-	+	+
Anthraquinones	-	-	-	-
Hydroxylmethyl anthraquinones	-	-	-	-

Table 3: Quantitative analysis of the phytochemicals (%)

Phytochemicals	**Leaves**	**Pulp**
Alkaloids	2.14±0.20	3.13±0.41
Glycosides	1.29±0.15	1.74±0.34
Saponins	1.41±0.20	1.56±0.24
Flavonoids	7.89±0.34	15.47±1.11
Reducing compounds	6.12±0.81	5.54±0.74
Polyphenol	22.45±1.21	18.38±1.78

Table 4: GC-MS of the leaves of *T. tetraptera*

S/N	Compounds	Leaves
1	Terpenes	14.56
2	Phytosterol	5.13
3	Oxalate	1.71
4	Steroid	1.51
5	Tannin	10.08
6	Phenol	8.42
7	Saponin	2.34
8	Alkaloid	21.67
9	Coumarin	0.83
10	Anthocyanins	1.58
11	Flavonoids	8.81
12	Phytate	12.39
13	Cardiac glycosides	4.24
14	Cyanogenic glycosides	2.01

Table 5 : GC-MS of the pulp of *T. tetraptera*

S/n	**Chemical group**	**Compounds**	**Concentration (%)**
1	Alkaloid	benzenesulfonamide, N-[4-[[4-methoxy-3-[(methylsulfonyl)amino]phenyl]amino]-3-nitrophenyl]-2,4,6-tris(1 -methylethyl)- F	5.780
2		Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-2-phenyl-, hydroxide, inner salt	4.566
3		1-(4-Phenylphenyl)-5-(2-dimethylaminoethenyl)-1H-tetrazole	6.121
4		Benzene, 4,6-difluoro-1,2,3,5-tetrakis(phenylthio)-	4.370
5		4H-1,2,4-Triazole-3-carboxamide, 4-(4-bromophenyl)-N-(4-methoxyphenyl)-N-(2-oxo-1,2-diphenylethyl)-5-phen	3.742
6		N-[(3,4-Dimethoxyphenyl)[2-(1-ethyl-2-oxopropyl)-4,5-dimethoxyphenyl]methylene] propionohydrazine	4.172
7		Loprazolam	3.810
8		hexanamide, N-[3-(acetylamino)-2-hydroxyphenyl]-2-[2,4-bis(1,1-dimethylpropyl)phenoxy]	5.446
9	Organic acid and Fatty	DL-3,4-Dehydroproline methyl ester	3.987
10	acid	Benzoic acid, 4-chloro-, 1-(4-methoxyphenyl)hydrazide	3.691

11		2-Butenedioic acid, 2-[methyl(5,6,7,8-tetrafluoro-1-naphthalenyl)amino]-, dimethyl ester, (Z)	4.849
12		L-Cystine, N,N'-bis[(phenylmethoxy)carbonyl]-, diethyl ester	5.274
13		4H-1-Benzopyran-8-carboxylic acid, 3-methyl-4-oxo-2-phenyl	7.567
14		9H-fluorene-4-carboxylic acid, 9-(dicyanomethylene)-2,7-dinitro-, hexyl ester	3.654
15	Flavonoid	Erythro-1,2,3,4-tetraphenylbutan-1,4-dione	4.591
16		1,3-Oxathiolane, 2-[[(2-chloroethyl)thio]methyl]-2-methyl	9.017
17		1H,7H-Furo[3,2-c:5,4-f']bis[1]benzopyran-7-one, 2,3-dihydro-10-hydroxy-3,3-dimethyl	4.671
18		Permethyl 2"-O-rhamnosylisovitexin	4.487
19		Ethanone, 2-(methylsulfinyl)-1-[octahydro-5,6-dimethoxy-4,7-dimethyl-3-[(methylsulfinyl)methyl]cyclopenta[c]py	5.402
20	Terpene	Diacenaphtho[1,2-j:1',2'-l]fluoranthene	3.874

Table 6: Antimicrobial sensitivity

Phytochemical	Leaves		Pulp		Chloramphenicol
	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	
Proteus species	15.63±0.12	13.50±0.01	15.60±0.30	13.25±0.11	22.50±0.13
E. coli	16.20±0.22	14.80±0.02	16.33±0.24	14.33±0.13	23.10±0.17
S. aureus	14.30±0.32	12.78±0.03	17.34±0.34	14.56±0.21	24.30±0.14

Discussion

T. tetraptera is a well known spices that is common to Southern Nigeria and is widely consumed as spice and used as medicine in various capacities (Ebana et al., 2016; Ogbunugafor et al., 2017). In our study, the nutritional analysis and antimicrobial activity of the leaves and fruit pulp of *T. tetraptera* freshly harvested in Calabar, Cross River State, Nigeria. The pulp is edible and it is used as a sweetening agent in the popular "white soup" delicacy common to Southern Nigeria. In our study nutrient analysis showed the presence of proximate nutrients (carbohydrate, moisture, protein, fat, fibre and ash), vitamins (A, C (total and soluble), and B (riboflavin, thiamin and niacin) and minerals (Na, K, CA, P, Mg, Zn, Fe, and Cu) in the fruit pulp and leaves, respectively. Ezeonu et al (2017) showed the presence of similar proximate nutrients (%) and these were protein (1.47 to 2.74), crude fat (0.52 to 1.07), ash (0.92 to 1.74), and moisture (89.71 to 91.05). Furthermore, they reported the presence of vitamins B1, B2, B6, and C, and minerals (calcium, iron, zinc and magnesium) in the samples. In another study, Ajiboye et al (2014) obtained crude protein, crude fat, ash, crude fibre, carbohydrate, and moisture content with concentrations that were 3.70%, 0.31%, 1.50%, 5.60g, 0.21% and 88.69 %, respectively. Furthermore, Akin-Idowu et al (2011) reported ash, crude protein, sugar, and starch concentrations that were 3.17–3.48, 5.13–8.65, 3.29–39.63, and 7.56–29.10, respectively in dry weight of the *T. tetraptera*. In addition, they reported the presence of minerals such as iron, zinc,

copper, magnesium, manganese, sodium, calcium, potassium, and boron in various concentrations. Godfrey (2015) showed the presence of proximate nutrients in the pulp of *T. tetraptera* to include carbohydrate (40.12±2.57), moisture (19.90±1.65), ash (17.84±1.63), crude protein (14.10±1.77), fats and oil (8.69±1.82) and crude fibre (6.84±0.31) (%) dry weight. Ogugor et al (2018) evaluated the bark of *T. tetraptera* fruits for proximate and heavy metals. Heavy metals analysis revealed the presence of zinc, arsenic, mercury, lead, cadmium and copper but in concentrations lower than those allowable by the World health organization. Their proximate analysis result showed the presence of ash, crude fibre, moisture, proteins and carbohydrate. Compared to our findings, although we observed the presence of these nutrients, vitamins and minerals obtained by the aforementioned authors were different from our findings in terms of concentration. However, our findings were more similar to those of our earlier study with the same plant collected from Akwa Ibom State (Ebana et al., 2016a). These differences obtained in the various nutrients and minerals could be as a result of the different climatic or edaphic factors or the methodologies employed in the various studies (Adusei et al., 2019).

Although, the leaves of the study plant are not known to be edible in Southern Nigeria, the pulp is edible. The abundance of nutrients in both samples suggests that the leaves could also be used as an edible vegetable. Furthermore, the result of the anti-nutrients shows the presence of anti-nutrients such as total and soluble, oxalate contents and hydrocyanic in levels that are comparable to those of other edible vegetable such as *Dennettia tripetala* and *Lasianthera africana* (Ebana et al., 2016b). This is collaborated by Igwe and Akabuike (2016) that showed that *T. tetraptera* is an excellent nutriceutical with antioxidant potential and antimicrobial activities due to the abundance of phytochemical and vitamins. Nutritional studies of *T. tetraptera* revealed that it is rich in nutrient justifying the edible nature of the fruit pulp. In an earlier study, it has been shown that their addition improves fermentation and the shelf-life of a local Ghanaian drink. Furthermore, their nutrients and phytochemical also add a number of health enhancing factors to the drink such as antioxidant, nephro- and hepato-protective, renal/diuretic, anticholesterol, antidiabetic, and antihypertensive effects (Adadi and Kanwugu, 2020).

Phytochemicals are plant based chemicals that are linked to their medicinal and pharmacological properties. Assob et al (2011) showed that the presence of phytochemicals in medicinal plant extracts such as alkaloids, triterpenes, sterols, tannins, and glycosides could be responsible for antimicrobial activity against microorganisms. In our study samples of *T. tetraptera*, show the presence of alkaloids, glycosides, flavonoids, reducing compounds, and polyphenol as overlapping compounds in both samples. On quantification, the most abundant phytochemical was polyphenol with an abundance of 22.45 and 18.38%, respectively in the leaves and pulp, respectively. In order to obtain a robust analysis, the phytochemicals in the study samples were further evaluated using gas chromatography mass spectrophotometer. This revealed the presence of terpenes, phytosterol, oxalate, steroids, tannin, phenol, saponin, alkaloid, coumarin, anthocyanins, flavonoids, phytate, cardiac glycosides and cyanogenic glycosides in the leaves and total of 20 compounds phytochemical in the fruit pulp and these were alkaloids, organic and fatty acids, flavonoids and terpenes.

In previous studies, Godfrey (2015) in their qualitative screening revealed the presence of saponin, tannin, steroids, terpenoids, alkaloids, flavonoid, resin and glycosides in varying amounts. Ekwenye and Okorie (2010), revealed the presence of saponin, tannin, steroids, triterpens, cyanogenic glycoside, and flavonoid. In an earlier study, Achi (2006), phytochemicals obtained from phenol and cold water extracts were tannin, saponin, and glycosides in addition to proteins and carbohydrates. However, tannin and glycosides were the most abundant in the whole pod extracts. These phytochemicals were also obtained from our study but in varying amounts. Adadi and Kanwugu (2020) showed the presence of phytochemicals such as total

polyphenol, flavonoids, saponins, tannin and phytate with concentrations (mg/100g) of 1604.03, 258.55, 551.90, 636.17 and 3245.33. Furthermore, earlier studies show the presence of various phytochemicals such as gallic, catechin, chlorogenic, caffeic and ellagic acid (Irondi et al., 2016), tannin, total flavonoids and total phenol (Irondi et al., 2013). Similar phytochemicals were also obtained in the seeds of *T. tetraptera* by Igwe and Akabuike (2016) and Adusei et al (2019) in ethanolic and aqueous extracts of pulp, seeds and whole fruits of *T. tetraptera* and in the bark of the fruits (Ogugor et al., 2018) while Koma et al (2016) showed the presence of alkaloids, phlobatanins, flavonoids, volatile oils and tannins in the crude extracts of the bark and leaves of *T. tetraptera.* The variation in the types and quantity are due to difference in climatic, edaphic and methodological factors pointed out previously (Adusei et al., 2019). An earlier study that evaluated the essential oil of the seeds using GC-MS showed acetic acid and various organic acids as the most abundant compound (Udourioh and Etokudoh, 2014). Compared to our fruit pulp sample, different organic and fatty acids were obtained

In our study samples, the extracts showed antimicrobial activities against the test clinical isolates. Our zones ranged from 14.30 to 15.63 mm and 15.60 to 17.34mm for the ethanolic extracts of the leaves and pods, and 12.78 to 13.50mm and 13.25 to 14.56mm, respectively for the aqueous extracts. Furthermore, the zones were only slightly lower than those of chloramphenicol used in this study. Ogugor et al (2018), in their study utilized aqueous, methanolic and ethanolic extracts of *T. tetraptera* that gave zones of inhibitions that ranged from 0.2 to 9mm, 6 to 12mm and 0.2 to 13mm respectively and these were lower than the zones reported for their control, ciprofloxacin. Achi (2006) reported zones of inhibitions that ranged from 16.40 to 28.10 mm for *S. aureus, Bacillus subtilis, P. aeruginosa* and *E. coli*. Ekwenye and Okorie (2010) reported zones for *Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeruginosa* that ranged from 11.00 to 20mm for the aqueous extract and 14.00 to 24.8 for the ethanolic extract, and the MIC for various isolates that ranged from 100 to 200mg. Igwe and Akabuike (2016) using the seed extract reported significant antimicrobial activity (7.00 – 20.00 mm) and the zones of inhibition were comparable with that of ciprofloxacin as standard (15.00 – 28.00 mm) used in their study. Our findings were also very similar to those reported previously (Olajuyigbe and Afolayan, 2018) but lower than those reported earlier (Njimoh et al., 2015). Igwe and Akabuike (2016) reported zones that ranged from 7 to 20.00mm against *S. aureus, E. coli, P. aeruginosa, C. albicans, Aspergillus niger,* and *P. notatum*. Ogunsile *et al* (2020) asserted that phytochemicals from *T. tetraptera* show therapeutic promise. The findings in this study confirms its therapeutic potential against clinical isolates.

Conclusion

The results obtained from various authors and as well this study is an indication that the study plant is a promising alternative for management of disease caused by pathogens. There is need for study based on molecular docking that can evaluate the various bioactive compounds further in terms of their individual or combined potential to inhibit key steps of the pathogenesis of pathogens such as binding to the host epidermis tissues.

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