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Chemical Characterization and Therapeutic Potentials of *Tetrapleura Tetraptera* (Aidan) Seed Oil

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ABSTRACT
Medicinal plants are fundamental elements in primary healthcare systems worldwide, especially in developing countries due to limited access to modern medicine. Identification and chemical characterization of these plants provide insight into their therapeutic potentials and possible incorporation into synthetic drugs. This study investigated the chemical characterization and therapeutic potentials of *Tetrapleura tetraptera*, a popular, but under studied medicinal plant. Oil extraction and chemical analyses were respectively done using soxlet method and HPLC with UV-DAD detector. The extraction yielded 26.36% oil. Twenty secondary metabolites belonging to various classes were identified. The most prominent were flavonoids (kaemmferol, Rutin, flavan-3-ol, epicatechin, naringenin, naringin, flavonones, flavone and anthocyanin) followed by alkaloids (lunamarin, spartein, epihedrine and rebalinidine). Others include glycosides (cardiac glycosides and cyanogenic glycosides), steroids and steroid derivatives (steroids and sapogenin) and non-flavonoid polyphenol (resveratrol). Antinutrients (phytate and oxalate) were equally identified. Free fatty acids present in the oil included six saturated (dodecanoic, tetradecanoic, hexadecanoic, octadecanoic, eicosanoic and docosanoic acids), one monounsaturated (Cis-9-octadecanoic acid) and four polyunsaturated fatty acids that included cis-9,12-octadecanoic, cis-9,12,15- octadecanoic, all-cis-5, 8,11,14-eicosatraenoic and all-cis-7, 10,13,16-docosatetraenoic acids. Twelve essential and six non-essential amino acids were equally present in significant concentrations. The essential amino acids present included valine, threonine, isoleucine, leucine, lysine, methionine, phenyl alanine, histidine, tryptohan, arginine, tyrosine and cystine. Glycine, alanine, serine, proline, aspartate and glutamate are the non-essential amino acids identified. Asparagine and glutamate were not detected. The chemical analyses indicate that *Tetrapleura tetraptera* seed oil has diverse chemical profile with pharmacological potential for treatment of common ailments.

Keywords:
Tetrapleura tetraptera,
Seed Oil,
Chemical Characterization,
Phytotherapy

INTRODUCTION

Medicinal plants have remained key element in primary healthcare systems worldwide, especially in developing nations where access to modern medicine is often difficult. The increasing global demand for therapeutics of plant origin has extensively redirected research towards bioactive phytochemicals with specific attention to ones present in edible and medicinal plants (El-Saadony *et al*., 2025). Among such plants is *Tetrapleura tetraptera* (Schumach. & Thonn.). The plant is known as Aidan tree (English), Uyayak (Ibibio), Aridan (Yoruba), and Oshosho (Igbo). It is a member of the family Fabaceae and has gained notable attention due to its ethnomedicinal importance, nutritional value, and diverse pharmacological activities (Mensah *et al.*, 2024). It is a tropical plant native to West Africa and has been widely utilized in countries such as Nigeria, Ghana, and Cameroon, where its dried fruits and seeds are traditionally employed as culinary spices and herbal remedies for common ailments. In Eastern Nigeria, it is widely used in treating postpartum bleeding, convulsions, leprosy, rheumatism, flatulence and jaundice. The use of its fruits and seeds in the treatment of hypertension, diabetes, inflammation,

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asthma, infectious diseases and as a nutritional spice has also been reported (Dongmo *et al.,* 2022). Due to the unique aroma of *T. tetraptera* fruit pod, it is utilized in making variety of culinary delights, perfumes, pomades, alcoholic beverages and biscuits. The dry pod is used in making pepper soup and yam pottage, especially for women that newly gave birth (Effiong *et al*., 2014). The myriads of reported medicinal applications of the plant have been linked to its rich bioactive compounds as revealed in different phytochemical studies (Anyamele, *et al*., 2022).

Recently, seed oils from medicinal plants have become an emerging area of pharmaceutical research with focus on their potentials as sources of nutraceuticals and therapeutic agents. Seed oils are known to be rich in essential fatty acids, fat-soluble vitamins, and bioactive lipophilic secondary metabolites (Navigato *et al*., 2025). Beyond these volatile components, seed oils are known to contain nutritionally and therapeutically relevant fatty acids such as linoleic, oleic, and palmitic acids, which are known to influence lipid metabolism, inflammation, and membrane integrity. Polyunsaturated fatty acids (PUFAs), in particular, play essential roles in cardiovascular health and immune regulation, while lipid-soluble antioxidants such as tocopherols contribute to oxidative stability and disease prevention (Zio *et al*., 2025). These arrays of attributes suggest that *T. tetraptera* seed oil may possess similar and promising therapeutic applications.

Analytical techniques like high-performance liquid chromatography (HPLC) have enhanced the identification and quantification of phytochemical compounds, providing insights into their therapeutic relevance. Thus, phytochemical analyses do not only reveal the qualitative and quantitative constituent of bioactive compounds in a sample, but help in establishing correlations between identified chemical constituents and biological activities (Kavya *et al*., 2024).

Despite documented ethnopharmacological applications and emerging phytochemical data on other parts of *Tetrapleura tetraptera* such as leaf, stem bark, root and whole pod, comprehensive studies integrating chemical characterization and therapeautic evaluation of the seed oil remain scarce. Existing published literature have largely focused on crude extracts, with limited information on the physicochemical properties, fatty acid profile, and bioactivity correlations of the seed oil. Again, variations in geographical source, extraction and analytical methods have resulted in inconsistencies in reported chemical compositions, necessitating the need for further investigations and standardization. Advances in HPLC technique now provide robust tools for accurate characterization of plant oils and their bioactive constituents, enabling a deeper understanding of their biological mechanisms (Wang *et al*., 2023)

In view of the above, the present study aims to chemically characterize the seed oil of *Tetrapleura tetraptera* using HPLC and evaluate its therapeutic profile with a view to bridging the existing knowledge gaps and provide scientific evidence supporting the various uses of the plant parts in treating common ailments in traditional medicine. Additionally, characterizing the bioactive components will give insight into their potential applications in drug development, functional food and complementry medicine.

MATERIALS AND METHODS

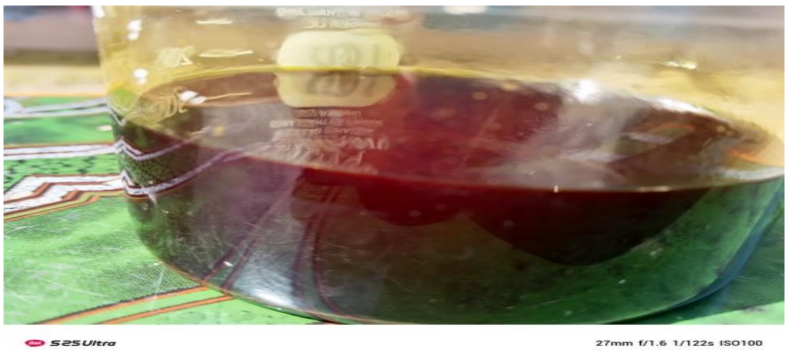
Plant materials

Tetrapleura Tetraptera (aidan) seed

Tetrapleura tetraptera **plant**

Dry Pods of *Tetrapleura tetraptera*

Dry Seeds of *Tetrapleura tetraptera*



***Tetrapleura tetraptera seed* oil.**

Chemicals and reagents

n-hexane 97% JHD, China
Ethanol 98% JHD China
Ethyl acetate BDH, England
Acetonitrile JHD China
Reference standards

The chemicals and reagents were of good analytical grades.

Equipment and instruments

Soxlet apparatus (Bionics India)
Refrigerator (Nexus)
HPLC -20 DAD system (Japan)

The equipment and instruments were all in maximum working condition.

Procurement and authentication of plant material

Dry pods of *Tetrapleura tetraptera* used were purchased from Nwofe market in Izzi Local Government Area of Ebonyi State. The sample was identified and authenticated in the plant taxonomy department of the Faculty of Agriculture and Natural Resources Management (FARM), Ebonyi State University, Nigeria.

Sample preparation

The dry pods were washed, allowed to dry and then crushed in a mortar to separate the seeds from the pod. The hard coated brown seeds were immersed in boiling water for 60 minutes. The swollen husks (seed coats) were separated manually from the endosperm. The endosperm was further air-dried to reduce moisture content. The dry endosperm was ground to fine powder using manual blender.

Oil extraction

A fixed quantity (2000 g) of the powder was extracted in batches of 200 g in muslin cloth using soxlet extractor with n-hexane (98%) as solvent. After each cycle of extraction, the weight of the powder was measured and recorded. The extracted oil was exposed for 72 hours to allow any trace of the solvent to escape. The oil was stored in air-tight container and preserved in a refrigerator (Menkiti *et al*., 2015).

Chemical analyses

The oil-extract was analyzed using high performance liquid chromatography (HPLC) with diode array detector (DAD) to identify bioactive compounds, free fatty and amino acids composition in line with the protocol adopted by Baros *et al.* (2009).

The HPLC instrument with an attached UV-DAD detector (Shimadzu Nexera mx) was set up with capcell pak C-18 reverse phase chromatographic column with dimensions adjusted to 100 mm length with internal diameters and thickness of 4.6 mm and 7 µm respectively. The mobile phase consisting of water and acetonitrile was constituted in the ratio of 70:30 (v/v). The analytical wavelengths were set at 280 nm phytochemicals, 210 nm for free fatty acids and 254 nm for amino acids and were separately set within the visible region with pump pressure of 15 MPa. After the setup, standards (phytochehemicals, fatty acids and amino acids) were separately injected to create chromatograms with specified peaks and profiles. Following the production of standard caliberation curves, 5 µL of the seed oil (sample) was then injected (loaded) using autosampler into the HPLC, setting the gradient elution at a constant flow rate of 2 mL/min. Peaks were identified after a run time of 30 minutes followed by comparison of retention times and UV spectra with reference standards. The linear regression equation y = mx + c was used to determine the concentration of each analyte. The analytes concentrations were then calculated using:

$$\textbf{Concentration} = \frac{Peak\ Area - c}{m}$$

m = slope; c = intercept

RESULTS AND DISCUSSION

Extraction Yield (%)

W_1 = 2000 g W_2 = 1472.76 g

W_3 = W_1 - W_2 = 527.24 g (weight of oil)

Percentage oil yield = $\frac{527.24}{20000}$ x 100 = 26.36%

Figure 1: HPLC Chromatogram of *Tetrapleura tetraptera* Seed Oil

Table 1: Bioactive Compounds Identified in the *Tetrapleura tetraptera* Seed Oil.

S/N	Molecular formular	Molecular mass	Retention time	Area	Height	Units	Bioactive Compound
1.	$C_{18}H1_5NO_4$	309.32	0.280	3400.3980	117.455	ug/ml	Lumarin
2	$C_{27}H_{32}O_{14}$	580.53	2.390	12252.8106	301.042	ug/ml	Naringin
3	$C_{29}H_{44}O_{12}$	584.65	4.120	6344.5478	157.158	ug/ml	Cardiac glycoside
4	$C_{15}H_{14}O_2$	226.27	6.016	18154.0688	442.689	ug/ml	Flavin-3-ol
5	$C_{15}H_{11}O^+$	207.25	7.470	8442.9838	206.428	ppm	Anthocyanin
6	$C_{15}H_{17}NO_4$	275.3	10.366	19598.0668	476.646	ug/ml	Ribalinidine
7	$C_{15}H_{12}O_5$	272.25	12.970	6238.3258	152.341	ug/ml	Naringenin
8	$C_{15}H_{26}N_2$	234.38	15.460	4967.5639	121.273	ug/ml	Spartein
9	$C_{27}H_{30}O_{16}$	610.52	17.966	11339.2568	276.424	ug/ml	Rutin
10	$C_{16}H_{19}NO_8$	353.32	27.536	11458.0104	280.295	ug/ml	Kaempferol
11	$C_{15}H_{10}O_3$	238.24	32.996	14337.0482	348.877	ug/ml	Phytate

12	$C_{26}H_{44}N_2O$	400.64	34.600	9573.1408	147.836	ug/ml	Flavone
13	$C_{15}H_{10}O_6$	286.24	42.276	3473.1416	85.310	ug/ml	Sapogenin
14	$C_{15}H_{14}O_6$	290.27	44.170	10509.6768	256.782	ug/ml	Epihedrine
15	$C_6H_{18}O_{24}P_6$	660.04	20.313	12756.4840	307.630	ug/ml	Cyanogenic glycoside
16	$C_{15}H_{10}O_2$	222.24	22.730	6059.7940	233.186	ug/ml	Flavonone
17	$C_2O_4^{--}$	88.02	25.650	10008.8176	245.115	ug/ml	Steroids
18	$C_{14}H_{12}O_3$	228.24	36.876	6988.5601	170.310	ug/ml	Oxalate
19	$C_{27}H_{44}O_2$	400.64	39.200	10234.6024	249.263	ug/ml	Resveratol
20	$C_{10}H_{15}NO$	165.23	29.860	5478.4406	133.723	Ug/g	Epicatechin

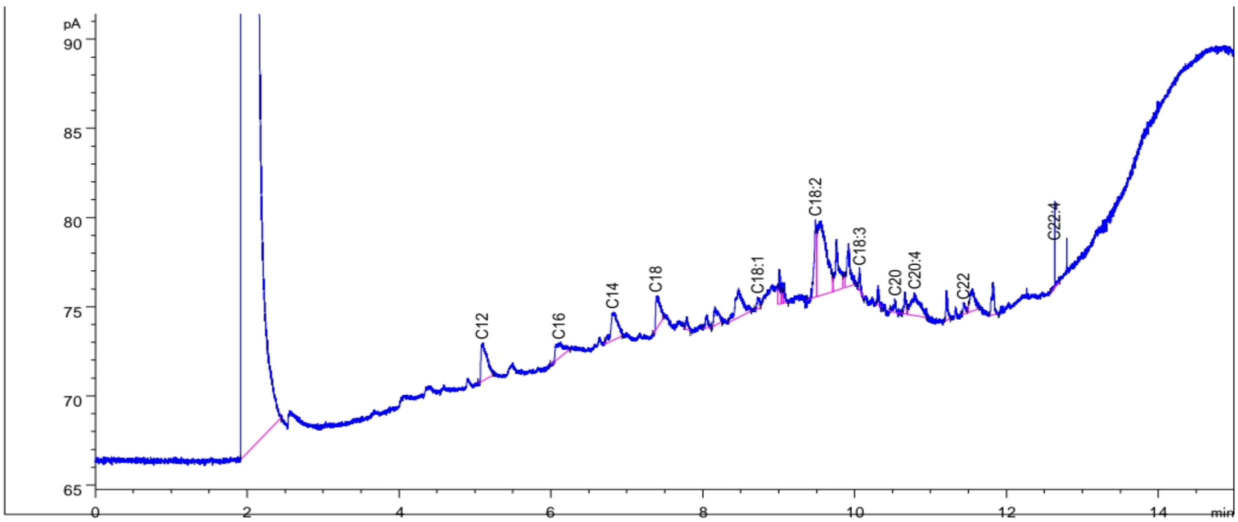


Figure 2: Free Fatty Acid Chromatogram of *Tetrapleura tetraptera* Seed Oil

Table 2: Free Fatty Acids Composition of Tetrapleura *tetraptera* Seed Oil

S/N	Retention time (min)	Area (pA*s)	Amount (ppm)	FFA Notation	Name
1	5.100	11.50274	4.37337	C12	Duodecanoic (Lauric) acid
2	6.106	6.01647	5.25291	C16	Hexadecanoic(palmitic) acid
3	6.820	10.03448	3.80224	C14	Tetradecanoic (myristic) acid
4	7.388	7.09191	8.66677	C18	Octadecanoic (stearic) acid
5	8.721	1.49380	5.10963e-1	C18:1	Cis-9-octadecaenoic (oleic) acid
6	9.489	9.01848	8.40761	C18:2	Cis-9,12-octadecadienoic (Linoleic) acid
7	10.067	1.42917	4.81879e-1	C18:3	Cis-9,12,15-octadecatrienoic (α-Linoleic) acid
8	10.534	1.48669	5.03413e-1	C20	Eicosanoic (arachidic) acid
9	10.791	9.62673	3.55580	C20:4	all-cis-5,8,11,14-eicosatetraenoic acid
10	11.442	1.19812	3.92985e-1	C22	Docosanoic (Behenic) acid
11	12.639	1.49048	4.99802e-1	C22:4	all-cis-7,10,13,16-Docosatetraenoic acid

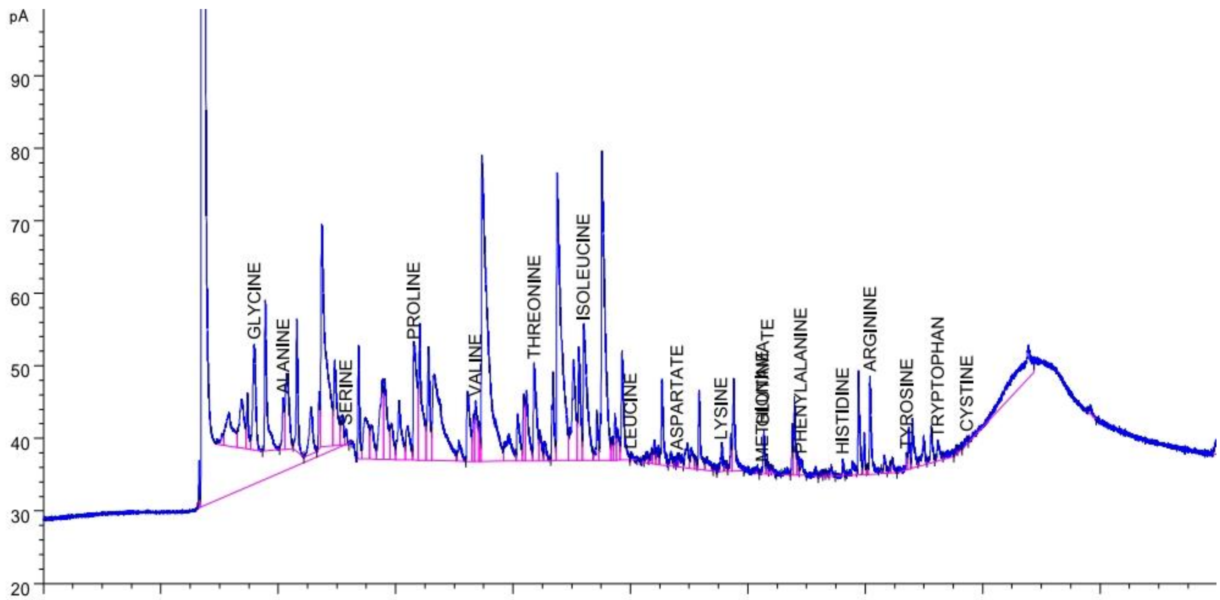


Figure 3: Amino Acids Chromatogram of *Tetrapleura tetraptera* Seed Oil

Table 3: Amino Acid Composition of *Tetrapleura tetraptera* Seed Oil

S/N	Retention time	Area (pA*s)	Amount (ppm)	Name
1	3.594	52.48975	4.46720	Glycine
2	4.090	16.97433	4.66209	Alanine
3	5.160	4.71648	1.28056	Serine
4	6.317	69.80251	9.23352	Proline
5	7.366	27.06929	7.44889	Valine
6	8.364	46.53328	2.81943	Threonine
7	9.202	86.96768	3.97011	Isoleucine
8	10.007	2.50128	6.67849e-1	Leucine
9	10.800	3.61911	9.76341e-1	Aspartate
10	11.554	10.78058	2.92541	Lysine
11	12.159	2.38175	6.32801e-1	Methionine
12	12.269	11.4542	3.13833	Glutamate
13	12.902	6.75205	1.83913	Phenylalanine
14	13.616	3.50366	9.41731e-1	Histidine
15	14.077	31.05949	8.54635	Arginine
16	14.704	5.32016	1.41222	Tyrosine
17	15.233	9.54886	2.60871	Tryptophan
18	15.729	3.73396	1.00452	Cystine

The extraction yield of 26.36% from 2000g seed powder of Tetrapleura *teraptera* can be viewed as been considerably high. Although there is no available literature indicating previous seed oil extraction yield from the plant, oil yield can be generally influenced by species genetics, seed maturity, environmental condition, solvent polarity and extraction method (Ominowa *et al.*, 2024). Other studies may demonstrate close or high range variability from ours due to the above reasons. For instance, solvent extraction of oil from the seed of

Trichosanthes cucumerina reportedly yielded approximately 27.8%, *Hura crepitans*, 51.3% and *Tevetia nerifolia*, 67% still pointing to the aforementioned plant and evironmental parameters as critical determinants of oil yield even when the same extraction methods are adopted (Fowomola and Akindahunsi, 2007; Mathiarasi and Partha, 2015; Manimaran *et al.*, 2020).

HPLC chromatogram of the seed oil revealed twenty (20) peaks (Figure 1), corresponding to 20 secondary metabolites as listed on table 2. The identified compounds fall under plant bioactive compound classes including flavonoids, alkaloids, glycosides, steroids and derivatives, phenolic compounds (non-flavonoid polyphenols) and antinutrients/ organic acids. Although, the compounds are well spread in all the classes, flavonoids remained dorminant with nine (9) compounds including kaemmferol, Rutin, flavan-3-ol, epicatechin, naringenin, naringin, flavonones, flavone and anthocyanin. Four (4) of the compounds belong to the class of alkaloids and included Lunamarin, spartein, epihedrine and rebalinidine. Two (2) are glycosides and included cardiac glycosides and cyanogenic glycosides. Two (2) others fall under the class of steroids and steroid derivatives such as steroids and sapogenin. Other compound classes include one (1) non-flavonoid polyphenol (resveratrol also called stilbene polyphenol) and two (2) antinutrients/organic acids (phytate and oxalate).

Though there was no available literature on previous bioactive compound profiling of Tetrapleura *tetraptera* seed oil, our result shows slight variation from those of previous researchers who profiled whole fruit pod and other parts using either GC-MS or HPLC. GC-MS analysis of the dry fruit pod by Bebia *et al*. (2024) revealed a total of twenty-eight bioactive compound belonging to similar compound classes as ours but with terpenes dominating in terms of number. A previous phytochemical profiling using HPLC/LC-MS identified twenty-four bioachive compounds with polyphenol specifically flavonoids revealed in highest abundance (Dzah, 2022). In a similar chemical profiling of the plant's stem bark ethanol-extract using HPLC, Imade *et al*. (2024) identified eleven bioactive compounds which were predominantly polyphenols. The report of Okechukwu *et al*. (2022) indicated a total of twenty-seven compounds in essential oil from the plant in a GC-MS analysis, which is also at variance with ours, although the two results agree in terms of dorminance of phenolic compounds. The observed differences in the number and number of identified compounds across studies can be attributed to extraction method, plant part used, solvent polarity, geographical source, sensitivity of anlytical instruments among others.

The number of compounds detected from the HPLC analysis of our seed oil is not only an indication that the sample has a rich reservoir of biologically active phytochemicals capable of demonstrating therapeautic effects, but also points to the sensitivity of our instrument as well as solvent efficiency. Plant falvonoids such as the one from our extract have documented pharmacological activity such as antioxidant and anti-inflammatory effects which are important in the management of several health challenges associated with age such as benign prostatic hyperplasia (Ebenyi *et al.* 2022). Reportedly, naringenin has strong bioactive properties and therapeutic potentials against neurological, cardiovascular, gastrointestinal, rheumatological, metabolic, and malignant disorders (Rani *et al*., 2016). The presence of Kaempferol in significant amounts in the seed oil suggest its possible in neuroproection where it is belived to modify inflammatory signalling pathways like β-catenin, p38MAPK, NF-κB AKT.KPF and its glycosylated derivatives that modulate diseases of the CNS diseases (Silva dos Santos *et al*., 2021). Naringenin and Naringin are effective against hepatotoxicity induced by alcohol, CCl4, and high-fat diets. Naringenin modulates lipid metabolism, enhances antioxidant enzyme expression, and reduces steatosis and inflammation experimental models as reported in a previous study (Flores-Peña *et al*., 2025). The role of flavonoids against diabetic neuropathy pathways, including the inhibition of α-glucosidase enzyme has been reported markig them as potent antidiabetic agents (Sood *et al*., 2025)

Generally, flavonoids act as exogenous antioxidants due to their ability to reduce by radicals directly produce less oxidants via different mechanisms like the inhibition of nitric-oxide synthase activity, inhibition of xanthine oxidase activity, modulation of channel pathway or interacting with other enzyme systems (Kumar *et al*. 201; Onwuka *et al*., 2026). Kong *et al*. (2013) demonstrated that kaempferol at 30-150 mg/kg/day increased antioxidant enzyme superoxide dismutase, and decreased lipid-peroxidation marker MDA in high-cholesterol rabbits. Thus, the established antioxidant activity of flavonoids may underscore their roles in phytomedicine, especially in the treatment of oxidative stress mediated ailments (Muscolo *et al*., 2024). Prochazkova *et al*. (2011) documented that the antioxidant capacity of flavonoids is higher than that of vitamin C and vitamin E recommending regular regularly inclusion of those fruits and vegetables rich flavonoids in daily food. Other reports have also established that plant-based flavonoids enhance bone, vascular and cardiovascular health, preventing chances of stroke through their antioxidant and anti-inflammatory properties (Terao, 2017). The anti-inflammatory effects of some of the identified phytocompounds like naringenin, kaempferol, rutin, epicatechin and flavan-3-ol have been established, implying that they may play a role in the management of health challeges involving inflammation such as benign prostatic hyperplasia, cancer, cadiovascular diseases.

They have been reported to demonstrate potential for obesity management, nephro and neuroprotection (Rasheed *et al*., 2026). Lunamarin, naringenin, flavan-3-ol and spartein also present in our seed oil have reported beneficial activity on brain, eye and cardiovascular health, enhancing blood flow through antioxidant and anti-inflammatory mechanisms as reported by Rashimi *et al*.(2018), Shi *et al*. (2025). The health significance of rutin as a potent inhibitor of 5α-reductase activity, the key enzyme implicated in benign prostatic hyperplasia has been documented (Nguyen *et al*., 2024; Azizi *et al*., 2021), standing the phytoflavonoid out as an anti-BPH compound. Steroids, sapogenin, flavonones and resveratrol which constitute significant component of our seed oil have been reported to modulate androgen/estrogen pathways which known to be linked with development and progression of benign prostatic hyperplasia, where they are believed to interfer with receptors associated with the disease (Krishnamoorthi *et al*., 2024).

Figure 2 displays the free fatty acid (FFA) chromatogram showing eleven (11) peaks corresponding to 11 free fatty acids (Table 2b). Each peak reflects the number of carbons and double bonds present in the FFA. All the identified free fatty acids are of the long chain group and consists of saturated (SFAs), momounsaturated (MUFA) and polyunsaturated fatty acids (PUFAs). The saturated FFA included doodecanoic (lauric) with reported anti-inflammatory and immunomodulatory effects, hexadecanoic (myristic acid) involved in protein signaling, palmitic acid known for its role in lipid signling and inflammation and dococdecanoic (behenic) acid) which is a structural lipid with low inflammatory potential (Abe *et al*., 2009). The pharmacological relevance of other FFAs in our sample such as lauric and palmitic acid also extends to their reported potential to inhibit 5α-reductase activity in the prostate epithelium and stroma homogenates (Abe *et al.* 2009). The unsaturated fatty acid identified from our oil sample included, oleic acid, linolenic acid, arachidonic acid, eicosatetraenoic acid and docosatetraenoic. These FFAs, particularly oleic acid, play significant role in managing BPH through anti-androgenic, anti-inflammatory, and anti-proliferative mechanisms (Raynaud *et al*., 2002), They also reportedly inhibit the two isoforms of 5α-reductase (Types 1 & 2) and α_1-adrenergic receptor-binding, minimizing dihydrotestosterone (DHT) binding to androgen receptors and consequently, decreasing smooth muscle contraction in the prostate as observed by Liu *et al*. (2009) and Abe *et al.* (2009).

Further chemical analysis of the *Tetralpeura tetraptera* seed oil revealed the presence of eighteen (18) primary amino acids in substantial quantities (Figure 3 and Table 3). The amino acids consist of twelve (12) essential (valine, threonine, isoleucine, leucine, lysine, methionine, phenyl alanine, histidine, tryptohan, arginine, tyrosine and cystine) and six (6) non-essential (glycine, alanine, serine, proline, aspartate and glutamate). Two (2) amino acids, asparagine and glutamine were not detected in our sample. This rich profile of amino acids from the seed oil-extract may underscore the use of the fruit pod deconcotion in the treatmemt of nutritional disorders like Kwashiokor. Apart from important nutritional roles of some of the identified amino acids, leucine, isoleucine and valine may specifically play essential role in general protein synthesis involving tissue repair, enzyme and hormone production. Tyrosine can serve as precursor for synthesis of catecholamines and thyroid hormones. Tryptophan also plays pharmacological role as starting material for the synthesis of serotonin and malatonin. Cystine is a component of glutathione and may play vital role in oxidative stress prevetion. Arginine and glutamate can enhance body immunity and may play preventive role in anti-inflammatory activity. The amino acid glutamate, glycine and alanine have been reported to reduce oedema of the prostate gland (Werbach, 1993; Gabby and Wright, 1999).

CONCLUSION

From findings of the study, it can be concluded that *Tetrapleura tetraptera* seed oil contains diverse phytochemicals with therapeutic roles. The identified secondary metabolites, free fatty acids and amino acids with reported antioxidant, anti-inflammatory and disease modulation potentials may support applications of the various parts of the plant in traditional medicine. However, further experimental studies to establish detailed mehanism of action of these phytochemicals including their toxicity profile and clinical successes are suggested.

Conflict of interest: The authors declare no known competing financial or personal interests.

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