American Journal of Biomedical Science & Research

www.biomedgrid.com

ISSN: 2642-1747

Research Article

Phytochemical Profile and Multi-Target Enzyme Modulation by Tetrapleura Tetraptera (Schumach. & Thonn.) Taub. Pod Extract in Metabolic, Vascular, And Neurodegenerative Disorders

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To Cite This article: *Anietie Eyo Robert*, Cletus Anes Ukwubile*, Kenneth Onwe Tochukwu et al, Phytochemical Profile and Multi-Target Enzyme Modulation by Tetrapleura Tetraptera (Schumach. & Thonn.) Taub. Pod Extract in Metabolic, Vascular, And Neurodegenerative Disorders. Am J Biomed Sci & Res. 2026 29(6) AJBSR.MS.ID.003865,*

DOI: 10.34297/AJBSR.2026.29.003865

Received: January 23, 2026; **Published:** January 30,2026

Abstract

Tetrapleura tetraptera pod is widely used in West African traditional medicine for managing diabetes, neurodegenerative disorders, cardiovascular dysfunction, and erectile dysfunction. This study aimed to perform comprehensive phytochemical profiling and evaluate the antioxidant and multi-target enzyme inhibitory potential of *T. tetraptera* pod extract. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were quantified as 802.21 mg Gallic Acid Equivalents (GAE)/g and 116.05 mg Quercetin Equivalents (QE)/g extract, respectively. High-performance liquid chromatography revealed ten phenolic compounds, including kaempferol (4.68%) and quercetin-3-O-galactoside (23.33%), indicating the extract's rich bioactive composition. Antioxidant activity assessed via DPPH and FRAP assays showed IC_{50} values of 24.34µg/mL and 26.56µg/mL, comparable to ascorbic acid (16.80µg/mL and 12.44µg/mL). Enzyme inhibition studies demonstrated dose-dependent inhibition of α-amylase (IC_{50}=16.01µg/mL) and α-glucosidase (IC_{50}=12.56µg/mL), comparable to acarbose (6.12µg/mL and 8.24µg/mL). Moreover, the extract inhibited acetylcholinesterase (IC_{50}=8.14µg/mL), angiotensin-converting enzyme (IC_{50}=8.06µg/mL), arginase (IC_{50}=8.01µg/mL), ecto-5′-nucleotidase (IC_{50}=8.28µg/mL), phosphodiesterase-5 (IC_{50}=18.45µg/mL), monoamine oxidase (IC_{50}=6.44µg/mL), and catechol-O-methyltransferase (IC_{50}=38.56µg/mL). These results suggest that *T. tetraptera* pod extract possesses significant antioxidant capacity and multi-target enzyme inhibitory activity, supporting its ethnomedicinal applications in managing metabolic, vascular, neurological, and erectile dysfunction disorders. The findings provide a mechanistic basis for its therapeutic potential and highlight its promise as a source of multi-target phytotherapeutics.

Keywords: *Tetrapleura tetraptera;* phytochemicals; antioxidant; α-amylase; multi-target enzyme inhibition.

Introduction

Non-Communicable Diseases (NCDs) represent one of the most pressing global health challenges of the 21st century, accounting for most of morbidity and mortality worldwide. Among these, metabolic disorders such as type 2 diabetes mellitus (T2DM), cardiovascular diseases, neurodegenerative conditions, and sexual dysfunctions are increasingly prevalent and frequently coexist within the same patient population. T2DM alone affects more than 537 million adults globally, with projections indicating a continued and substantial rise in disease burden over the coming decades, particularly in low- and middle-income countries [1]. Importantly, these disorders are not isolated entities but are mechanistically interconnected

through shared pathophysiological pathways, including persistent oxidative stress, chronic low-grade inflammation, endothelial dysfunction, impaired neurotransmission, and dysregulated enzyme activity [2]. Oxidative stress plays a central role in the initiation and progression of these chronic conditions by promoting cellular damage, mitochondrial dysfunction, and aberrant signaling cascades. Excessive production of Reactive Oxygen Species (ROS) alters vascular homeostasis, impairs nitric oxide bioavailability, and accelerates atherosclerotic processes, thereby contributing to endothelial dysfunction and cardiovascular complications.

In the context of diabetes, oxidative stress exacerbates insulin resistance and β-cell dysfunction while simultaneously promoting secondary complications such as peripheral neuropathy, cognitive decline, and erectile dysfunction. These complications are further amplified by dysregulated enzymatic pathways, including carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase), Angiotensin-Converting Enzyme (ACE), Acetylcholinesterase (AChE), Monoamine Oxidase (MAO), Catechol-O-Methyltransferase (COMT), phosphodiesterase type 5 (PDE-5), and arginase, all of which play critical roles in glucose metabolism, vascular tone, neurotransmitter balance, and erectile physiology. Current pharmacological interventions for these conditions are largely single target in nature and often require polypharmacy to manage disease progression and associated complications. While such approaches may offer symptomatic relief, they are frequently associated with adverse effects, drug-drug interactions, high costs, and poor long-term compliance. Moreover, they fail to adequately address the complex, multifactorial nature of chronic diseases driven by oxidative stress and enzymatic dysregulation. These limitations have intensified scientific interest in natural products and medicinal plants as alternative or complementary therapeutic strategies, particularly those capable of exerting antioxidant and multi-target enzyme inhibitory effects simultaneously.

Tetrapleura tetraptera (Schumach. & Thonn.) Taub., a member of the Fabaceae family, is a perennial tropical tree widely distributed across West Africa. Commonly referred to as Aidan or Aridan, its fruits are extensively used both as culinary spices and as remedies in traditional medicine for the management of diabetes, hypertension, inflammation, epilepsy, postpartum disorders, and other chronic ailments [1,3]. The prominence of *T. tetraptera* in ethnomedicine suggests the presence of pharmacologically active constituents capable of modulating multiple biological targets. Phytochemical investigations of *T. tetraptera* fruits have identified a diverse array of secondary metabolites, including polyphenols (notably flavonoids and tannins), saponins, triterpenoids, alkaloids, and coumarinic compounds such as scopoletin. These phytoconstituents are well-recognized for their antioxidant, anti-inflammatory, enzyme-modulatory, and vasoprotective properties. Experimental studies have reported significant antioxidant activity of *T. tetraptera* extracts, evidenced by their ability to scavenge free radicals and inhibit lipid peroxidation [4]. Anti-inflammatory and antihyperglycemic effects have also been demonstrated in both in vitro and in vivo models, supporting its traditional use in metabolic disorders [1].

Notably, hydroethanolic fruit extracts of *T. tetraptera* have shown pronounced anti-insulin resistance, anti-lipidemic, anti-obesity, and anti-inflammatory effects in high-carbohydrate, high-fat diet-induced obese and type 2 diabetic animal models, with outcomes comparable to standard antidiabetic agents such as metformin [1]. In addition, ethanol leaf extracts have exhibited neuroprotective properties through antioxidants, anti-inflammatory, and anti-apoptotic mechanisms in experimental models of neurotoxicity [5,6]. These findings collectively highlight the broad pharmacological potential of *T. tetraptera* and underscore its relevance in managing diseases driven by oxidative stress and enzymatic dysfunction. Despite this growing body of evidence, critical gaps remain in the current understanding of the mechanistic basis underlying the therapeutic effects of *T. tetraptera.* Most previous studies have focused on isolated biological activities or whole-animal outcomes without integrating comprehensive phytochemical profiling with systematic evaluation of enzyme-targeted actions. In particular, the inhibitory potential of *T. tetraptera* extracts against key enzymes that bridge diabetes, endothelial dysfunction, neurodegeneration, and erectile dysfunction have not been thoroughly investigated. This is a significant limitation, as enzymatic dysregulation represents a convergent mechanism linking these conditions.

For example, excessive activity of α-amylase and α-glucosidase contributes to postprandial hyperglycemia, while upregulation of ACE promotes vasoconstriction and endothelial dysfunction. In the nervous system, altered activities of AChE, MAO, and COMT disrupt neurotransmitter homeostasis, contributing to cognitive impairment and neurodegeneration. Similarly, erectile dysfunction in diabetic patients is closely associated with oxidative stress-induced endothelial damage, reduced nitric oxide signaling, increased arginase activity, and dysregulated PDE-5-mediated cyclic GMP metabolism. These interconnected pathways suggest that a single therapeutic agent capable of modulating multiple enzymes while simultaneously mitigating oxidative stress may offer substantial advantages over conventional single-target drugs. In this context, the present study adopts a multi-target natural product discovery strategy to evaluate the therapeutic potential of *Tetrapleura tetraptera* fruit extract comprehensively. By integrating detailed phytochemical profiling, including HPLC-based characterization of major bioactive constituents, with robust in vitro antioxidant assays and enzyme inhibition studies, this work seeks to elucidate the mechanistic foundations of the plant's ethnomedicinal applications. The biological evaluation focuses on enzymes directly implicated in glucose metabolism (α-amylase and α-glucosidase), cardiovascular and endothelial regulation (angiotensin-converting enzyme), neurodegenerative processes (acetylcholinesterase, monoamine oxidase, catechol-O-methyltransferase), and erectile function (phosphodiesterase type 5 and arginase). Through this integrated approach, the study aims to provide a unifying mechanistic framework that links the antioxidant capacity and enzyme inhibitory properties of *T. tetraptera* to its traditional use in managing complex chronic diseases. The findings are expected to advance scientific understanding of the plant's multi-target mode of action, support its rational development as a phytotherapeutic or nutraceutical candidate, and contribute valuable evidence toward

the validation of African medicinal plants in modern drug discovery pipelines.

Materials and Methods

Chemicals, Reagents, and Equipment

Analytical-grade chemicals and reagents, including Folin-Ciocalteu reagent, Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), sodium carbonate, potassium acetate, aluminium chloride, rutin, gallic acid, ascorbic acid, acarbose, Trichloroacetic Acid (TCA), 5,5′-Dithiobis-(2-Nitrobenzoic Acid) (DTNB), 1,1-Diphenyl-2-Picrylhydrazyl (DPPH), potassium ferricyanide, ferric chloride, and commercial enzymes (α-amylase, α-glucosidase, acetylcholinesterase, phosphodiesterase-5), were procured from JoeChem Nigeria Ltd (Sigma-Aldrich®, St. Louis, MO, USA). Chromatographic analysis was performed using an Agilent 1100 series HPLC system (Agilent Technologies, USA) equipped with a Diode Array Detector (DAD). All other reagents used in this study were of analytical grade.

Plant Collection and Authentication

Dried pods of *Tetrapleura tetraptera* were purchased from Itam Market, Itu Local Government Area, Akwa Ibom State, Nigeria, in January 2025. Botanical authentication was carried out by *Dr. Imoh I. Johnny,* Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria. A voucher specimen was deposited at the herbarium with accession number UUPH18AI.

Preparation of Plant Extract

The dried pods were thoroughly cleaned, shade-dried for one week, and pulverized into a fine powder using an electric blender (Panasonic®, Nigeria). A total of 180g of powdered material was subjected to cold maceration in ethyl acetate for 72h with intermittent agitation. The extract was filtered and concentrated under reduced pressure to yield a dark-brown residue weighing 30g, corresponding to a percentage yield of 16.6%. The extract was stored at 4°C until further analysis.

Preliminary Phytochemical Screening

Qualitative phytochemical screening of the ethyl acetate extract was carried out using standard procedures described by Harborne [7] to detect the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones, sterols, and triterpenes.

HPLC-UV Phytochemical Profiling

High-performance liquid chromatography analysis was performed following the method of *Kanu et al* [8] with slight modifications. One gram of extract was dissolved in methanol, sonicated, diluted, filtered (0.45µm), and injected (20µL) into the HPLC system. Separation was achieved on a Zorbax Eclipse XDB-C18 column (150mm × 4.6mm, 5µm) using isocratic elution at a flow rate of 0.5mL/min. The mobile phase consisted of acetonitrile: methanol: aqueous formic acid (50:30:20, v/v/v; pH 3.5). Detection was carried out at 280nm, and peak integration was performed using Agilent ChemStation software.

Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin-Ciocalteu colorimetric method [7]. Absorbance was measured at 760nm, and results were expressed as mg gallic acid equivalents (GAE)/g extract.

Determination Of Total Flavonoid Content (TFC)

Total flavonoid content was quantified using the aluminium chloride method as described by *Patel et al,* [9], with quercetin as standard. Absorbance was recorded at 415nm, and results were expressed as mg quercetin equivalents (QE)/100g extract.

In Vitro Antioxidant Assays

4.8.1.DPPH Radical Scavenging Assay: The DPPH radical scavenging activity of the extract (10-100µg/mL) was evaluated following Molole and Abdissa [10]. Absorbance was measured at 517 nm, using ascorbic acid as reference standard. Percentage scavenging activity was calculated using standard equations.

4.8.2.Ferric Reducing Antioxidant Power (FRAP) Assay: FRAP activity was assessed according to *Ajibade et al,* [11]. Absorbance was measured at 700nm, and increased absorbance indicated higher reducing power.

Enzyme Inhibitory Assays

Alpha-Amylase Inhibition Assay: Alpha-Amylase inhibitory activity was determined using the DNSA method described by Harborne [7]. The extract (31.25-500µg/mL) was tested against porcine pancreatic α-amylase, with acarbose as the positive control. Absorbance was read at 540nm.

Alpha-Glucosidase Inhibition Assay: The α-glucosidase inhibition was evaluated according to *Li et al,* [12]. The liberated glucose was quantified using the anthrone reagent, and absorbance was measured at 640nm.

Acetylcholinesterase (Ache) Inhibition Assay: The AChE inhibitory activity was assessed using Ellman's method as modified by *Akomolafe et al* [13]. Absorbance was measured at 412 nm, and percentage inhibition was calculated relative to the control.

Ecto-5-Nucleotidase Activity: The effect of the extract on ecto-5-nucleotidase activity was evaluated following *Heymann et al* [14] and *Ademiluyi et al* [15]. Liberated inorganic phosphate was quantified using the Fiske-Subbarow method [16].

Phosphodiesterase-5 (PDE-5) Inhibition Assay: PDE-5 inhibitory activity was determined using the method of Kelly and Butler [17]. Sildenafil served as the reference drug, and absorbance was read at 400nm.

Data analysis

All assays were performed in triplicate. Results were expressed as mean ± SEM, and percentage inhibition was calculated using standard formulae.

Results

Total Phenolic and Flavonoid Contents

The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of the pod extract of *Tetrapleura tetraptera* were quantified using standard colorimetric assays. The TPC was determined as 802.21 mg Gallic Acid Equivalents (GAE)/g extract, calculated from the gallic acid calibration curve (y = 0.0068x + 0.084; R^2 = 0.9418) Figure 1. Similarly, the TFC of the extract was 116.05 mg quercetin equivalents (QE)/g extract, derived from the quercetin calibration curve (y=0.0068x + 0.8426; R^2= 0.9628) Figure 1. The calibration curves for phenolic and flavonoid estimations are presented in (Figures1, 2).

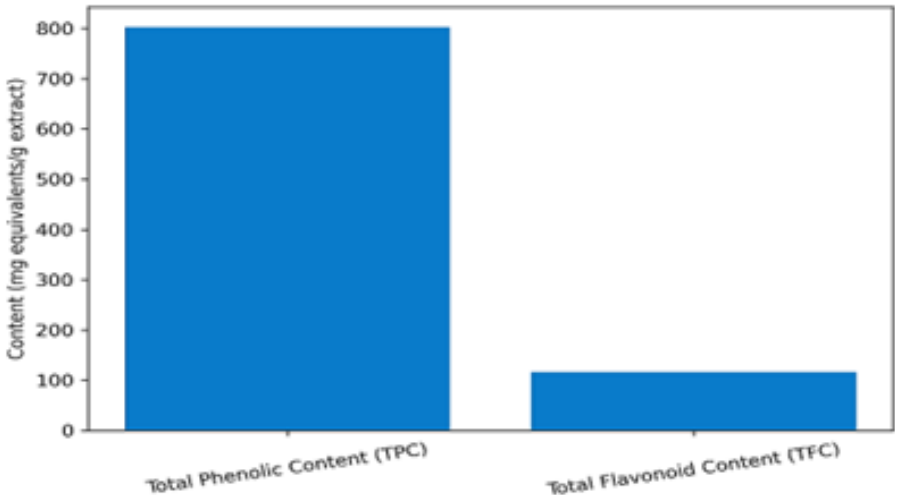

Figure 1: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of T. tetraptera pod extract. TPC is expressed as mg Gallic Acid Equivalents (GAE)/g extract, while TFC is expressed as mg quercetin equivalents (QE)/g extract.

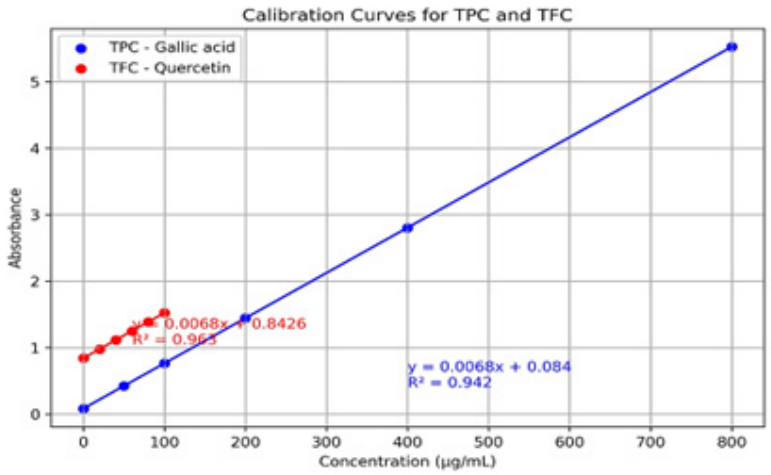

Figure 1: Combined calibration curves for Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) determination. TPC was measured using gallic acid (blue), and TFC was measured using quercetin (red). Regression equations and R^2 values are shown for each standard curve.

Qualitative Phytochemical Screening

The qualitative phytochemical analysis of the pod extract of *T. tetraptera* revealed the presence of several bioactive secondary metabolites Table 1. Detected constituents included alkaloids, flavonoids, tannins, saponins, triterpenoids, phytosteroids, cardiac glycosides, proteins, polyphenols, and oxalates. In contrast, fat/oils and anthraquinones were absent from the extract (Table 1).

Table 1: Qualitative phytochemical constituents of *Tetrapleura tetraptera* pod extract.

Phytochemical class	Inference
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Triterpenoids	+
Phytosteroids	+
Cardiac glycosides	+
Proteins	+
Polyphenols	+
Oxalates	+
Fats and oils	–
Anthraquinones	–

Note*: (+) Present; (–) Absent.

HPLC Phytochemical Profiling

High-Performance Liquid Chromatography (HPLC) was employed to characterize the phenolic profile of the pod extract of *T. tetraptera.* The chromatographic analysis revealed the presence of multiple phenolic and bioactive compounds, with a total of 15 peaks detected in the chromatogram (Figure 2). Among these, ten major compounds were tentatively identified, including paxilline, rocaglamide, pentanetriol, bastadin, kaempferol, quercetin-3-O-galactoside, citreoisocoumarin, and naammine, based on retention times and UV absorbance characteristics. Quercetin-3-O-galactoside exhibited the highest relative peak area (23.33%), followed by a major unidentified peak at 21.32 min (25.24%). The detailed retention times, peak heights, areas, relative areas, and compound classifications are summarized in Table 2, while the overall chromatographic distribution confirms the chemical complexity and phenolic richness of the extract (Table 2).

Table 2: HPLC phenolic profile of *T. tetraptera* pod extract.

Peak No.	Retention Time (min)	Peak Height (Mau)	Peak Area (Mau·Min)	Relative Area (%)	Identified Compound
1	1.23	2691.43	382.13	13.91	Paxilline
2	10.9	139.18	51.91	1.89	Rocaglamide
3	12.11	355.21	117.94	4.29	Pentanetriol
4	13.26	395.1	129.92	4.73	Bastadin
5	15.56	214.63	111.75	4.07	Unidentified
6	16.16	117.19	36.25	1.32	Unidentified
7	17.98	149.26	87.4	3.18	Unidentified
8	18.64	550.47	128.52	4.68	Kaempferol
9	19.39	101.62	17.03	0.62	Rutin
10	19.83	1364.65	640.89	23.33	Quercetin-3-O-galactoside
11	21.32	1475.29	693.46	25.24	Unidentified
12	22.84	255.16	111.87	4.07	Unidentified
13	24.05	822.14	206.16	7.5	Citreoisocoumarin
14	35.47	84.32	14.39	0.52	Naamine
15	38.52	52.78	17.71	0.64	Unidentified
Total	—	8768.42	2747.31	100	—

Antioxidant Activity

Table 3: Antioxidant activity of *T. tetraptera* pod extract.

Sample	DPPH IC_{50} (µg/Ml)	ABTS IC_{50} (µg/Ml)
Pod extract	24.34±0.82	26.56±1.14
Ascorbic acid	16.80±0.63	12.44±0.57

Note*: Values are mean ± SD (n = 3). No significant difference compared with control ($p > 0.05$).DPPH: 1,1-diphenyl-2-picrylhydrazyl; ABTS: 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid).

The antioxidant potential of the pod extract of *T. tetraptera* was evaluated using DPPH and ABTS radical scavenging assays. As shown in Table 3, the extract demonstrated strong antioxidant activity with IC_{50} values of 24.34µg/mL (DPPH) and 26.56µg/mL (ABTS). Although ascorbic acid exhibited lower IC_{50} values (16.80µg/mL for DPPH and 12.44µg/mL for ABTS), the antioxidant activities of the extract were not significantly different ($p>0.05$) from the standard, indicating comparable radical scavenging efficiency (Table 3).

A-Amylase Inhibitory Activity

The inhibitory effect of the pod extract of *T. tetraptera* on α-amylase activity was concentration-dependent. The extract exhibited an IC_{50} value of 16.01µg/mL, which was insignificantly higher than that of the standard inhibitor acarbose (6.12µg/mL, $p > 0.05$). As presented in Table 4, percentage inhibition increased

progressively with extract concentration (50-800µg/mL), reaching a maximum inhibition of 72.44% at 800µg/mL (Table 4).

Table 4: Alpha-amylase inhibitory activity of *T. tetraptera* pod extract.

Concentration (µg/Ml)	Extract Inhibition (%)	Acarbose Inhibition (%)
50	24.18±0.71	45.14±1.02
100	28.34±0.64	48.66±0.88
200	45.67±0.93*	68.24±1.14
400	56.02±1.21*	78.22±1.09
800	72.44±1.35*	81.11±1.26
IC_{50}	16.01±0.54	6.12±0.29

Note*: Values are mean ± SD (n=3); *p > 0.05 vs. acarbose.

Alpha-Glucosidase Inhibitory Activity

Similarly, the pod extract demonstrated dose-dependent inhibition of α-glucosidase activity. The IC_{50} value of the extract was 12.56µg/mL, compared to 8.24µg/mL for acarbose, with no statistically significant difference between the two (p>0.05). The percentage inhibition values across the tested concentration range (50-800µg/mL) are summarized in Table 5, confirming the extract's strong inhibitory potential against α-glucosidase (Table 5).

Table 5: Alpha-glucosidase inhibitory activity of *T. tetraptera* pod extract.

Concentration (µg/Ml)	Extract Inhibition (%)	Acarbose Inhibition (%)
50	28.22±0.86	45.14±0.95
100	34.56±1.02	48.66±1.07
200	52.11±1.14	68.24±1.22
400	63.08±1.27*	78.22±1.18
800	68.24±1.31*	81.11±1.25
IC_{50}	12.56±0.47*	8.24±0.36

Note*: Values are mean ± SD (n=3); *p > 0.05 vs. acarbose.

Inhibition Of Other Enzyme Targets

Table 6: IC_{50} values for enzyme inhibition by *T. tetraptera* pod extract.

Sample / Standard	ACE	AChE	Arginase	Ecto-5 - NTD	PDE-5	MAO	COMT
T. tetraptera	8.06	8.14*	8.01	8.28	18.45*	6.44	38.56
Lisinopril	0.08	—	—	—	—	—	—
Nicotinic acid	—	0.82*	—	—	—	—	—
L-citrulline	—	—	2.03	—	—	—	—
Simvastatin	—	—	—	4.06*	—	—	—
Sildenafil	—	—	—	—	6.02*	—	—
Clorgyline	—	—	—	—	—	3.08	—
Entacapone	—	—	—	—	—	—	12.66

Note*: IC_{50} values expressed in µg/mL. Standards showed significantly lower IC_{50} values than the extract (p < 0.05).

The inhibitory effects of the pod extract of *T. tetraptera* against key enzymes implicated in cardiovascular, neurodegenerative, metabolic, and erectile dysfunction pathways were evaluated. As shown in Table 6, the extract inhibited Angiotensin-Converting Enzyme (ACE), Acetylcholinesterase (AChE), arginase, ecto-5′-nucleotidase (Ecto-5-NTD), Phosphodiesterase-5 (PDE-5), Monoamine Oxidase (MAO), and Catechol-O-Methyltransferase (COMT) in a concentration-dependent manner. Although the IC_{50} values of the reference standards (e.g., lisinopril, sildenafil, clorgyline, entacapone) were significantly lower than those of the extract, the observed multi-target enzyme inhibition highlights the broad pharmacological relevance of *T. tetraptera* pod extract (Table 6).

Discussion

This study provides a detailed phytochemical characterization and multi-target biological evaluation of Tetrapleura tetraptera pod extract, revealing its therapeutic potential in conditions characterized by oxidative stress and enzymatic dysregulation, including diabetes, endothelial dysfunction, neurodegeneration, and erectile dysfunction. The extract demonstrated a high total phenolic content (802.21mg GAE/g) and total flavonoid content (116.05mg QE/g), consistent with the presence of abundant polyphenols and flavonoids known for their antioxidant, anti-inflammatory, and enzyme-modulatory activities [18-27]. The HPLC analysis confirmed the presence of ten phenolic compounds, including kaempferol (4.68%) and quercetin-3-O-galactoside (23.33%), which have been previously reported to scavenge free radicals, modulate key signaling pathways, and inhibit enzymes implicated in metabolic and neurodegenerative disorders [26,27]. The antioxidant potential of the extract was demonstrated through DPPH (IC_{50}=24.34µg/mL) and FRAP (IC_{50}=26.56µg/mL) assays, showing activities comparable to ascorbic acid (DPPH IC50=6.80µg/mL; FRAP IC_{50}=12.44µg/mL). These findings suggest that *T. tetraptera* pods contain bioactive compounds capable of mitigating oxidative stress, a central contributor to the pathophysiology of diabetes, endothelial dysfunction, neurodegenerative processes, and erectile dysfunction [28,29]. Oxidative stress promotes insulin resistance, endothelial nitric oxide (NO) depletion, lipid peroxidation, neuronal damage, and impaired penile smooth muscle function [30,31]. By neutralizing reactive oxygen species (ROS), the extract may prevent or attenuate these pathogenic events, aligning with prior studies that identified polyphenol-rich plant extracts as effective modulators of oxidative stress-mediated diseases [32].

The enzyme inhibition assays further demonstrated the multi-target pharmacological potential of *T. tetraptera.* The extract inhibited α-amylase (IC_{50}=16.01µg/mL) and α-glucosidase (IC_{50}=12.56µg/mL) in a dose-dependent manner, comparable to acarbose, a standard antidiabetic agent (IC_{50}=6.12 and 8.24µg/mL, respectively). Inhibition of these carbohydrate-hydrolyzing enzymes delays glucose absorption and reduces postprandial hyperglycemia, a key therapeutic strategy in type 2 diabetes management [33]. Notably, the extract's inhibition of both enzymes supports its potential as a natural alternative or adjunct to synthetic inhibitors, with the advantage of a broader safety profile and simultaneous antioxidant activity [34]. Beyond glycemic control, the extract exhibited significant inhibitory effects

on enzymes implicated in vascular and endothelial function. Angiotensin-Converting Enzyme (ACE) was inhibited with an IC50 of 8.06µg/mL, suggesting potential antihypertensive activity. ACE catalyzes the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor that contributes to hypertension and endothelial dysfunction [35]. By inhibiting ACE, the extract may promote vasodilation, improve endothelial NO bioavailability, and attenuate vascular oxidative stress, thus offering a mechanistic basis for the traditionally reported hypotensive effects of *T. tetraptera* [36]. Similarly, the inhibition of arginase (IC_{50}=8.01µg/mL) may preserve L-arginine availability for NO synthesis, further supporting endothelial function and vascular health [37].

Neuroprotective potential was evidenced by inhibition of acetylcholinesterase (AChE, IC_{50}=8.14µg/mL), monoamine oxidase (MAO, IC_{50}=6.44µg/mL), and catechol-O-methyltransferase (COMT, IC_{50}=38.56µg/mL). Dysregulation of these enzymes contributes to neurotransmitter depletion, oxidative neuronal injury, and cognitive impairment [38,39]. AChE inhibitors prolong acetylcholine action, improving synaptic transmission in cholinergic pathways, while MAO and COMT inhibition prevent excessive catecholamine breakdown, mitigating oxidative stress in the nervous system [40]. These results suggest that *T. tetraptera* may confer multi-modal neuroprotection through enzyme modulation and antioxidant mechanisms, complementing previous in vivo studies reporting reduced neurotoxicity and improved behavioral outcomes in rodent models treated with *T. tetraptera* extracts [41].

Erectile dysfunction (ED), particularly in the context of diabetes, involves oxidative stress, endothelial dysfunction, reduced cyclic GMP signaling, and increased PDE-5 activity [42]. The extract inhibited phosphodiesterase-5 (PDE-5, IC_{50}=18.45µg/mL), an enzyme that degrades cyclic GMP in penile smooth muscle, thereby promoting vasodilation and erection [43]. Coupled with arginase inhibition and antioxidant activity, this multi-target effect supports the potential role of *T. tetraptera* in managing diabetic ED, corroborating traditional uses and highlighting a pharmacological rationale for clinical investigation [44]. The multi-target enzyme inhibitory profile of the extract underscores the concept of polypharmacology in natural products. Unlike conventional single-target drugs, which often require combination therapy to address complex diseases, plant extracts such as *T. tetraptera* may exert synergistic effects through simultaneous modulation of multiple pathways. Phenolic compounds, flavonoids, saponins, and other secondary metabolites likely interact to produce additive or synergistic effects on oxidative stress and enzyme activities, enhancing therapeutic efficacy while reducing adverse effects [45,46].

Collectively, these findings provide mechanistic insight into the ethnomedicinal applications of *T. tetraptera,* linking its rich phytochemical composition to measurable biological activities relevant to metabolic, vascular, neurodegenerative, and sexual health disorders. The results advocate for further in vivo studies, bioactive compound isolation, pharmacokinetic profiling, and safety assessment to fully establish its translational potential as a multi-target phytotherapeutic agent.

Conclusion

The present study demonstrates that *Tetrapleura tetraptera* pod extract is a rich source of phenolic and flavonoid compounds with potent antioxidant capacity and multi-target enzyme inhibitory activities. The extract effectively inhibited α-amylase, α-glucosidase, ACE, arginase, AChE, PDE-5, MAO, and COMT, suggesting potential therapeutic utility in managing diabetes, vascular dysfunction, neurodegenerative disorders, and erectile dysfunction. The integration of phytochemical profiling with enzyme inhibition assays provides a mechanistic framework linking its ethnomedicinal uses to observable pharmacological effects. These results underscore the promise of *T. tetraptera* as a multi-target phytotherapeutic agent and warrant further studies, including in vivo evaluation, safety assessment, and bioactive compound isolation, to fully harness its clinical potential.

Acknowledgement

We are grateful to Mr. Babagana Yusuf of the Department of Pharmacology and Toxicology, University of Maiduguri, for his technical assistance.

Competing Interest

We have none to declare.

Funding

Not applicable.

Ethical Considerations

Not applicable.

Data Availability

Data will be made available on genuine request.

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