A QUANTITATIVE ASSESSMENT OF THE ANTIBACTERIAL ACTIVITY, PHYTOCHEMICAL AND NUTRIENT COMPOSITION OF THE LEAVES OF *Tetrapluera tetraptera* (MIMOSACEAE)

ISSN: 2141 – 3290
www.wojast.com

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ABSTRACT

The leaves of *Tetrapluera tetraptera* (Uyayak) were screened for antibacterial activity, phytochemical and nutrient composition using standard procedures. Investigation using GC-MS revealed the presence of fourteen (14) components in varying amounts namely alkaloids (21.67 mg/100g); terpene (14.56 mg/100g); phytate (12.39 mg/100g) and tannins (10.08 mg/100g). Other components present include flavonoid (8.81 mg/100g), phenol (8.42 mg/100g), phytosterol (5.13 mg/100g), cardiac glycosides (4.24 mg/100g), saponins (2.34 mg/100g), cyanogenic glycosides (2.01 mg/100g), oxalate (1.71 mg/100g), anthocyanins (1.58 mg/100g), steroids (1.51 mg/100g) and coumarin (0.83 mg/100g). Results of the antimicrobial susceptibility test indicated that only the aqueous extract was potent as it inhibited *Escherichia coli*, *Staphylococcus aureus* and *Proteus* species. The highest inhibition was on *Proteus* species (17.38mm). The leaves were also shown to contain essential elements such as Na (4.92mg/100g); K (3.90mg/100g) and Ca (2.08m/100g). The leaves were abundant in vitamins especially vitamin A (295.72 IU/100g) and vitamin C (34.60mg/100g). Though nutritious, the plant also contained detectable levels of anti-nutrients such as phytic acid (248.73 ± 0.01mg/100g), total oxalate (67.84 ± 0.01mg/100g), soluble oxalate (20.26 ± 0.02mg/100g) and hydrocyanic acid (5.40 ± 0.1mg/100g). The importance of *Tetrapluera tetraptera* plant leaf as a potential source of drug and food supplements which could be exploited to meet the health needs of developing countries cannot be overemphasized.

INTRODUCTION

Although a number of new antibiotics have been produced in the last three decades, resistance to these drugs by microorganisms has increased tremendously (Nascimento *et al* 2000). The resistance factor in these microorganisms is a cause for concern, considering the number of patients with suppressed immunity, and the emergence of new multi-resistant bacterial strains worldwide (Doughari *et al* 2009).

In most rural African communities, Western health care supplies are not readily assessed due to poverty. This coupled with the increasing incidences of resistance to commonly used antibiotics has necessitated the search for herbal remedies as a cheap and available alternative. Plants have been an integral part of life in many indigenous communities. Apart from providing building materials, fodder, weapons and other commodities, plants are especially important as traditional medicines in the treatment of infectious diseases (Bussmanu *et al* 2006). Plants produce phytochemicals to protect themselves; however, studies have shown that these chemicals have therapeutic properties (Ebana *et al* 2016, Anyanwu and Nwosu 2014).

Tetrapluera tetraptera (Mimosaceae) is generally found in the lowland forests of tropical Africa. The dry fruit has a characteristics aroma which makes it a popular seasoning spice in Southern and Eastern Nigeria. It contains a lot of phytochemicals, vitamins and minerals. It also has antioxidant and anti-inflammatory properties which makes it useful in traditional medicine (Aladesanmi 2006). In some West African countries, it is used to flavour soups taken as a general tonic and stimulant or as part of post- partum diet therapy. Other uses include treatment of skin diseases and gastro intestinal disorders regulation of blood sugar, lowering of blood pressure, arthritic and rheumatoid pains etc. (Abii and Elegalam 2007).

A lot of studies have been carried out on the fruits of *Tetrapluera tetraptera* (Achi, 2006, Ekwenye and Okorie 2010, Ebana *et al* 2016 and Gbadamosi and Yekini, 2016). However, there is dearth of information on the leaves. Therefore, this study was carried out to assess the anti-microbial, phytochemical and nutrient composition of the leaves of this useful plant.

MATERIALS AND METHODS

Sources of Plant

The leaves of *T. tetraptera* were collected from the forest at Obong Ntak and identified by Taxonomist in Department Botany and Ecological Studies, University of Uyo, Nigeria.

Collection and Identification of Test Bacteria

The test organisms were isolates from clinical specimens obtained from the Microbiology Laboratory of the University of Uyo Teaching Hospital, Uyo, Nigeria. The isolates were characterized and identified using standard microbiological methods (Cheesebrough, 1998). They included *Staphylococcus aureus, Proteus* species and *Escherichia coli*

Proximate, Elemental and Anti-nutrient Composition

The proximate components were analyzed according to the method of A.O.A.C 2010). The elements K and Na were analyzed using the flame photometry method while Ca,Mg,P,Fe,Zn,Cu were determined by the methods of UNEP(2004). The antinutrients were estimated using the methods of Bassir (1969)

Determination of Vitamins Content

The methods of Association of Vitamin Chemists as described by Kirk and Sawyer (1989) was adopted for determination of vitamins A (retinol), C (ascorbic acid), B_2 (Riboflavin), B_1 (Thiamine) and Niacin.

Preparation of Plant Extracts and Phytochemical Screening

The aqueous extract was obtained by weighing 10g of oven-dried, and powdered sample into 100ml of sterile distilled water in a beaker. The beaker was wrapped with aluminum foil and kept for 72 hours. After this, it was filtered using Whatman's No 1 filter and the filtrate heated in a water bath at 70ºC until a slurry was obtained and stored in McCartney bottles at 4º until required for use. The ethanol extract was also obtained as described above but 75% ethanol replaced sterile distilled water.

The extracts were screened for the following phytochemicals namely: alkaloids, tannins, saponins, flavonoids, glycosides, polyphenol, Anthraquinones, hydroxyl methyl anthraquinones and reducing compounds using the methods described by Trease and Evans (1989) and Ebana *et al.* (2016).

Antimicrobial Sensitivity Assay

This was carried out using the method of CLSI (2014). Briefly, filter paper discs (5mm in diameter) wrapped in aluminum foil were sterilized in the oven (40º for 30 minutes). A discrete colony of the test isolate was sub-cultured on nutrient broth and incubated at 37ºC for 6hours. This was inoculated on freshly prepared Mueller Hinton Agar plates. Then using aseptic techniques, the sterile filter paper discs were soaked in the respective extracts and placed on the plates. Incubation was at 37ºC for 24 hours after which mean values of the zones of inhibition were determined and recorded.

Statistical Analysis

The replicate data obtained were analysed using SPSS (Statistical Package for Social Sciences) version 21. The results were presented as mean ± standard deviation. Values with P values < 0.05 were considered significant at 95% confidence level.

RESULTS

Proximate, Elemental and Antinutrient Properties of *Tetrapluera tetraptera* Leaf

Table 1 shows the results of the proximate components of the plant. Carbohydrate was 89.26 ± 0.02**%**, moisture 84.50 ± 0.1**%**, while protein content was 4.62 ± 0.02**%**. Others were fibre (2.70 ± 0.1**%**), ash (2.52 ± 0.02**%**) and lipids (0.72 ± 0.01**%**). The elemental composition of the leaves presented in Table 2 has shown that sodium was the most abundant (4.92 mg/100g dry matter) followed by Potassium (3.90 mg/100g dry matter) and Calcium (2.08 mg/100g dry matter) and Magnesium (1.45 mg/100g dry matter). Others were present in very minute quantities.

The anti-nutrient components of the leaves of *T. tetraptera* are presented on Table 3. Phytic acid was the highest component (248.73 ± 0.01mg/100g) followed by total oxalate (67.84 ± 0.01mg/100g), soluble oxalate (20.26 ± 0.02mg/100g) and hydrocyanic acid (5.40 ± 0.1mg/100g).

Table 1: Proximate composition of *Tetrapluera tetraptera* leaf

Components	% composition
Moisture	84.50 ± 0.1
Ash	2.52 ± 0.02
Crude protein	4.62 ± 0.02
Lipids	0.72 ± 0.01
Fibre	2.70 ± 0.1
Carbohydrates	89.26 ± 0.02

Each value represents the mean of 3 replicates ± SD

Table 2: Elemental composition of *Tetrapluera tetraptera* leaf

Elements	Composition (mg/100g Dry matter)
K	3.90±0.01
Na	4.92±0.04
Ca	2.08±0.02
Mg	1.45±0.01
Fe	0.07±0.01
Zn	0.03±0.01
Cu	0.02±
P	0.71±0.01

Each value represents the mean of 3 replicates

Table 3**:** Anti-nutrients composition of *Tetrapluera tetraptera* leaf

Anti-nutrient	Composition (mg/100g)
HCN	5.40 ± 0.1
Total Oxalate	67.84 ± 0.01
Soluble Oxalate	20.26 ± 0.02
Phytic Acid	248.73 ± 0.01

Each value represents the mean of 3 replicates ± SD

Vitamins Content of *Tetrapluera tetraptera* Leaf

The results of the vitamin composition of the leaves of *T. tetraptera* are shown on Table 4. Vitamin A was the most abundant (295.75 ± 0.02 IU/100g) while vitamin C was next (82.46 ± 0.01 mg/100g). The least vitamin was Niacin (0.12 ± 0.02mg/100g).

Table 4: Vitamin Composition of *Tetrapluera tetraptera* Leaf

Vitamins	Composition
Vitamin A (IU/100g)	295.72 ± 0.02
Total Vitamin C (mg/100g)	82.46 ± 0.01
Soluble Vitamin C (mg/100g)	34.60 ± 0.01
Riboflavin (mg/100g)	0.43 ± 0.01
Thiamin (mg/100g)	0.18 ± 0.02
Niacin (mg/100g)	0.12 ± 0.02

Each value represents the mean of 3 replicates ± SD

Antioxidative Properties of *Tetrapluera tetraptera* Leaf

The quantitative estimates (mg/100g) using GC-MS (Table 5) revealed fourteen (14) components. Alkaloid was the most abundant component (21.67) followed by terpenes (14.56 mg/100g); phytate (12.39 mg/100g); tannin (10.08 mg/100g), flavonoid (8.81 mg/100g) and phenol (8.42 mg/100g). Others were phytosterol, cardiac glycosides, saponin, cyanogenic glycoside, oxalate, anthocyanin, steroid and coumarin.

Table 5: Phytochemical components of *Tetrapluera tetraptera* Leaf

	Compounds	Concentration (mg/100g)
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 Glycoside	2.01

Antibacterial Potential of *Tetrapluera tetraptera* Leaf

Table 6 show the antibacterial activities of the leaf extracts. For the bacterial isolates, the aqueous extract inhibited all three organisms with the highest inhibition on *Proteus* species (17.38mm). Others were *S. aureus* (17.30mm), and *E. coli* (16.30mm). The ethanolic extract did not show any inhibition of growth on the test organisms.

Table 6: Antibacterial activity of leaf extracts of *T.tetraptera*

Organism	Zones of inhibition (mm) Ethanolic	Aqueous
E.coli	0	16.30 ± 0.03
S. aureus	0	17.30 ± 0.03
Proteus	0	17.33 ± 0.02

± standard deviation from 3 replicates.

DISCUSSION

Tetrapleura tetraptera popularly known as (Uyayak) by the Ibibios and Efiks of South-South Nigeria, has been shown to contain essential nutrients. Previous study of the dry fruits (Ebana *et al* 2016) revealed a fibre content of 11.38% while the present study shows that the leaves contain 2.7% of fibre. However, the carbohydrate and moisture contents were higher in the leaves. Studies have also showed that both the dry fruits and the leaves had negligible lipids content and this is of health benefits.

The leaves of *Tetrapleura tetraptera* have been shown to contain important vitamins and minerals. Uyoh *et al* (2013) showed that the dry fruits contained six vitamins with vitamins A and E slightly higher than others. Also, Akin-Idowu *et al* (2011) showed that the dry fruits contained potassium and calcium in abundance. Other minerals were Fe,Zn,Cu,Mg,Mn,Na. Also, Uyoh *et al* (2013) also found that calcium and potassium were very high, magnesium and phosphorus moderate while zinc and sodium were minimal. However, in the leaves, sodium was the most abundant followed by potassium and calcium. Mineral nutrition is very important to human health. The electrolytes, including potassium are involved in the maintenance of normal pH balance and work in conjunction with calcium and magnesium to maintain normal nerve transmission, muscle contraction and relaxation (Akpabio and Akpakpan 2012).

Gbadamosi and Yekini (2016) investigated the free radical scavenging activity of different parts of this plant and found that the leaves had the highest quantity of ash (11.2%); crude fibre(20.1%) and crude protein(18.9%) compared to other parts of the plant. In our study, the components were not present in such large quantities. This could be due to different methods used for analysis. Quantitative analysis of the phytochemical components of the leaves showed that alkaloid was the most abundant followed by terpenes and phytate. Achi (2006) showed that in the dry fruits, the main components were tannins and glycosides. Also, Ekwenye and Okorie (2010) revealed alkaloids and flavonoids as the major components of the fruits. However, Mboto *et al* (2013) showed that the fruits contain more flavonoids than alkaloids.

Alkaloids and their synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and antibacterial activities (Kaur and Arora 2015). Alkaloids and glycosides are also known to complex with bacterial protein thereby leading to disruption of the cell. Mala *et al* (2009) had observed that the terpenoids contain essential oil derivatives which are inhibitory to bacteria, leading to destruction of the cellular membrane by first penetrating the extensive meshwork of peptidoglycan in the cell wall.

Naturally occurring substances in plants often play an important role in controlling the growth of spoilage and pathogenic microorganisms in food (Burt 2004). In this study, the extract produced clear inhibitory effect on *E. coli, S. aureus* and *Proteus* species. Similar results were obtained by Achi (2006) on foodborne pathogens indicating that the extracts could be used as alternative to chemical preservatives of food .Other researchers (Ekwenye and Okorie (2010), Mboto *et al* (2013) also observed large zones of inhibition on other organisms such as *Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Chromobacterium violesium, Bacillus* species, *Streptococcus faecalis* and *Micrococcus*. Also, the dry fruits have been shown to have activity against *Aspergillus*, *Mucor*, and *Penicillium* species (Effiong *et al 2018).*

CONCLUSION AND RECOMMENDATION

The present study has shown that the leaves of *Tetrapleura tetraptera* is rich in nutrients, vitamins and antioxidants which can be exploited by the indigenous people for their nutrient and antimicrobial properties especially when the dry fruits are not in season.

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