

ANTIMICROBIAL ACTIVITY, PROXIMATE AND AMINO ACIDS ANALYSIS OF *TETRAPLEURA TETRAPTERA*

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Abstract: The fruits of an African spice and medicinal plant, *Tetrapleura tetraptera*, was analysed for its antimicrobial property, proximate composition and amino acid composition using standard procedures. The sensitivity screening revealed that the fruits of *Tetrapleura tetraptera* exhibited antimicrobial activity against *Salmonella typhi*, *Escherichia coli*, *Shigella spp* and *Staphylococcus aureus* isolates. The best activity was observed on *Escherichia coli* at a concentration of 250mg/ml having a zone of inhibition of 21mm and the least activity was observed on *Shigella spp*. with a zone of inhibition of 15mm at the same concentration. On the other hand, at a lower concentration of 31.25mg/ml, *Salmonella spp* was more susceptible with a zone of inhibition of 12mm while the least activity was observed on *Shigella spp* at a concentration of 31.25mg/ml producing a zone of inhibition of 5mm. these reveals that *Shigella spp* is more resistant to the extract compared with the other isolates. Upon proximate analysis, *Tetrapleura tetraptera* had 5% moisture, 14% ash, 11% crude protein, 8% fats and 62% crude fibre. Also, amino acid analysis of the plant revealed that the plant contains about 17 amino acids whose concentrations were expressed in g/100g protein. Among this amino acids, there were 9 essential amino acids which includes Cystine(2.45), Isoleucine(6.21), Leucine (5.57), Lysine (5.97), Methionine (0.83), Phenylalanine(4.05),Threonine(4.75), Valine (5.50) and Tyrosine (3.65) while the remaining 8 were Non-Essential Amino Acids which includes Alanine(6.15), Arginine (6.39), Aspartic acid(11.41), Glutamic acid (13.10), Glycine (6.15), Histidine(3.47), Proline (3.15) and Serine (5.86). This study therefore indicates that *Tetrapleura tetraptera* fruits could be useful in the treatment of diseases caused by the test organisms. Furthermore, the plant could effectively serve as dietary condiment, particularly at this time when the economies of most nations are on the decline.

Keywords— *Tetrapleura tetraptera*, amino acids, proximate analysis, spice, antimicrobial, herbalism.

Introduction

Heterotrophs require food to carry out essential functions (metabolism), which include growth, development and reproduction. Plants are the ultimate source of food, and also provide shelter and medicinal agents (Ngaski, 2006). The conventional food plants provide most nutrients needed for energy, body building, maintenance and

(regulation of body processes. The classes of conventional plant food includes cereals, oils, vegetables, tubers, stems (e.g sugarcane) fruits and seeds. (Gibson, 1998)

Dietary proteins aids in tissue repair and replacement thereby functionally promoting growth, and are needed for the synthesis of enzymes, hormones and antibodies (Cheesbrough, 1987). Carbohydrates and oils provide the energy need of the body for physical, physiological

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and metabolic activities. Minerals serve a wide variety of essential physiological functions, ranging from structural components of body tissues to essential components of many enzymes and other biologically important molecules. Recently, studies showed that wild or semi-wild plants are nutritionally important because of high vitamins, minerals, essential fatty acids and fibre contents (Meagher and Thomson, 1999). Some of the plants also enhance taste and colour in diets (Bianco *et al.*, 1998; Meagher and Thomson, 1999)

Plants also contain important variables that also confer its medicinal characteristics. (Satyanand *et al.*, 2010). The medicinal values of plants are due to the presence of certain variables called phytochemicals (Mamta *et al.*, 2013). Phytochemicals are a large group of plant-derived compounds that is believed to be responsible for much of the disease protection provided by diets high in fruits and vegetables, beans, cereals, and plant based beverages such as tea and wine (Arts *et al.*, 2005; Silva *et al.*, 2007; Tapas *et al.*, 2008).

Tetrapleura tetraptera is a species of flowering plant in the pea family native to Western Africa. The plant is called Prekese in the Twi language of Ghana. *Tetrapleura tetraptera* is also one of the medicinal plants in Nigeria usually called Aridan (or Aidan) in western Nigeria. The tree has many uses. Its sweet fragrance is highly valued; its fruit is used to spice dishes such as Banga soup, and its bark is used for medicinal purposes. The major constituents are tannins, flavonoids and starch. The biological or pharmacological activities are found to be molluscicidal, cardiovascular, neuromuscular, hypotensive, anti-conversant, antiulcerative, anti-bacterial and anti-inflammatory. The pods notably have an appealing culinary use for mothers from the first day of delivery to post parturition and as a lactation aid (Enwere,

1998). At the same time, in herbalism, it is believed that the fruit is adopted for the management of convulsion, leprosy, inflammation and rheumatoid pains (Dalziel, 1948). Consumers' desire for fresher and more natural additive-free products is on the increase (Nychas, 1995). Therefore, this research was aimed at obtaining the antimicrobial activity, proximate composition and amino acid analyses of the plant, with a view to ascertaining its use as an antibacterial agent and dietary supplement.

Materials and Methods Plant sample collection and preparation

Tetrapleura tetraptera fruit was collected from Onitsha in Anambra State, Nigeria and identified at the department of biological sciences of the Federal University of Agriculture, Umudike. The sample was transported to the laboratory for preparation and analysis.

The plant extract was prepared using the soxhlet apparatus with ethanol as solvent. The extract obtained was dried to obtain a 2.5g concentration which was dissolved in 10ml of distilled water in order to obtain a concentration of 250mg/ml. Subsequently, a double fold serial dilution was carried out using 250mg/ml concentration as the working stock to obtain 125mg/ml, 62.5mg/ml and 31.25mg/ml

Antimicrobial susceptibility testing

Cultures (24h) of *Salmonella typhi*, *Escherichia coli*, *Shigella spp* and *Staphylococcus aureus* obtained from the Anthony Van Leuwenhoek research laboratory at Nekede, Imo State were used for the antimicrobial susceptibility testing using the agar well diffusion technique (Cheesbrough, 2009). The microorganisms were standardized to 0.5 McFarland's standard. Each organism was inoculated into the Mueller Hinton agar using the spread plate method. Sterile cork borers

were used to produce wells into a seeded Mueller Hinton agar. The different concentrations of the plant extract were introduced into each well at a volume of 0.1ml. The resulting plate was incubated at a temperature of 37°C for 24 hrs. The zones of inhibition were measured using a metre rule to the nearest mm.

Proximate analysis

The moisture, crude protein, crude fat, total ash and crude fibre contents of the sample was determined using Standard methods of the Association of Official Analytical Chemists (AOAC, 2006). Moisture content was determined by heating 2.0g of each fresh sample to a constant weight in a crucible placed in an oven maintained at 105°C. The dry matter was used in the determination of the other parameters. Crude protein (% total nitrogen x 6.25) was determined by the Kjeldahl method, using 2.0g samples; crude fat was obtained by exhaustively extracting 5.0g of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40-60°C) as the extractant. Ash was determined by the incineration of 10.0g samples placed in a muffle furnace maintained at 550°C for 5h. Crude fibre was obtained by digesting 2.0g of sample with H₂SO₄ and NaOH and incinerating the residue in a muffle furnace maintained at 550°C for 5h. Moisture content was determined by heating 2.0g of each sample to a constant weight in a crucible placed in an oven maintained at 105°C. Each analysis was carried out in duplicates.

Determination of amino acid profile

The amino acid profile in the known sample was determined using methods

described by Benitez (1989). The sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the technicon sequential Multi-sample Amino acid Analyser (TSM).

Defatting the sample

The sample was defatted using chloroform methanol mixture of ratio 2:1. 4g of the sample was put in extraction thimble and extracted for 15 hours in the soxhlet extraction apparatus. (AOAC, 2006)

Nitrogen determination

A small amount, (200mg) of ground sample was weighed, wrapped in whatman filter paper (No. 1) and put in the Kjeldhal digestion flask. Concentrated sulfuric acid 10ml was added. Catalyst mixture (0.5g) containing sodium sulphate (Na₂SO₄), Copper sulphate (CuSO₄) and selenium sulphate (SeO₂) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put into the Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (100ml) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected. The distillate was then treated with standardized 0.01N HCl to grey coloured

$$\text{Percentage Nitrogen} = \frac{(a - b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

where:

a = titre of the digested sample; *b* = titre value of the blank sample;
V = volume after dilution(100ml); *W* = weight of dried sample (mg);
C = Aliquot of the sample used(10ml); 14 = nitrogen constant in mg

Hydrolysis of the sample

A known weight of the defatted sample was weighed into glass ampoule. 7ml of 6NHCl was added and Oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°C ± 5°C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humus. It should be noted that tryptophan is destroyed by 6NHCl during hydrolysis. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which are kept in the freezer.

Loading of the hydrolysate into TSM analyzer

The amount loaded was between 5 to 10 microlitre. This was dispensed into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 76 minutes.

Method of calculating amino acid values from the chromatogram peak

An integrator attached to the analyzer calculates the peak area proportion to the concentration of each of the amino acids. Alternatively, the net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and width of the peak on the half height was accurately measured and recorded. Approximately area of each peak was then obtained by multiplying the height with the width at half height. The Norleucine Equivalence (NE) for each amino acid in the standard mixture was calculated using the formula

$$NE = \frac{\text{Area of norleucine}}{\text{Area of each amino acid}}$$

A constant S_{std} was calculated for each amino acid in the standard mixture:

$$\text{where } S_{std} = NE_{std} \times \text{molecular weight} \times \mu\text{MAA}_{std}$$

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the formula

$$\text{Concentration} \left(\frac{g}{100g} \text{ protein} \right) = \frac{NH \times W @ NH/2 \times S_{std} \times C}{\text{dilution} \times 16}$$

$$\text{where, } C = \frac{\text{smple wt}(g) \times N\% \times 10 \times \text{Vol. loaded}}{NH \times W(nleu)}$$

where NH = net height; W = width @ half height and nleu = Norleucine

Results

The antimicrobial susceptibility screening revealed that *Tetrapleura tetraptera* fruit extracts exhibited inhibitory activity against the four tested bacterial isolates including *Salmonella typhi*, *Escherichia coli*, *Shigella spp* and *Staphylococcus aureus*. The highest activity recorded at 250mg/ml concentration was against *Escherichia coli* (21mm) while the least at the same concentration was against *Shigella* species (15mm) (table 1).

The amino acids analysis of *Tetrapleura tetraptera* revealed that the plant contains variable quantities of essential and non-essential amino acids. The essential amino acids present in the plant sample include Cystine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Valine and Tyrosine while the non-essential amino acids were found to be Alanine, Arginine, Aspartic acid, Glutamic acid, Glycine, Histidine, Proline and Serine. Table 2 shows the tabulated results following the amino acids analysis carried out in this research. Table 3 depicts the quantified essential amino acids while table 4 consists of the quantified non-essential amino acids. The result of the proximate analysis is also shown in figure 1.

Table 1: antimicrobial property of ethanolic-extracts of *Tetrapleura tetraptera* on selected organisms.

CONC. (mg/ml)	ZONE OF INHIBITION			
	<i>Salmonella typhi</i> (mm)	<i>Escherichia coli</i> (mm)	<i>Shigella spp</i> (mm)	<i>Staphylococcus aureus</i> (mm)
250	19	21	15	18
125	16	19	13	15
62.5	14	15	9	13
31.25	12	10	5	10

Table 2: amino acid analyses of *T. tetraptera*

Wt. of sample hydrolyzed=5.0000g; Dilution= ×5 and %N (fat free)=0.40.

1	2	3	4	5	6=(2×4×5×C)
Amino acid	Net height	NH/2(mm)	Width@NH/2 (mm)	S _{std}	Concentration: g/100g protein
Lysine	110.5	55.25	4	13	5.97
Histidine	55	27.5	4	15.18	3.47
Arginine	37	18.5	18	9.23	6.39

Aspartic acid	184	92	3.5	8.53	11.41
Threonine	86	43	3.5	7.59	4.75
Serine	108	54	3.5	7.46	5.86
Glutamic acid	86.5	43.25	6	12.15	13.10
Proline	15.5	7.75	7	13.99	3.15
Glycine	128	64	4	5.78	6.15
Alanine	42	21	4	9.14	3.19
Cystine	18.5	9.25	7	9.10	2.45
Valine	95	47.5	3	9.29	5.50
Methionine	16	8	2.5	10.03	0.83
Isoleucine	98	49	3.5	8.72	6.21
Leucine	102	51	10	7.51	5.57
Norleucine	70	35	5.5		
Tyrosine	11.5	5.75	10	15.28	3.65
Phenylalanine	24	12	8	10.15	4.05

Table 3: essential amino acids in *T. tetraptera*

Concentration: g/100g protein	
ESSENTIAL AMINO ACIDS (EEA)	
AMINO ACIDS	CONCENTRATION
Cystine	2.45
Isoleucine	6.21
Leucine	5.57
Lysine	5.97
Methionine	0.83
Phenylalanine	4.05
Threonine	4.75
Valine	5.50
Tyrosine	3.65

Table 4: Non-essential amino acids in *T. tetraptera*

NON ESSENTIAL AMINO ACIDS	
Alanine	6.15
Arginine	6.39
Aspartic acid	11.41
Glutamic acid	13.10
Glycine	6.15
Histidine	3.47
Proline	3.15
Serine	5.86

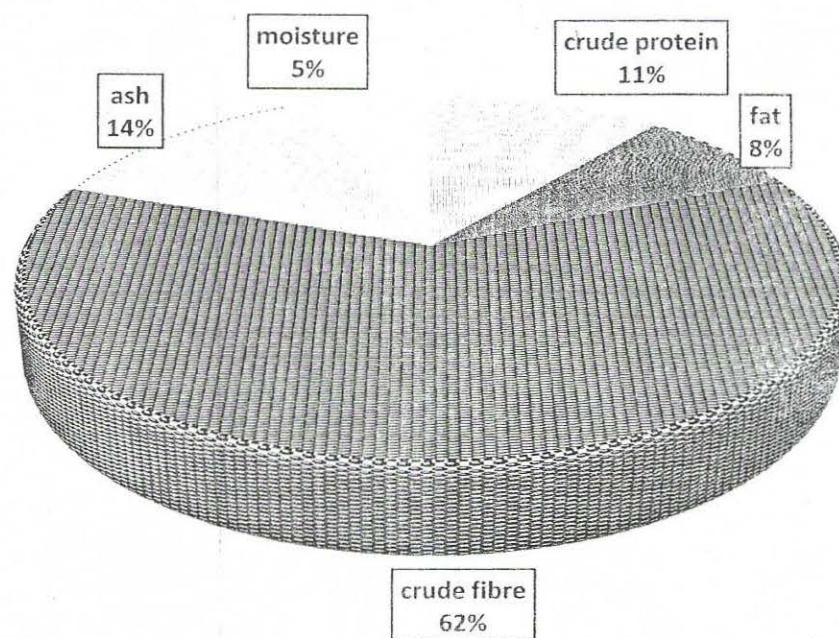


Figure 1: percentage proximate composition of *T. tetraptera*

Discussion

Tetrapleura tetraptera fruits had an antimicrobial activity on the test organisms even at low concentrations. The best activity was observed on *Escherichia coli* at a concentration of 250mg/ml while the least activity was observed on *Shigella spp* at all concentration (table 1). This is in agreement with previous findings in which *Tetrapleura tetraptera* fruit extracts (both aqueous and ethanolic) were reported to have antibacterial activity on *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* (Achi, 2006; Sunday *et al.*, 2010; Uchechi and Chigozie, 2010; Mbotto *et al.*, 2013). Amino acids are associated with conferring physiological effects on consumers since they are precursors for the synthesis of secondary metabolites such as alkaloids, which provide chemical defense for plants. Alkaloids provide protection to plants from a variety of herbivores, and some of them possess significant pharmacological activity

such as analgesic, antibacterial and antibiotic (Croteau *et al.*, 2000). It is for this reason that the plant has been recently explored for its medicinal activities (Okoronkwo and Echeme, 2012; Sunday *et al.*, 2012; Uchechi and Chigozie, 2010; Mbotto *et al.*, 2013; Sunday *et al.*, 2014)

The results (figure 1) also revealed that the proximate composition of *T. tetraptera* are 5% moisture, 14% ash, 11% crude protein, 8% fats and 62% crude fibre contrary to Abii and elegalam (2007) which obtained 9% ash, 45% fibre, 4% oil, 3% moisture, 5.6% crude protein on analysis of the plant fruit. The probable reason for this variation could be the difference in the sources of the plant fruits.

The amino acid analysis (table 2 and 3) reveals that the plant contains essential and non-essential amino acids in varied amounts. Table 2 shows the different distribution of essential and non-essential amino acids that are present in the plant sample. The results reveal that the plant

contains glutamic acid as the highest amino acid concentration while methionine was the least in concentration. Among the amino acids, Lysine, being present with a concentration of 5.97 g/100g protein is of paramount importance. Lysine facilitates the adequate absorption of calcium; helps collagen biosynthesis; aids in the production of antibodies, hormones as well as enzymes. (Poneros-Schneier and Erdman, 1983) Reviews have also shown that Lysine may be an effective treatment against herpes by improving the balance of nutrients that reduce viral growth. It is involved in a number of processes, including the biosynthesis of carnitine in the liver and kidneys (Gaby, 2006). Carnitine may also be synthesized from methionine, another essential amino acid, although it is present in meagre amounts in *Tetrapleura tetraptera*. Conversion of lysine or methionine into carnitine is dependent on an adequate level of vitamin C. Therefore, an infusion of *T. tetraptera* with a source of vitamin C will aid production of carnitine if consumed. A deficiency of Lysine may result in fatigue, inability to concentrate, irritability, bloodshot eyes, retarded growth, hair loss, anaemia, and reproductive problems (Lall and Anderson, 2005).

If *Tetrapleura tetraptera* is consumed for its nutritional value, it will be needed to add other supplements that could be rich in methionine since methionine is an essential amino acid. Methionine is a principal supplier of sulphur which prevents disorders of the hair, skin and nails. It increases the liver's production of lecithin thereby lower cholesterol levels. It reduces liver fat, protects the kidneys, and acts as a natural chelating agent for heavy metals, it also regulates the formation of ammonia and creates ammonia-free urine which reduces bladder irritation, and promotes hair growth (Watanabe *et al.*, 1983). Phenylalanine (4.05 g/100g) of protein in *Tetrapleura tetraptera* is used by the brain to produce Norepinephrine, a chemical that transmits signals between nerve cells and the brain. It helps keep a person awake and alert, reduces hunger pangs. It also functions as an antidepressant and helps

improve memory (Watanabe *et al.*, 1983). Threonine (4.75 g/100g protein in *Tetrapleura tetraptera*) is an important constituent of collagen, elastin, and enamel protein. It helps prevent fat build-up in the liver, helps the digestive and intestinal tracts function smoothly, and assists metabolism and assimilation of nutrients. (Watanabe *et al.*, 1983)

Valine (5.50 g/100g protein in *Tetrapleura tetraptera* promotes mental vigor, muscle coordination, and calm emotions. Leucine and Isoleucine provide ingredients for the manufacturing of other essential biochemical components in the body, some of which are utilized for the production of energy, stimulants to the upper brain, and promote alertness. Arginine may improve immune responses to bacteria, viruses and tumour cells. It also promotes wound healing and regeneration of the liver. It is involved in the release of growth hormones and is considered crucial for optimal muscle growth and tissue repair. (Watanabe *et al.*, 1983)

Aspartic acid was the second highest in quantity with a concentration of 11.41g/100g protein. Although it is a non-essential amino acid, this amino acid is essential for purine, pyrimidine, asparagine and inositol synthesis. Glutamic acid and glycine increase the antioxidant capacity of the plant by participating in the synthesis of glutathione. Valine maintains the balance of branched chain amino acids, whereas alanine is involved on hepatic autophagy, gluconeogenesis and transamination. Leucine regulates the protein turnover (mTOR signaling) and gene expression (Wu, 2009; Akram *et al.*, 2011). Glycine, lysine, threonine and glutamate help to maintain intestinal integrity and health (Rhoads *et al.*, 2009; Wang *et al.*, 2009). Amino acids, such as aspartic, are related to detoxification and excretion of ammonia (Rhoads *et al.*, 2009).

Conclusion

Tetrapleura tetraptera fruits is a good source of essential and non-essential amino acids. Moreover, it could be supplemented with vitamin C for carnitine deficiency, or a source of methionine since its methionine

component is trace. Owing to the widespread use of this spice in native medicine and as a food spice, understanding the nutritional value is of paramount importance. Analysis of its vitamin components should be encouraged or reviewed. *Tetrapleura tetraptera* fruits had an antimicrobial activity on the test organisms even at low concentrations. The best activity was observed on *Escherichia coli* while the least activity was observed on *Shigella spp.* More research is therefore encouraged on the plant material.

REFERENCES

- Abii, T.A and Elegalam, A (2007). investigation into the chemical composition of dry fruits of (ubukirihu). *Journal of Food Technology*. 5(3):229-232
- Achi, O.K (2006). Composition and antibacterial activities of *T. tetraptera* Taub. Pod Extracts. *Research Journal of Microbiology*. 1(5): 416-422.
- Akram, M., Asif, H., Uzair, M, Akhtar, N., Madni, A., Shah, S.M.A., Ul-Hasan, Z and Ullah, A (2011). Amino acids: A review article. *J Med Plants Res* 5: 3997-4000.
- AOAC (2006) Official methods of analyses of the Association of Official Analytical Chemists (AOAC) (W. Horwitz editor) eighteenth edition, Washington D.C.
- Arts, I.C and Hollman, P.C (2005). Polyphenols and disease risk in epidemiologic studies, *Am J Clin Nutr*. 81: 317-325.
- Benitez, L.V (1989). Amino acids and fatty acid profiles in aquaculture nutrition studies, p, 23-35. In S.S. De Silva (ed.) *Fish nutrition Research in Asia. Proceedings of the third Asian fish nutrition networking meeting. Asian fish society special publication*. 4, 166p. Asian fisheries society, manila Philippines.
- Bianco, V.V., Santa Maria, P and Elia, A (1998). Nutritional value and nitrate content in edible wild species used in Southern Italy. Proceeding 3rd on diversification of vegetable crops. *Acta Horticulture* 467: 71 – 87.
- Cheesebrough, M (2009). *District Laboratory Practice in Tropical Countries*. 2nd ed. Cambridge: Butterworth-Heineman Ltd.
- Croteau, R., Kutchan, T.M and Lewis, N.G (2000). Natural products (secondary metabolites): *Biochemistry and Molecular Biology of plants* pp. 1250-1318.
- Dalziel, J.M (1948). *The Useful plants of tropical West Africa*. (Crown Agents for overseas Government and Administration) London. Pp.223-224.
- Enwere, N.J (1998). Foods of Plant Origin. *International Journal of Microbiology* 9 (94): 329-334
- Gaby, A.R. (2006). Natural remedies for Herpes simplex. *Altern Med Rev* 11:93-101.
- Gibson, E.L., Wardel, J and Watts, C.J (1998). Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children. *Appetite* 31: 205-228.
- Lall, S.P and Anderson, S (2005). Amino acid nutrition of salmonids: Dietary requirements and bioavailability. *Cahiers Options Méditerranéennes* 63: 73-90.
- Mamta, S., Jyoti, S., Rajeev, N., Dharmendra, S and Abhishek, G (2013). Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry*. 1 (6):168-182.
- Mboto, C.I., Jacob, I.O and Ndem, E.E (2013). Phytochemical properties and antibacterial potentials of *Tetrapleura Tetraptera* Fruits (Uyayak) on some medical important bacteria. *J. Microbiol. Biotech. Res.*, 3 (6):82-92
- Meagher, E and Thomson, C (1999). Vitamin and Mineral Therapy. In *Medical Nutrition and Disease*, 2nd ed., G Morrison and L Hark, Malden,

- Massachusetts: Blackwell Science Inc, 33-58.
- Ngaski, M.M (2006). Phytochemical screening and proximate analysis of *Cassia siamea* leaves. M.Sc. Dissertation (Unpublished). Submitted to postgraduate school, Usmanu Danfodiyo University, Sokoto
- Nychas, G.S (1995). Natural antimicrobial from plant. In *New methods of food preservation*. Blackie Academic and Professional. Gould, G.W (Ed.) London, Pp.58-59.
- Okoronkwo, N.E and Echeme, J.O (2012). Cholinesterase and microbial inhibitory activities of *Tetrapleura tetraptera* Journal of Applied and Natural Science. 4 (2): 156-163
- Poneros-Schneier, A.G and Erdman, J.W (1989). Bioavailability of calcium from sesame seeds, almond powder, whole wheat bread, spinach and nonfat dry milk in rats. *J Food Sci* 5:150-153.
- Rhoads, M.J and Wu, G (2009). Glutamine, arginine, and leucine signaling in the intestine. *Amino Acids* 37: 111-122.
- Satyanand, T., Gunjan, S., Anamika, S. and Gulshan, A (2010). Phytochemicals as candidate Therapeutics: An Overview. *International Journal of Pharmaceutical Sciences Review and Research* 3(1): 012
- Silva, E.M., Souza, J.N.S., Rogez, H., Rees, J.F and Larondelle, Y (2007). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chemistry*, 101: 1012-18.
- Sunday, E.A., Ojochenemi, E.Y., Mubarak, L.L. and Dorothy, U.I. (2014). Effect of methanolic extract of *Tetrapleura tetraptera* (schum and thonn) taub leaves on hyperglycemia and indices of diabetic complications in alloxan-induced diabetic rats, *Asian Pac J Trop Biomed* 4(4): 272-278
- Sunday, O.A., Imoh, E.U and Herbert, O.C.M (2010). Antibacterial effects of the aqueous and ethanolic extracts of tetrapleura tetraptera pods on the pathogens in nosocomial wound infections *ijpi's Journal of Pharmacognosy and Herbal Formulations*. 1:2 18-23
- Tapas, A.R., Sakarkar, D.M., Kakde, R.B. (2008). Flavonoids as Nutraceuticals: A Review. *Tropical Journal of Pharmaceutical Research*, 7: 1089-1099.
- Uchechi, U.E and Chigozie, F.O (2010). Antibacterial activity of *Tetrapleura tetraptera* taub.pod extracts. *International Journal of Pharma and Bio Sciences* 1(4): 734-741.
- Wang, W., Qiao, S and Li, D (2009). Amino acids and gut function. *Amino Acids* 37: 105-110.
- Watanabe, T., Kitajima, C and Fujita, S (1983). Nutritional values of live organisms used in Japan for mass propagation offish: review. *Aquaculture* 34:115-143
- Wu, G (2009). Amino acids: metabolism, functions, and nutrition. *Amino Acids* 37: 1-17.