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Phytochemical Characterization, Biochemical Analysis, and Antimicrobial Activity of *Tetrapleura tetraptera* Fruit in Traditional Treatment of Skin Infections

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Abstract

The high cost of conventional medicines and the prevalence of substandard pharmaceutical products in many African communities have driven interest in locally available medicinal plants with potential pharmacological benefits. *Tetrapleura tetraptera* fruits are increasingly used in the traditional treatment of skin lesions, despite limited empirical validation. This study investigated the phytochemical composition, vitamins A, C, and E content, antioxidant potential, and antimicrobial activity of ethanol and n-hexane extracts of *T. tetraptera* fruits, with the aim of establishing a scientific basis for their therapeutic application in pathological skin conditions. Phytochemical screening, reducing power, and free radical scavenging assays were conducted using standard methods, while vitamins A, C, and E were quantified with established analytical protocols. Antimicrobial activity was assessed using the agar diffusion method. Both extracts contained appreciable levels of phytochemicals; however, the ethanolic extract demonstrated superior antioxidant and free radical scavenging activities, as well as higher concentrations of vitamins C and E, compared to the n-hexane extract. The abundance of these vitamins may account for the fruit's reported efficacy in managing skin morbidities, supporting its ethnomedicinal use by traditional practitioners. While *T. tetraptera* presents a promising, affordable alternative to costly orthodox medicines, toxicity evaluation is recommended to define its safety margins. Further studies on its therapeutic potential and safety profile are encouraged.

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Introduction

Medicinal plants play a significant role in the health of humanity (Sofowora *et al.,* 2012). As an avenue to unveil the hidden potential of medicinal plants and also advance research in ethnopharmacology, information update on medicinal plants had aided the frontiers of research. Review updates on different plants such as *Tetrapleura tetraptera* by Onansanwo *et al*., (2011) among others had been published in different scientific journals. The aim of this study is to establish a scientific basis or otherwise for the use of the fruit of *Tetrapleura* tetraptera in the treatment of skin abnormalities by the determination of the phytochemical composition, antioxidant capacity, free radical scavenging potential and antimicrobial effects of *Tetrapleura tetraptera* fruit using extracts by two solvents. *Tetrapleura tetraptera* (taub) is a single stemmed, robust, perennial tree of 15—20 m in height, with dark green leaves and thick, woody base and spreading branches. The plant has a wide natural distribution over a large part of tropical Africa, especially in the rain forest belt of West, Central and East Africa. The four-winged, mature pods, which constitute the fruits of the plant, are about 15—27 cm long and 4—5 cm wide, and dark-brown when fully ripe (Ojewole and Adewunmi, 2004). The fruit as reported by Aladesanmi, (2006) possesses a fragrant, characteristically pungent aromatic odour, which is attributed to its insect repellent property. Its fruit is highly cherished in West and Central Africa, where it is native and used as spice, medicine and dietary supplement and is traditionally used in Cameroonian folk medicine to treat cancer (Kuete *et al*., 2014). The plant has a wide natural distribution over a large part of tropical Africa, especially in the rainforest belt of West, Central and East Africa (Ojewole and Adewunmi, 2004). *Tetrapleura tetraptera* (Mimosaceae), locally referred to as "Aidan Toro" in Yoruba, "Ura I-Ira" in Igbo, is a flowering plant of the pea family native to West Africa. The dried fruit is used as a seasoning spice in the Southern, Eastern and Western part of Nigeria and in the Niger Delta areas. The pod, fruit and seeds are used as spices. A neuroprotective effect of *Tetrapleura tetraptera* has been reported in scopolamine-induced amnesic rats (Nwidu *et al*., 2019). It is one of the molluscicidal

medicinal plants of Nigeria, also useful in the management of convulsions, leprosy, inflammation and/or rheumatoid pains (Aladesanmi, 2006). It is a rich source of phytochemicals which contribute to its documented biological and pharmacological activities, including cardiovascular, anti-inflammatory, hypoglycemic, hypotensive, neuromuscular, anti-convulsant, molluscicidal, trypanocidal, hirudinicidal, anti-ulcerative, ecotoxicity, anti-microbial, emuIsifying property, birth control, food value and the control of intestinal parasites (Agomuo *et al*., 2011; Aladesanml, 2006; Kuate *et al*, 2005). Due to its antimicrobial properties, it is used in making traditional black soaps (Adebayo *et al*, 2000), which is good for skin inflammation and irritation. The fruit of *Tetrapleura tetraptera* is frequently used in Tropical African traditional medicine for the management and/or control of an array of human ailments, including arthritis and other inflammatory conditions, asthma, diabetes mellitus, hypertension, epilepsy and schistosomiasis (Ojewole *et al*, 2004). Phytochemical analysis refers to series of chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study. Phytochemicals are the chemicals produced by plants which may have an impact on health, flavour, texture, smell or colour of plants but are not required by humans as essential nutrients. These phytochemicals have been classified as either primary or secondary metabolites and these depend on their role in plant metabolism. Primary metabolites include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids etc. Secondary metabolites are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics and glucosides (Koche *et al*., 2016). Phytochemicals have multiple health benefits for metabolic disorders such as cancer, cardiovascular disease, neurodegenerative diseases, and obesity. Plants with a higher concentration of phytochemicals play a role in protection against free radical damage (Al-Ishaq *et al*., 2020).

Materials and Methods

Study area

Phytochemical screening and vitamin content determinations were conducted in the Department of Biochemistry Laboratory, Faculty of science, Federal University Otuoke, Bayelsa State. While the antioxidant and antimicrobial studies were undertaken at Enis biomedical laboratory, Igbogene, Bayelsa State. Bayelsa State is located within latitude 4^0/5/N and Latitude 5^0/23 South in the Federal republic of Nigeria (Agoro *et al*., 2017).

Sample Collection and Authentication

The samples (fruits) of *Tetrapleura tetraptera* were bought from a seller in Swali market in Yenagoa and authenticated/identified by a botanist in the Department of Biology, Faculty of Science, Federal University Otuoke, Bayelsa State.

Sample Processing and Extraction

The samples were washed to get rid of any extraneous materials, dissected into small pieces, dried under room temperature for 3 weeks and pulverized by grinding in a blender into fine particles. The dried materials were stored in glass jars until analysis. 100% of the ethanolic extract of *T. tetraptera* fruit was prepared by weighing 750 g of the pulverized sample and mixing it with 2.5 L of ethanol as solvent and allowing the mixture to stand for 24 hours. This process was repeated using n-hexane as solvent to get 100% the n-hexane extract. The mixtures were filtered and concentrated using a rotary evaporator to obtain the ethanolic and n-hexane extracts used for the analysis.

Sample Analysis

Each of the extracts were subjected to the standard qualitative and quantitative phytochemical screening protocols to identify the phytochemicals present using standard methods. The vitamin A content of the sample extracts were determined using the method of Rutkoski *et al*. (2006). Ascorbic acid content of the sample extracts was determined according to Klein and Perry (1982). The vitamin E content of the sample extracts were analyzesed using the method of Rutkoski *et al*. (2005). The reducing power of the extracts were determined according to the method of Barros *et al*., (2007). Stable 22-diphenyl-l-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the sample extracts. This was assayed using the method of Ebrahimzadem, *et al*., (2009). The agar diffusion method was carried out to analyze the antimicrobial activity of the plant extracts. All determinations were performed in triplicates with standards.

Statistical Analysis

The data obtained from the experiments were analyzed using statistical package for social sciences (SPSS) (version 21) software for Windows (SPSS Inc. Chicago Illinois, USA). Data were expressed as mean ± standard error. Statistical analysis of data was performed using student's T-test and analysis of variance (ANOVA) to determine if significant differences exist between the experimental groups. The limit of significance was set at $p<0.05$.

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Results

Table 1: Results of phytochemical screening of the extracts

Phytochemicals	Qualitative analysis (ethanol extract)	Quantitative analysis (ethanol extract)	Qualitative analysis (n-hexane extract)	Quantitative analysis (n-hexane extract)
Alkaloids	+++	22.2%	+++	53.1%
Saponin	++	8.4%	++	17.1%
Anthraquinone glycoside	+	4%	+	9.9%
Tannin	+	4.9%	++	7.7%
Steroid	-	-	-	-
Terpene	-	-	+	1.5%
Phenol	+	3.6%	++	5%
Flavonoid	+	2.9%	++	5.9%

Table 2: Average phytochemical composition of the extracts

Phytochemicals	Ethanol extract (mg/g)	Hexane extract (mg/g)
Alkaloids	23.03±1.55	22.20±0.52
Saponins	7.53±0.41	8.37±0.45
Anthraquinone glycoside	4.20±0.55	3.97±0.24
Tannins	7.70±0.61	4.93±0.22
Phenol	4.97±0.09	3.60±0.40
Flavonoids	5.93±0.23	2.93±0.23
Terpenes	1.47±0.32	-

Table 3: Characterization of the extracts

Parameters	Value (Ethanol extract)	Value (n-hexane extract)
% Oil yield	25.81%	35%
Saponification	18.71mgKOH/g	20.2mgKOH/g
Acid value	17.95mgKOH/g	13.82mgKOH/g
Ester value	16.4mgKOH/g	18.1mgKOH/g
Iodine	87.63gh, 100g	50.2gh, 100g
Peroxide value	16.00meq/kg	17.3meq/kg
Specific gravity	0.902	0.082
Free fatty acid (as oleic acid%)	9.02	12.1

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Table 4: Vitamins A, C and E analysis of the extracts

Vitamins	Ethanol extract (Mean ± Standard error)	N-hexane extract (Mean ± Standard error)	P(T<=t) two-tail
Vit A (mg/g)	2.93±0.13	1.40 ± 0.13	0.013088
Vit. C (mg/g)	6.42 ± 0.55	0.00 ± 0.00	0.00714
Vit. E (mg/g)	29.61 ± 1.88	7.63 ± 1.92	0.012283

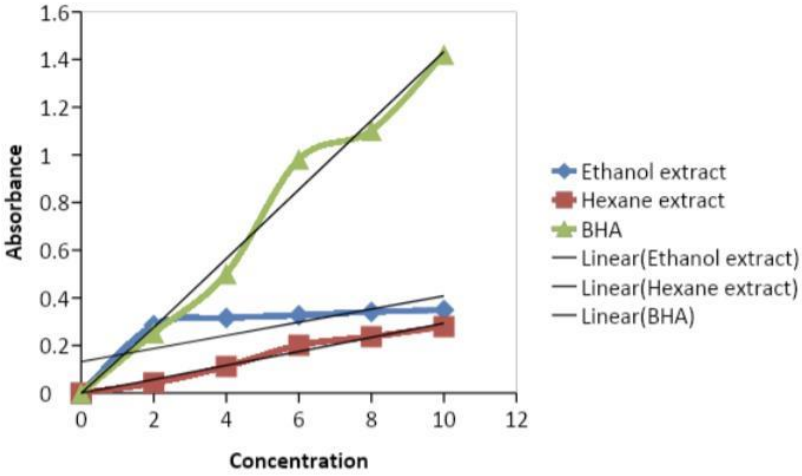

Figure 1: Graph of the absorbance of the extracts and BHA against concentration
BHA: Butylated hydroxy anisole

Table 5: Statistical representation of the extracts and BHA in ascending order of absorbance.

Samples Extracts	Lower EC50 (µg/mL)
BHA	3.6
Ethanol	13.6
N-hexane	17.3

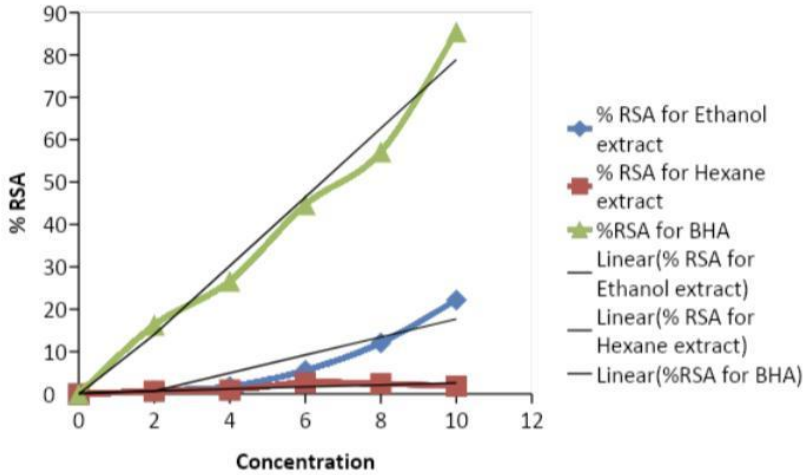

Figure 2: Graph showing the free radical scavenging effects of the extracts and BHA at different concentrations.

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RSA: Radical scavenging activity.

Table 6: Statistical representation of the extracts and BHA in ascending order of absorbance

Samples extracts	%RSA (50%) (µg/mL)
BHA	6.4
Ethanol	25.2
N-hexane	215.8

Table 7: Antimicrobial activity of the ethanolic extract of *T. tetraptera* by the zone of inhibition of three microorganisms

Pathogens	Ethanolic extract of *T. tetraptera* (400mg/ml)	Streptomycin (20mg/ml)	DMSO
Escherichia coli	5.7mm	20.7mm	-
Staphylococcus aureus	4mm	15.3mm	-
Candida spp.	4.3mm	-	-

Discussion

Tetrapleura tetraptera is a is well distributed tree plant along the forest zones of Nigeria and commonly used by African traditional healers. The study investigated the phytochemical, vitamin A, C & E composition, free radical scavenging activity and antimicrobial properties of ethanol and n-hexane solvent extracts of the fruits of *Tetrapleura tetraptera* with a view to establishing the scientific rationale or otherwise for its utility in the treatment of pathological skin conditions. Tannins, alkaloids, saponins, terpenes, phenols, anthraquinone glycosides and flavonoids were found to be present in both extracts of *T. tetraptera* fruits studied while others were absent. The medicinal effects of *Tetrapleura tetraptera* plants have been ascribed to the presence of polyphenol components such as phenols, flavonoids and saponins. The fruit of *Tetrapleura tetraptera* has been shown to have antihypertensive, anticonvulsive, and cognitive enhancing properties. In West Africa, *Tetrapleura tetraptera* is mainly used as a spice, medicine and as a dietary supplement rich in vitamins (Osei-Tutu *et al*., 2010). Aladesanmi (2007) reported the usage of the fruits, seeds and flowers in the preparation of perfumes, production of some alcoholic beverages and as flavouring for biscuits in Ghana. The plant is mostly used in the management of convulsion, leprosy, inflammation and rheumatic pains, schistosomiasis, asthma, hypertension and also recommended for fast relief of ailment such as malaria fever. The medicinal value of this plant lies in the bioactive phytochemical constituents that produce certain physiological action on the human body (Akinmoladun *et al*., 2007).

As defined by Schofield *et al*., (2001), Tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin etc.), alkaloids, nucleic acids and minerals. They are water soluble phenolic compounds with molecular weight of more than 500 Da. They have the ability to precipitate proteins from aqueous solutions (Akande *et al*., 2010). They are found commonly in fruits such as grapes, persimmon, blueberry, tea, chocolate, legume forages. On the basis of their structural characteristics, it is therefore possible to divide the tannins into four major groups: Gallotannins, ellagitannins, complex tannins and condensed tannins (Koche, 2016). The structural complexity of saponins results in a number of physical, chemical, and biological properties, only a few of which are common to all members of this diverse group (Güçlü-Üstündağ and Mazza, 2007). Saponins have been reported to possess a wide range of biological activities. The ability of saponins to swell and rupture erythrocytes causing a release of haemoglobin (the in vitro haemolytic activity) has been one of the most investigated properties of saponins (Oda *et al*., 2000).

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The characteristic feature of an antioxidant is ability to scavenge the free radicals due to their redox hydrogen donors and singlet oxygen quencher (Adusei *et al*., 2019). *Tetrapleura tetraptera* demonstrated appreciable free radical scavenging and antioxidant activity notably against the highly

damaging hydroxyl radical and against lipid peroxidation which is a major culprit in the etiology of many brain pathologies. Its effectiveness could be due to the presence of both hydrophilic and lipophilic antioxidants. Ojewole and Adewunmi (2004) conducted a study intended to evaluate the hypoglycemic effect of the fruit extract. The study revealed significant reductions ($P < 0.05–0.001$) in the blood glucose concentrations of both normal and fasted diabetic rats. The hypoglycaemic effect of the fruit extract became significant ($P < 0.05$) 1h following oral administration, reaching the peak of its hypoglycaemic effect 2–4 h after administration. However, the hypoglycaemic effect of the fruit extract was still significant 8 hrs after oral administration of the extract. In an in vivo study carried out in alloxan-induced diabetic rats by Atawodi *et al*., (2014), the *T. tetraptera* extract apparently decreased plasma glucose levels in diabetic rats than the standard drug, glibenclamide at the recommended dose. This seems to suggest that *T. tetraptera* has the capacity to reduce the risk of other complications associated with people suffering with diabetes, and hence validates its local use as a hypoglycemic agent by the Igala people of north central Nigeria.

In order to assess the anti-trypanosomal and anthelmintic activity of *Tetrapleura tetraptera*, Obeng *et al*., (2021) tested both for its anti-trypanosomal and anthelmintic activity using the extract of both the fruit and stembark. It was discovered that the fruit extract was more potent for its anti-trypanosomal and anthelmintic activity. The *Tetrapleura tetraptera* fruits have been reported to contain many chemical compounds such as triterpenoid glycoside (aridanin), coumarin (scopoletin), flavonoids and other phenolic compounds (Saliu *et al*., 2021). Anti-plasmodial screening of plants have implicated terpenes and flavonoids in this activity. Moreso, the observed antiplasmodial activity of the fruit extract might have contributed to the low parasitaemia rate reported for adults in parts of the Niger Delta region of Nigeria (Calabar area) (Okokon *et al*., 2007). The anticonvulsant effect of *T. tetraptera* fruit aqueous extract (TTE) was assessed by Ojewole (2005) in Wistar rats. The results revealed that the extract significantly delayed the onset of seizures induced by pentylenetetrazole (PTZ) and also significantly antagonized picrotoxin (PCT)-induced seizures.

Wound healing properties of the stem bark extract were investigated by Adesina et. al (2016). The authors found that topically-applied stem bark extract promoted healing of excision and incision wounds in rats. The study showed an excellent potential of the stem bark therapy on dermal wound healing with a possible mechanism of action related to epithelialization, contraction and tenable strength improvement. Atawodi *et al*., (2014) reported *Tetrapleura tetraptera* extract to decrease the HDL cholesterol level in serum of rats. Thus, potential health related functions of dietary plants such as *Tetrapleura tetraptera* was reported to include immune stimulation and nervous system action (John, 2001). To ascertain the phytochemical components responsible for the ethno-medicinal properties, a qualitative analysis of the on the aqueous and ethanol extracts of pulp, seeds and whole fruit of *T. tetraptera*, which was conducted by (Ochuko *et al*., 2017). Phytochemicals such as flavonoids, phenols, cardiac glycosides, and terpenoids which were detected in aqueous extract of *Tetrapleura tetraptera* have been reported to possess various pharmacological effects such as antioxidants, antidiabetic, antihypertensive and anti-Alzhemic activities. Flavonoids are vital in transduction pathways, suppression of oncogenes and stimulation of the immune system] and have the potential of inhibiting lipid peroxidation which contributes to oxidative damage by substituting into the phenolic C ring a sugar moiety. Tannins share its antioxidant activity with phenols and flavonoids but have been determined that they can enhance the uptake of glucose and inhibit adipogenesis, a potential drug for the treatment of non-insulin dependent diabetes mellitus (Adusei *et al*., 2019).

Table 3 shows the yield of the extracts. The result shows the n-hexane fruit extract had a higher percentage of oil yield (35%) when compared with that of the ethanolic fruit extract (25.81%). This may be attributed to n-hexane's hydrophobicity, its simple recovery and nonpolar nature and its low latent heat of vaporization (330 kJ/kg).

Free fatty acids (FFA) in plant oils are a quality feature for these oils. According to the CODEX Alimentarius standards, the maximum acceptable level of FFA in vegetable oils is 5.0% (as palmitic acid in virgin palm oil), 4.0% (as lauric acid in crude palm kernel oil) and 0.3% (as oleic acid in rice bran oil). As rancidity is usually accompanied by free fatty acid formation, determination of acid value is often used as a general indication of the condition and edibility of oils. The acid value of the ethanolic extract (17.95mg KOH/g) is higher than that of the n-hexane extract (13.82mgKOH/g). The iodine values of the ethanol extract (87.63gh, 100g) is higher than that of the n-hexane extract (50.2gh, 100g). This signals that the ethanolic extract is more unsaturated and it's likely to be more reactive, less stable, softer and more susceptible to oxidation. The saponification values of the ethanolic and n-hexane extracts were 18.71mgKOH/g and 20.2mgKOH/g respectively. In soap and ointment making, both oils would be

classified as soft oils (Mishra, 2013). These oils have substantial amounts of unsaturated acids namely: oleic, linoleic and linolenic acids. Other sources of these oils are: groundnut, cotton seed, fish and olive. Table 4 shows that the ethanolic extract showed significantly higher concentrations of vitamin E and C. Long storage conditions are a factor that contributes to the loss of vitamin C available in plants (Lee and Kader). The results of the antimicrobial activity of *T. tetraptera* showed that the ethanolic extracts exhibited significant antimicrobial activity at the studied concentrations, which aligns with the findings of Larbie *et al* (2020) who reported that it exhibited no activity at 100 mg/ml, 200 mg/ml and 300 mg/ml.

Conclusion

Both the ethanolic and n-hexane extracts of *T. tetraptera* were found to contain appreciable levels phytochemicals. The ethanolic extract exhibited a higher free radical scavenging and antioxidant effects and it is likely to be more reactive than the n-hexane extract. The ethanolic extract also contained a high quantity of vitamins C and E. The revealed large amounts of vitamins C and E constituents of *T. tetraptera* fruits explains their apparent potency in the treatment of skin morbidities and justifies its use in the management of skin traumas by traditional medicine practitioners. While its rich phytochemical content and significant antioxidant capacity may contribute to its other pharmacological properties. And its modest antimicrobial effects may be responsible for its antibiotic exploitation and its emerging use in combating skin infections. Although the fruit may be a good alternative to expensive orthodox medicines, subjecting it to toxicity tests would provide the margins of safety for its use. Hence, studies on the safe therapeutic utility of this species should be encouraged.

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