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Tetrapleura tetraptera of Ghanaian Origin: Phytochemistry, Antioxidant and Antimicrobial Activity of Extracts of Plant Parts

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CL, FCMR, EBQ and ROA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. All authors were involved in the experiments and data generation. Authors EBQ and ROA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The role of medicinal plants in meeting the healthcare needs of the populace, particularly in developing countries cannot be overemphasized. They provide holistic treatment and wellbeing due to a plethora of phytochemicals found in them. Among such is *Tetrapleura tetraptera* (Schum. & Thonn.) Taub., a multipurpose tree plant with varied biological activities. The current study was aimed at assessing the basic phytochemical constituents, free radical scavenging activity as well as the antimicrobial effect on various solvent extracts of leaves, fruit and stem bark of *T. tetraptera* of Ghanaian origin.
Materials and Methods: Aqueous, 50% hydroethanolic and methanolic extracts were made from

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the leaves, fruits, and stem bark of *T. tetraptera*. They were assessed for the qualitative and quantitative phytochemical constituents, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, antimicrobial activity as well as the spectroscopic properties (Ultraviolet – visible and Fourier-transform infrared spectroscopy). Compounds in the methanolic extracts were evaluated using the gas chromatography–mass spectrometry.

Results: Tannins, reducing sugars, coumarins and flavonoids were present in all extracts while hydroethanolic and methanolic extracts were rich in phenols, tannins and flavonoids, especially for the stem bark. Leaf extracts had better antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Proteus mirabilis*.

Conclusion: Generally, leaves and stem bark extracts of *T. tetraptera* are rich in biologically active phytochemicals, supporting their use in ethnomedicine and could be exploited further for other biological activities.

Keywords: Tetrapleura tetraptera; phytochemistry; antimicrobial; antioxidant; GC-MS.

1. INTRODUCTION

Over the years, plants, aside providing both humans and animals with shelter, also serve as an indispensable source of medicine to humans. Medicinal plants are used as spices and preservatives without them being acknowledged for their essentiality to the human body. Herbal formulations have globally been accepted as therapeutic, antidiabetic, hepatoprotective, lipid-lowering agents among others [1,2]. Recent years have seen significant growth in exploring plant-based natural antioxidants, especially the phenols and tocopherols [3,4]. Antioxidants generally inhibit oxidative damage to a target molecule by sequestering free radicals such as peroxide, hydroperoxide or lipid peroxyl, therefore inhibiting the oxidative mechanism which results in degenerative diseases. Medicinal plants, since time immemorial, have been a good source of antioxidants [3]. Functional foods from plant sources have also been given a closer look due to their safer and potential nutritional and therapeutic effects [5].

The plant under study, *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. (family Leguminosae) is a medicinal plant and a popular spice in Ghana and has proven efficacious in managing innumerable health conditions. *T. tetraptera* plant is popularly known in Ghana as "*Prekese*" [6], and as "*Aridan*", "*Abogolo*' and "*Dawo*" respectively among the Yorubas, Igalas, and Hausas of Nigeria [7]. The plant is mostly seen in the lowland forest of several tropical African countries and known to have a fleshy pulp fruit with small brownish-black seeds [7]. The dry ripe fruit gives off a nice fragrance and is therefore used in cooking as a spice in Western and Central Africa [8]. It is an essential plant as vital phytochemical constituents and nutrients are present and significant for the normal functioning of the body [9]. These include flavonoids, tannins, alkaloids, steroids, saponins and phenolic compounds. In Africa, the plant is used in ethnomedicine for treating several diseases such as intestinal parasites, diabetes mellitus, hypertension, arthritis, asthma, epilepsy, schistosomiasis, malaria, wound healing and for prevention of postpartum contraction [9,10].

The documented biological and pharmacological activities of the fruit are found to be molluscicidal, trypanocidal, hirudinicidal, control of schistosomiasis and intestinal parasites [7], cardiovascular and neuromuscular [11,12], hypotensive and anticonvulsant [13], anti-ulcerative, anti-inflammatory and hypoglycaemic [14], anti-microbial, birth control, analgesic and anticonvulsant [15] and anti-plasmodial [10]. The leaves have anti-diabetic [1] and antibacterial activities [16,17]. Figure 1 shows the *T. tetraptera* plant and parts.

This study therefore, sought to determine the basic phytochemical constituents and biological activities including antimicrobial activity and radical scavenging activity of the various parts of the *Tetrapleura tetraptera* plant found in Ghana.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

The plant materials (leaves, fruit, and stem bark) of *Tetrapleura tetraptera* were collected from a farm in the Ahafo region in Ghana and authenticated at the Department of Herbal Medicine in KNUST by comparing them with a voucher specimen (KNUSTHM1/2019/F002). The samples were washed to get rid of any extraneous materials, shade dried for 3 weeks and milled to fine particles.

Figure 1. *Tetrapleura tetraptera* plant and parts; (A) Tree (B) Fruit (C) Seeds

2.2 Extraction

The methanolic, 50% hydroethanolic and aqueous extracts of *T. tetraptera* parts were prepared by decoction. 500 g of pulverized materials was extracted with 2 L of solvent for 24 hours, repeated twice. The mixture was filtered and dried over a water bath to obtain extract designated as aqueous fruit extract (AFE), hydroethanolic fruit extract (HFE), methanolic fruit extract (MFE), aqueous leaves extract (ALE), hydroethanolic leaves extract (HLE), methanolic leaves extract (MLE), aqueous stem bark extract (ASE), hydroethanolic stem bark extract (HSE) and methanolic stem bark extract (MSE). The yield of the solvent extracts was calculated as a percentage of the total weight of the raw plant material used for extraction.

2.3 Qualitative and Quantitative Phytochemical Screening

All the extracts and raw plant materials were subjected to the standard qualitative phytochemical screening protocols to identify tannins, reducing sugars, alkaloids, coumarins, saponins, flavonoids, terpenoids and sterols using standard methods as described by Harborne [18] and Trease and Evans [19].

The total phenolic content was determined by the Folin-Ciocalteu method [20] with some modifications. Approximately, 50 µL of each extract was mixed with 3 mL of distilled water and 250 µL of the Folin-Ciocalteu reagent. The mixture was allowed to stand for a minimum of 5 minutes and 750 µL of 20% Na_2CO_3 was added. This mixture was vigorously vortexed for 2 min followed by 30 min incubation at room temperature. The absorbance of the solutions was measured at 760 nm using a UV-VIS spectrophotometer (Shimadzu Corporation, 1201, Kyoto, Japan). The total phenolic content was expressed as Gallic Acid Equivalent (GAE) (mg GAE/g extract).

The aluminium chloride colorimetric assay method [21] was employed to evaluate total flavonoid content (TFC) using quercetin as standard. An aliquot of 500 µL of each extract was mixed with 1.5 mL of 99.9% ethanol (EtOH), 100 µL of 1M potassium acetate, 100 µL of 10% aluminium chloride, and 3 mL of distilled water. The mixture was shaken vigorously and left to stand in dark at room temperature. The resulting mixtures were incubated for 30 min at room temperature and corresponding absorbance measured at 415 nm. The total flavonoid content of each extract was determined from the curve and the results recalculated and expressed as mg QE/100 g.

The amount of tannins in the plant extracts were determined by the Folin–Ciocalteu method with slight modifications [22]. 100 µL of each extract was added to 5 mL of distilled water, 500 µL of Folin–Ciocalteu reagent and 1 mL and of 35% Na_2CO_3 solution. The mixture was shaken well and kept at room temperature for 30 min and absorbance measured against the blank at 725

nm. The total tannin content was determined from the calibration curve, and the results expressed in terms of mg GAE/100 g. All determinations were performed in triplicates with standards (gallic acid and quercetin).

2.4 Determination of DPPH Radical Scavenging Activity

The free radical scavenging ability of the extracts against DPPH free radical was evaluated as described by Oliveira et al. [23], with some modifications as previously described by Donkor et al. [24]. Briefly, 200 µL of each extract was added to 3.8 mL of 0.004% DPPH methanolic solution, incubated at room temperature in dark for 60 min, and the absorbance was measured at 517 nm against a methanol blank. Each experiment was performed in triplicate.

Inhibition (I %) was calculated as follows:

$$I\% = \frac{Abs_0 - Abs_1}{Abs_0} \times 100$$

Abs_0 = absorbance of 0.004% DPPH without analyte
Abs_1= absorbance of 0.004% DPPH plus the test compound

2.5 UV and FTIR Spectroscopic Analyses

The different solvent extracts of fruits, leaves and stem bark of *T. tetraptera* were diluted to 1:10 with respective solvents. They were scanned in the wavelength ranging from 200 to 700 nm using a double-beam ultraviolet spectrophotometer (PerkinElmer, USA) to detect the characteristic peaks. 10 mg of the dried extracts were encapsulated in 100 mg of KBr pellet to prepare translucent sample discs. They were loaded in an FTIR spectroscope (UATR Spectrum 2, PerkinElmer, USA) with a scan range from 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1}. The peak values of the UV and FTIR were recorded. The functional groups present were determined by comparing with reference tables.

2.6 GC-MS Analyses of Methanolic Extracts

The methanolic extract of leaves, stem bark and fruits were analysed using the GC-MS to identify the major compounds present. GC-MS analyses of the samples was performed using a PerkinElmer GC Clarus 580 Gas Chromatograph interfaced to a Mass Spectrometer PerkinElmer (Clarus SQ 8 S) equipped with ZB-5HTMS (5% diphenyl/95% dimethyl polysiloxane) fused capillary column (30 × 0.25 µm ID × 0.25 µm DF). The oven temperature was programmed from 100°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C and holding for 22 min at 280°C. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.9999%) was used as a carrier gas at a constant flow rate of 1 mL/min, and an injection volume of 1 µL was employed. The injector temperature was maintained at 250°C, the ion-source temperature was 220°C. Mass spectra were taken at 70 eV; a scan interval of 1 s and fragments from 50 to 500 Da. The solvent delay was 0 to 3 mins, and the total GC/MS running time was 50 min respectively. The mass-detector used in this analysis was Turbo-Mass, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-6.1.0. Interpretation of mass-spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns.

2.7 Heavy Metal Analyses

To eliminate any form of interference in the analysis, the glassware was soaked overnight in 10% (v/v) solution of nitric acid and then washed with 10% (v/v) hydrochloric acid followed by rinsing with double-distilled water and oven drying. Each extract and raw samples of mass 1 g were weighed into correspondingly labelled Pyrex digestion tube. Each sample was wet-digested using a mixture of 1 mL H_2O, 2 mL HCl, 5 mL 1:1 HNO_3:$HClO_4$, and 2 mL of H_2SO_4 for 20 minutes on a heating block at 150°C. The digest was allowed to cool, transferred to a volumetric flask and diluted with distilled water to 50 mL. Heavy metals including lead, copper, nickel, zinc, and iron were analysed using an atomic absorption spectrometer (Varian AA 240FS) with a long path air acetylene burner and cathode lamp for respective metals.

2.8 Preparation of Inoculum

The assay factored six bacteria and yeast isolates. The stock cultures of *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC25922), *Salmonella typhi* (ATCC 19430), *Staphylococcus aureus* (ATCC 25923), *Proteus mirabilis* (ATCC 49565), *Klebsiella pneumonia* (ATCC 33495), and *Candida albicans* were subcultured onto freshly prepared nutrient agar

(Oxoid, CM0003, England) plates to obtain pure colonies. Each organism in single colonies was suspended in test tubes containing 5 mL of sterilized bacteriological peptone (Sigma-Aldrich, P0556, Germany) and incubated at a temperature of 37°C for 10–18 hours to reach turbidity of 0.5 McFarland standards [25]. The turbidity of the actively growing broth cultures which exceeded the standard was adjusted with sterile bacteriological peptone to obtain turbidity which can be optically compared to that of 0.5 McFarland standard (approximately 1 - 2 $\times 10^8$ CFU/mL) (WHO, 1999). All media were prepared according to the manufacturer's instructions.

2.9 Antimicrobial Activity

The agar diffusion method was carried out to analyse the antimicrobial activity of the plant extracts [26]. The inoculums were inoculated by swabbing onto sterile Mueller-Hinton agar. A sterilized cock borer of an internal diameter 4 mm was used to punch six holes in the medium. Stock solutions of extracts (100 mg/mL, 200 mg/mL, and 300 mg/mL) were reconstituted in 20% v/v DMSO (Sigma, D5879, Germany) resulting in percentage concentrations of 10%, 20% and 30% for the determination of antimicrobial activity. The plant extracts were dispensed into the holes to a volume of 100 µL. The positive control was an antibiotic disc of 30 µg chloramphenicol and 20% v/v DMSO was used as the negative control in a triplicate. The plates were refrigerated for about 4 hours to allow for absolute diffusion of the extract and incubated at 37°C for 24 hours, after which the diameter of each zone of inhibition was measured in millimetres (mm) with a sterilized ruler.

2.10 Data Analysis

The results were expressed as mean ± standard error mean (SEM) and compared by one-way ANOVA followed by Tukey's multiple comparison test at the 95% significant level using GraphPad Prism version 8.0 (La Jolla, CA, USA).

3. RESULTS

The raw plant materials (Fruits RF, Leaves RL and Stem bark RSB) were extracted with the water, 50% hydroethanol and methanol to obtain the following extracts; aqueous fruit extract (AFE), hydroethanolic fruit extract (HFE), methanolic fruit extract (MFE), aqueous leaves extract (ALE), hydroethanolic leaves extract (HLE), methanolic leaves extract (MLE), aqueous stem bark extract (ASE), hydroethanolic stem bark extract (HSE), methanolic stem bark extract (MSE).

3.1 Yield

Table 1 shows the yield of the extracts obtained from parts of *T. tetraptera*. The aqueous fruit extract had the highest yield (28.55% per 500 g of plant material) while the aqueous stem bark extract was least (4.35%).

3.2 Phytochemical Constituents

Table 2 shows the qualitative phytochemical constituents in the raw plant materials (leaves, fruit and stem bark) and the solvent extracts. Tannins, reducing sugars, coumarins and flavonoids were present in all extracts. Alkaloids, terpenoids, sterols and saponins were varied. Figure 2 shows the quantitative levels of phenols, tannins and flavonoids. Generally, the methanolic and hydroethanolic extracts of leaves and stem bark were richest in phenols, tannins and flavonoids with the stem bark extract having the highest tannin and phenol contents while the leaves was highest in flavonoids.

3.3 Radical Scavenging Activity of *T. tetraptera*

Figure 3 shows DPPH scavenging activity of extracts of *T. tetraptera*. Hydroethanolic and methanolic extracts of leaves and stem bark had the highest radical scavenging activities. The activity of aqueous extracts of all parts were low. The gallic acid standard had a percent inhibition of 94.15%.

3.4 Ultraviolet (UV) Absorption Spectra of Extracts of *T. tetraptera*

Figure 4 and Table 3 show the UV absorbances of extracts of *T. tetraptera* in respective solvents. Most extracts had a maximum absorbance between 190 and 285 nm.

3.5 Fourier-transform Infrared (FTIR) Spectra of Extracts of *T. tetraptera*

Figures 5 to 7 shows the FTIR spectra of extracts of *T. tetraptera*. The results revealed the presence of alcohols, phenols, alkanes, aldehydes, ketones, aromatics, aliphatic amines, aromatic amines, amides, carboxylic acids, esters, nitro compounds, alkynes, primary and secondary amines, and alkyl halides. These were

confirmed by FTIR spectroscopic analyses that predicted the presence of the following groups: C-Br, O-H, C-H, C=C, C=O, C≡C, N-H, C-H, C-N, C=O.

3.6 Chemical Composition of Methanolic Extracts by GC-MS

The various compounds were identified by comparing with the National Institute of Standard Technology (NIST) database (Tables 4-6). Figure 8 shows the spectra of the methanolic extract of fruit, leaves and stem bark.

3.7 Heavy Metal Content of Raw and Extracts of *T. tetraptera*

Table 7 shows the presence of Copper (Cu), Iron (Fe), Lead (Pb) and Zinc (Zn) in the raw and solvent extracts of *T. tetraptera* parts. Cu was high in the raw leaf (RL) and highest in the hydroethanolic stem extract (HSE). Fe was high in the RL and highest in the HSE. Lead was present in all raw and extracts with highest concentrations in methanolic stem extract (MSE). Zinc was present with highest levels in the aqueous leaf extract (ALE).

Table 1. Yield of solvent extracts of *T. tetraptera* parts

Part	Extract	Mass of extract (g)	Yield (%)
Fruit	AFE	142.75	28.55
	HFE	100.63	20.13
	MFE	75.13	15.03
Leaves	ALE	36.28	7.26
	HLE	59.50	11.90
	MLE	44.50	8.90
Stem bark	ASE	21.76	4.35
	HSE	55.00	11.00
	MSE	45.50	9.10

Table 2. Phytochemical constituents of methanolic, hydroethanolic and aqueous extracts of *Tetrapleura tetraptera* parts

Sample	Raw			Fruit			Leaves			Stem bark		
	RL	RF	RSB	AFE	HFE	MFE	ALE	HLE	MLE	ASE	HSE	MSE
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Reducing Sugars	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	-	+	-
Terpenoids	+	+	+	+	+	+	+	+	+	-	+	+
Sterols	+	+	+	-	-	+	-	+	+	-	-	+
Coumarins	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	-	-	+	-	+	+	+

Note: + present; - absent

Table 3. UV-Vis peaks and absorption range of *T. tetraptera*

Plant part	Extract	Number of peaks	Wavelength range (nm)
Fruit	Aqueous	2	201.0 – 285.1
	Hydroethanolic	2	203.0 – 280.0
	Methanolic	2	211.0 – 283.1
Leaf	Aqueous	1	278.9
	Hydroethanolic	2	205.0 – 281.0
	Methanolic	2	208.0 – 276.9
Stem Bark	Aqueous	2	194.0 – 283.1
	Hydroethanolic	2	205.0 – 283.1
	Methanolic	2	213.0 – 280.0

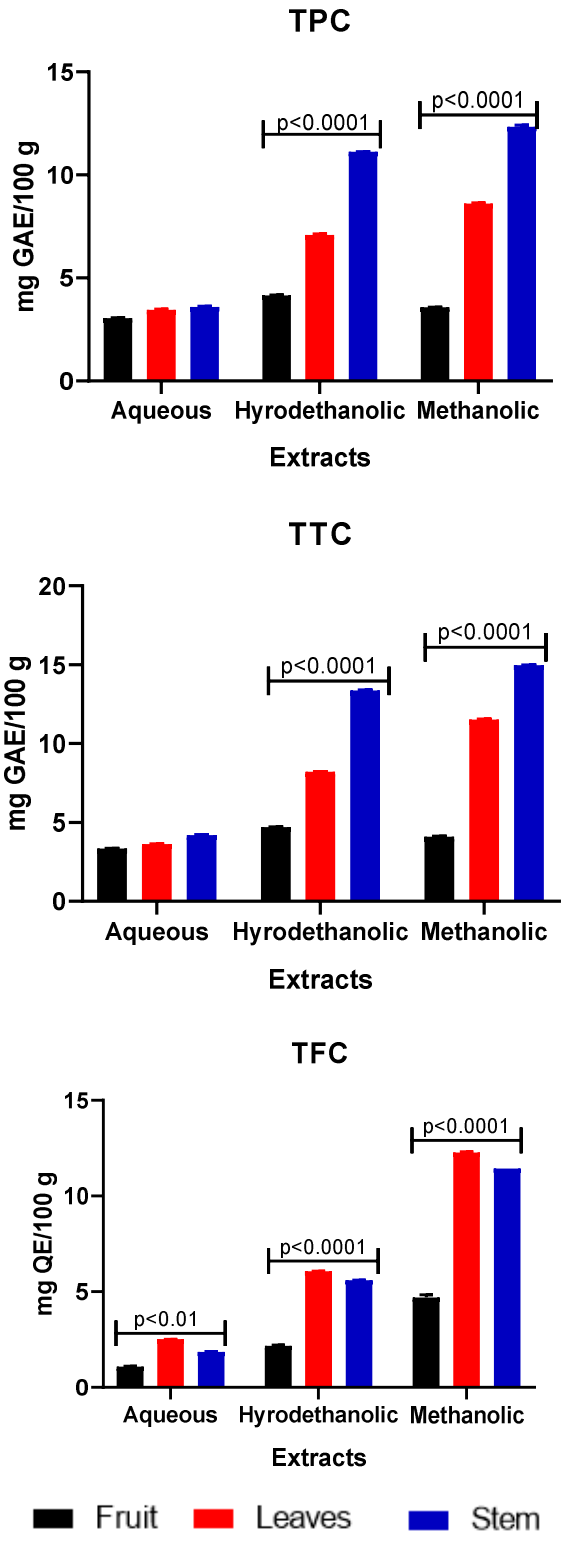

Figure 2. The total phenolic content (TPC), total tannin content (TTC), and total flavonoid content (TFC) of extracts of *T. tetraptera* parts

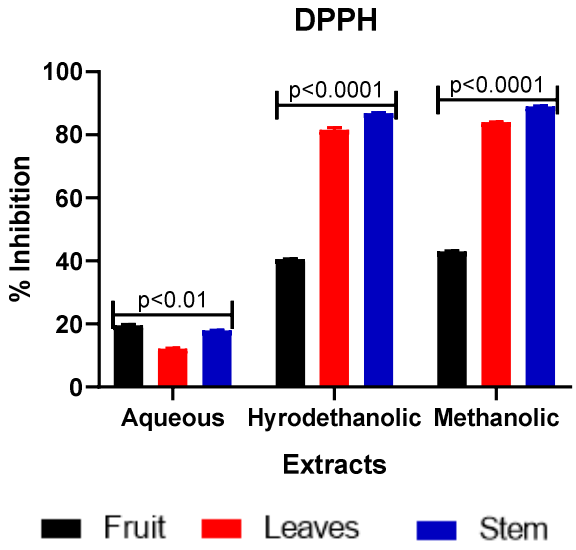

Figure 3. DPPH scavenging activity of extracts of *T. tetraptera*

Table 4. GC-MS analyses showing compounds in methanolic fruit extract of *T. tetraptera*

SN	RT	Area	% Composition	SI (%)	Chemical constituent
1	4.392	18502762.0	0.94%	82	Glyceraldehyde
2	4.759	76700624.0	3.90%	96.6	Benzoic acid
3	5.27	38613724.0	1.96%	85.3	5-ethoxydihydro-2(3H)-furanone
4	5.510	15593289.0	0.79%	72	Thiophene, 2,5-dihydro-
5	5.840	14760625.0	0.75%	81	Alpha-l-sorbopyranose
6	6.134	7549463.5	0.38%	88	2-Myristynoyl pantetheine
7	7.692	36494864	1.86%	87.98	Methyl 4-nitrohexanoate
8	8.407	255528896.0	13.00%	92.91	Sucrose
9	9.067	19689966.0	1.00%	98.92	(5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate
10	9.397	11095190.0	0.56%	98	Phenol, 2,4-bis(1,1-dimethylethyl)-
11	10.589	156201392.0	7.94%	91.70	3-Deoxy-d-mannoic lactone
12	10.919	228177488.0	11.61%	87.87	3-O-Methyl-d-glucose
13	11.598	783542336.0	39.85%	94.93	Laminitol (D-4-C-methyl-myo-inositol)
14	12.111	10159824.0	0.52%	66.8	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
15	12.533	6454183.0	0.33%	75	Benzocycloheptano[2,3,4-I,j]isoquinoline, 4,5,6,6a-tetrahydro-1,9-dihydroxy-2,10-dimethoxy-5-methyl-
16	14.641	34255656.0	1.74%	92.5	n-Hexadecanoic acid
17	17.043	22468070.0	1.14%	95.01	Oleic Acid
18	17.373	18374496.0	0.93%	79.93	Octadecanoic acid
19	17.63	17071814.0	0.87%	88.76	Hexadecanamide
20	20.252	160911648.0	8.18%	98.89	9-Octadecenamide, (Z)-
21	20.619	7949431.0	0.40%	61	Octadecanamide
22	22.38	16624953.0	0.85%	85	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
23	25.33	9471287.0	0.48%	89	Octadecanoic acid, 2,3-dihydroxypropyl ester

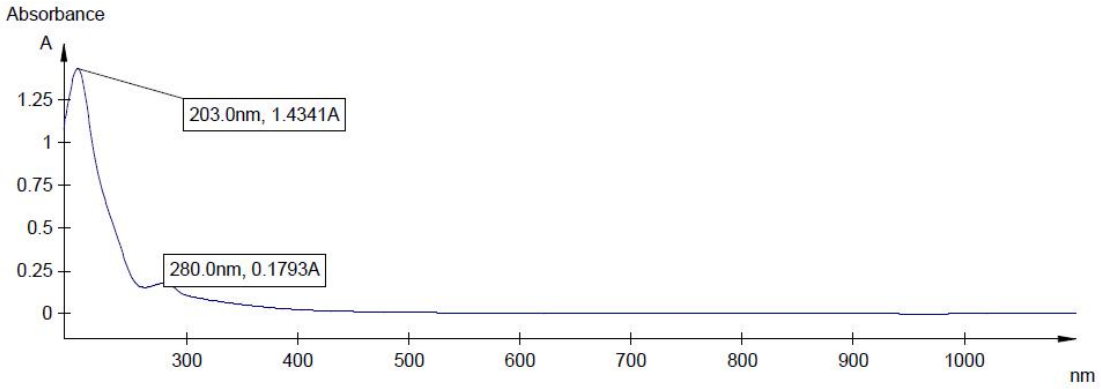

Figure 4. Representative UV spectra of the hydroethanolic leaves extract (HLE) in water

Table 5. GC-MS analyses showing compounds in methanolic leaves extract of *T. tetraptera*

SN	RT	Area	% Composition	SI	Chemical constituent
1	4.81	27522822.0	14.15%	79	Heptanediamide, N,N'-di-benzoyloxy
2	5.235	6405775.0	3.29%	91	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
3	9.09	2836670.0	1.46%	70	Curcumene
4	9.40	4202589.0	2.16%	91	Phenol, 2,4-bis(1,1-dimethylethyl)-
5	9.73	3638839.0	1.87%	99	3,3',4,4'-Tetrahydrospirilloxanthin
6	11.029	36082896.0	18.55%	95	3-O-Methyl-d-glucose
7	11.286	8878595.0	4.57%	88.5	Ar-tumerone
8	14.20	3604161.0	1.85%	95	9-octadecenoicacid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis
9	14.64	8846475.0	4.55%	94	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester
10	16.713	4881981.5	2.51%	85	Phytol
11	17.630	5729115.5	2.95%	86	9-Octadecenamide, (Z)-
12	20.252	67312032.0	34.61%	98	9-Octadecenamide, (Z)-
13	20.619	6210911.5	3.19%	85	9-Octadecenamide, (Z)-
14	22.966	8314082.5	4.28%	77	Diisooctyl phthalate

Table 6. GC-MS analyses showing compounds in methanolic stem bark extract of *T. tetraptera*

SN	RT	Area	% Composition	SI	Chemical constituent
1	3.952	3734716.2	3.55%	73	Undecane
2	5.24	1408059.0	1.34%	92	Octadecane, 3-ethyl-5(2-ethylbutyl)-
3	9.416	4955521.0	4.70%	94	Phenol, 2,4-bis(1,1-dimethylethyl)-
4	10.993	6302584.5	5.98%	92	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester
5	11.286	4989923.0	4.74%	72	Ar-tumerone
6	14.20	2646063.0	2.51%	90	Oxiraeundecanoicacid, 3-pentyl-,methyl ester,cis-
7	14.64	7014418.0	6.66%	100	3-Pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentam...
8	17.630	4837669.5	4.59%	79	Octadecanamide
9	20.270	64406172.0	61.14%	98	9-Octadecenamide, (Z)-
10	20.619	5039583.0	4.78%	74	Octadecanamide

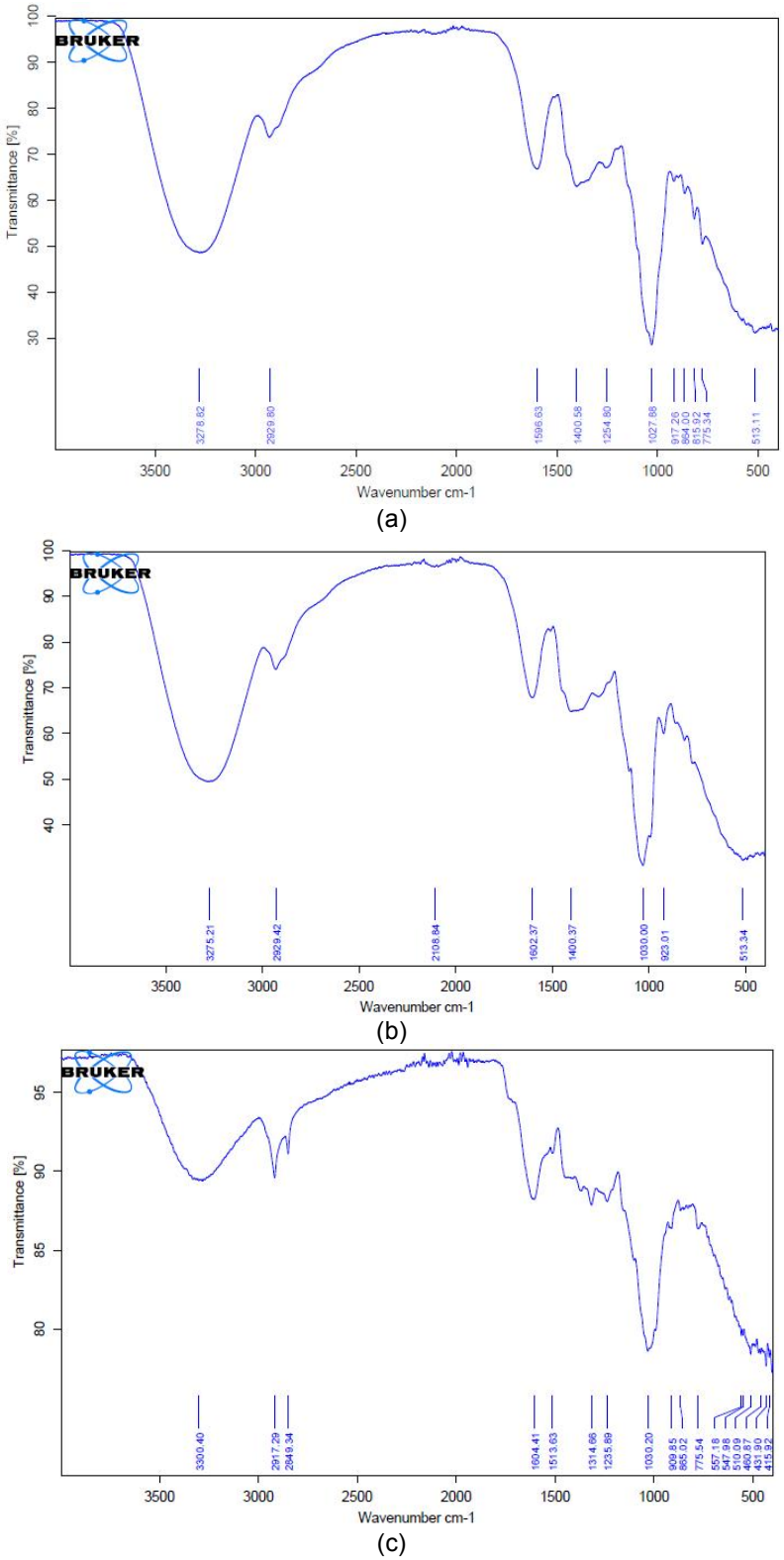

Figure 5. FTIR spectrum of fruit extracts of *T. tetraptera* (a) Aqueous (b) Hydroethanolic (c) Methanolic

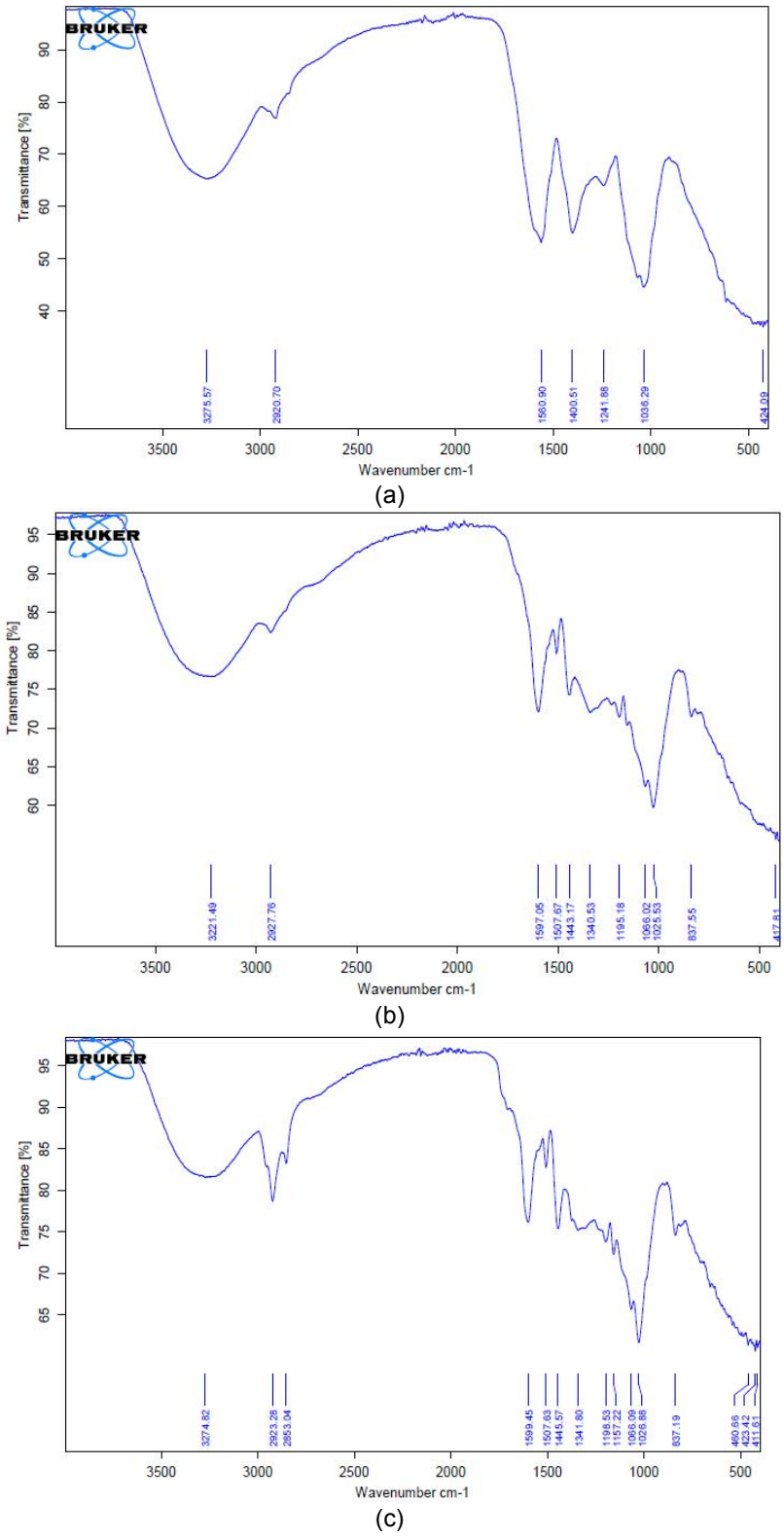

Figure 6. FTIR spectrum of leaves extracts of *T. tetraptera* (a) Aqueous (b) Hydroethanolic (c) Methanolic

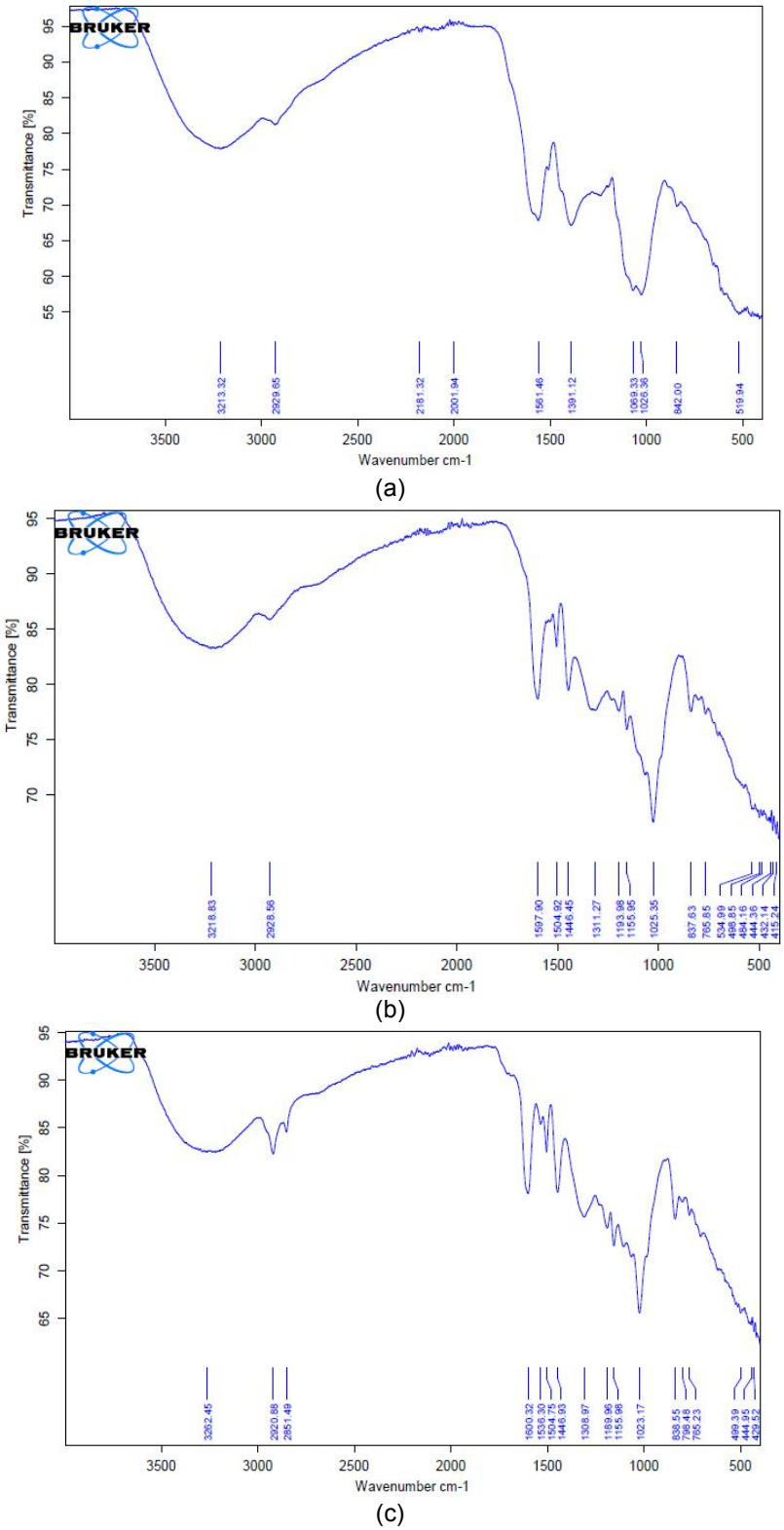

Figure 7. FTIR spectrum of stem bark extracts of *T. tetraptera* (a) Aqueous (b) Hydroethanolic (c) Methanolic

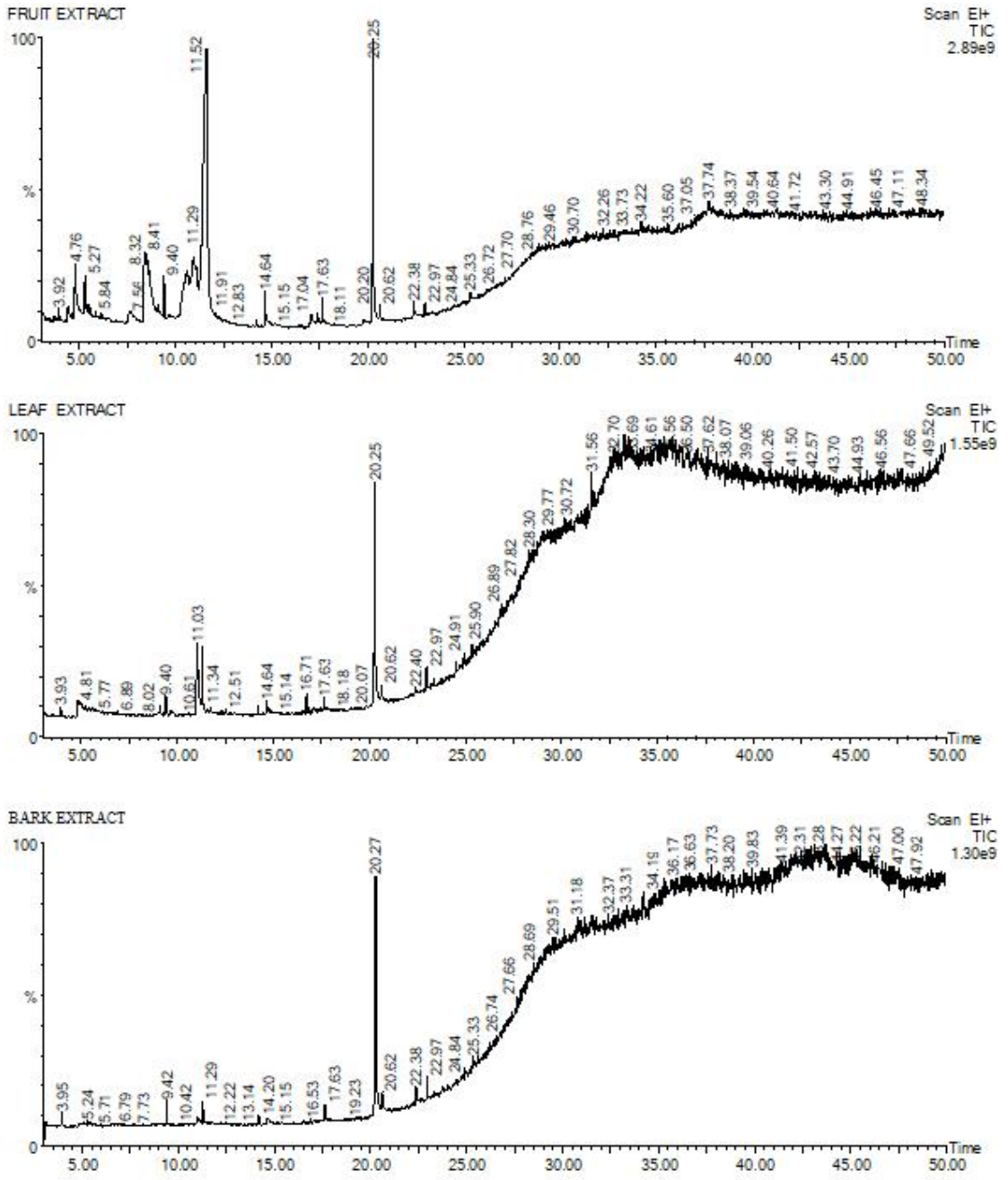

Figure 8. GC-MS spectra of methanolic extract of fruits, leaves and stem bark of *T. tetraptera*

3.8 Antimicrobial Activity of Extracts

Table 8 shows the antimicrobial effect of extracts on *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Proteus mirabilis*. Extracts of fruit of *T. tetraptera* showed no activity on all microbes studied. Hydroethanolic and methanolic extracts of the leaves showed a dose-dependent non-significant activity on all microbes. Only methanolic extract of stem bark had activity. These activities ranged from a minimum inhibition of 0.97 mm for 100 mg/ml hydroethanolic leaves extract to 1.63 mm for 300 mg/ml methanolic extract of stem bark.

Table 7. Heavy metal content of the raw materials and extracts of *Tetrapleura tetraptera*

	Samples	Concentration (mg/L)				
		Cu	Fe	Ni	Pb	Zn
Raw Sample	Leaves	0.29 ± 0.02	3.26 ± 0.06	B D L	1.71 ± 0.02	0.45 ± 0.01
	Fruit	0.22 ± 0.02	2.60 ± 0.05	B D L	1.61 ± 0.02	0.40 ± 0.01
	Stem bark	0.25 ± 0.02	1.87 ± 0.02	B D L	1.33 ± 0.02	0.58 ± 0.02
Fruit	AFE	0.17 ± 0.01	0.58 ± 0.01	B D L	2.69 ± 0.04	0.49 ± 0.02
	HFE	0.13 ± 0.01	0.22 ± 0.01	B D L	1.85 ± 0.03	0.40 ± 0.02
	MFE	0.11 ± 0.01	0.10 ± 0.01	B D L	1.85 ± 0.02	0.33 ± 0.01
Leaves	ALE	0.38 ± 0.04	3.09 ± 0.06	B D L	2.74 ± 0.05	1.17 ± 0.01
	HLE	0.30 ± 0.03	1.07 ± 0.03	B D L	2.73 ± 0.05	0.89 ± 0.03
	MLE	0.22 ± 0.02	1.69 ± 0.03	B D L	2.41 ± 0.04	0.57 ± 0.02
Stem bark	ASE	0.25 ± 0.03	0.78 ± 0.01	B D L	2.63 ± 0.04	0.40 ± 0.02
	HSE	0.55 ± 0.05	8.98 ± 0.12	B D L	2.68 ± 0.04	0.49 ± 0.02
	MSE	0.26 ± 0.02	0.37 ± 0.02	B D L	2.79 ± 0.04	0.35 ± 0.02

Note: BDL, below detection limit: detection limit, 0.00001 mg/L

Table 8. Antimicrobial properties of extracts of *T. tetraptera*

			Zone of Inhibition (cm)				
Plant parts	**Extract**	**Concentration (mg/mL)**	**TO 1**	**TO 2**	**TO 3**	**TO 4**	**TO 5**
Fruit	AFE	100	-	-	-	-	-
		200	-	-	-	-	-
		300	-	-	-	-	-
	HFE	100	-	-	-	-	-
		200	-	-	-	-	-
		300	-	-	-	-	-
	MFE	100	-	-	-	-	-
		200	-	-	-	-	-
		300	-	-	-	-	-
Leaves	ALE	100	-	-	-	-	-
		200	-	-	-	-	-
		300	-	-	-	-	-
	HLE	100	1.03 ± 0.03	0.97 ±0.03	1.03 ± 0.07	0.97 ± 0.03	1.17 ± 0.03
		200	1.13 ± 0.03	1.03 ± 0.03	1.13 ± 0.03	1.20 ± 0.06	1.30 ± 0.06
		300	1.30 ± 0.06	1.33 ± 0.03	1.27 ± 0.03	1.33 ± 0.03	1.43 ± 0.03
	MLE	100	0.09 ± 0.06	1.13 ± 0.03	1.00 ± 0.06	1.07 ± 0.03	1.27 ± 0.03
		200	1.13 ± 0.03	1.17 ± 0.03	1.17 ± 0.03	1.17 ± 0.03	1.37 ± 0.03
		300	1.37 ± 0.03	1.27 ± 0.03	1.33 ± 0.03	1.33 ± 0.03	1.50 ± 0.06
Stem Bark	ASE	100	-	-	-	-	-
		200	-	-	-	-	-
		300	-	-	-	-	-
	HSE	100	-	-	-	-	-
		200	-	-	-	-	-
		300	-	-	-	-	-
	MSE	100	1.03 ± 0.06	1.13 ± 0.03	1.03 ± 0.07	1.03 ± 0.03	1.20 ± 0.06
		200	1.37 ± 0.03	1.33 ± 0.03	1.27 ±0.03	1.30±0.06	1.30 ± 0.06
		300	1.53 ± 0.03	1.43 ± 0.03	1.47 ± 0.03	1.47 ± 0.03	1.63 ± 0.03
Controls	Ctrl N	20% v/v	-	-	-	-	-
	Ctrl P	30 µg	2.50 ± 0.15	2.60 ± 0.10	2.1 ± 0.15	1.7 ± 0.33	2.00 ± 0.23

Note: TO: Test organism; TO 1: Pseudomonas aeruginosa; TO 2: Staphylococcus aureus; TO 3: Escherichia coli; TO 4: Candida albicans; TO 5: Proteus mirabilis; Crtl N: Negative control – 20% v/v DMSO; Ctrl P: Positive control – 30 µg Chloramphenicol

4. DISCUSSION

Tetrapleura tetraptera is a common folkloric tree plant among traditional healers of Ghana and is well distributed along the forest zones of the country. The current study was aimed at comparing the phytochemical, free radical scavenging activity and antimicrobial properties of various solvent extracts of the leaves, fruits, and stem bark of the plant. Tannins, reducing sugars, coumarins and flavonoids were found to be present in all the raw plant materials and the various extracts of *T. tetraptera* while others were absent. Phytochemicals play vital roles in disease treatment, together with nutrients and dietary fibres. Alkaloids have been proven to possess an antioxidant property by reducing oxidative damage triggered by H_2O_2 [27] and inhibit protein synthesis in *Plasmodium falciparum* and as such have been attributed to have antimalarial activity [28]. Tannins, with their ability of chelating metals ions such as Fe (II) and obstructing a reaction step in the Fenton reaction, contribute to retarding oxidation [29]. The ability of saponins in scavenging hydrogen peroxide renders them antioxidant [29]. Coumarins are known to stimulate macrophages to degrade extracellular albumen, allowing for faster resorption of oedematous fluid [30]. These and other reported activities of phytochemicals work together in support of the various ethnopharmacological application of the specie. The current observations were similar to those reported for aqueous whole fruit extract [31,32], aqueous and ethanolic extract of the stem bark [33] and ethanolic extract of leaves [17]. The observed differences in the qualitative and quantitative levels of these phytochemicals could be attributed to geographical differences in plant samples used.

The FTIR result made known the presence of alcohols, alkanes, aldehydes, esters, ketones, ether, aromatics, phenols, aliphatic amines, aromatic amines, amides, carboxylic acids, nitro compounds, alkenes, alkynes (terminal), primary and secondary amines and alkyl halides with other peaks whose functional groups were unknown. The presence of the various characteristic functional groups may be responsible for the medicinal properties of *T. tetraptera*. GC-MS analyses of methanolic extracts of *T. tetraptera* showed various compounds which may have a synergistic effect for disease treatment. The methanolic fruits extract was predominantly laminitol (D-4-C-methyl-myo-inositol; 39.85%) and 9-Octadecenamide, (Z) for leaves (34.61%) and stem (61.14%) extracts which have been demonstrated to possess anti-inflammatory, antimicrobial and as an endogenous sleep-inducing agent [34,35,36].

Generally, the methanolic extracts of leaves and stem bark of *T. tetraptera* exhibited the highest free radical scavenging activity attributable to the high phenolic, flavonoids and tannin contents. A similar observation was made for the activity of hydroethanolic extracts. However, aqueous extracts were low in these phytochemicals making water an inappropriate solvent for extraction of polyphenols and flavonoids [37]. Phenolic compounds can act as antioxidants in various ways; it contains phenolic hydroxyl which are good hydrogen donors, able to react with reactive oxygen species and nitrogen species which chelate the cycle of new radicals [17] and can chelate metal ions involved in the production of free radicals. Phenolics could also inhibit some enzymes involved in the generation of radicals [38,39]. The antioxidant activity of flavonoids renders them very active constituents in possessing various biological activities which are essential for disease treatment including having anticancer properties. Further, flavonoids are vital in transduction pathways, suppression of oncogenes and stimulation of immune system [40] 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 [41].

Medicinal plants used in healthcare delivery expose humans to consuming trace and heavy metals which play a role in fighting several human diseases, however, beyond certain permissible limit, these metals may be deleterious to human health. This makes it necessary to evaluate their levels in medicinal plants. In the analyses of these metals, varying levels were observed in both raw and solvent extracts of *T. tetraptera,* except for nickel, which was below the detection limit. The metals included copper, iron, lead and zinc. The HSE had the highest copper (0.5455 mg/L) and Iron (8.9830 mg/L) concentration though below the permissible limit of 10 mg/L and 20 mg/L respectively recommended by WHO [42]. The

lead contact was highest in MSE (2.788 mg/L) while the ALE was highest with zinc (1.1650 mg/L). These values were within WHO limits. Fe is an important trace element, and iron-protein mixtures play a vital role in metabolic activities. It is essential for haemoglobin biosynthesis and transport of oxygen and electrons throughout the body [43]. Zn forms part of metalloenzymes, especially in nucleic acid metabolism and facilitates bone formation, wound healing, brain development, and normal growth processes [44]. Cu is an essential component of many enzymes, and it plays an important role in a wide range of physiological processes including Fe utilization, free radical elimination, bone and connective tissues development, and melanin production [43]. Thus, the use of *T. tetraptera* extracts may not pose any toxicity dangers for the measured metals.

The results of the antimicrobial activity of *T. tetraptera* showed all the aqueous extracts did not have any activity at the concentrations tested, while only the MSE, MLE and HLE has some inhibitory activity in a dose-dependent manner, though not significant. This could be attributed to the phytochemical present, with highest levels of phenols, tannins, and flavonoids. Further, the presence 3-Deoxy-d-mannoic lactone from *Clerodendrum viscosum* previously reported as antimicrobial [45], however, in the current study, the compound was detected in the MFE though no activity was observed.

5. CONCLUSION

Solvent extracts of plant parts of *Tetrapleura tetraptera* contain tannins, reducing sugars and coumarins. It also contains alkaloids, flavonoids, terpenoids, sterols and saponins but in a variable amount with significant radical scavenging activities. Copper (Cu), iron (Fe), lead (Pb) and zinc (Zn) were detected though they were all within the permissible range for medicinal plants. Variable antimicrobial activities were observed with the methanolic extracts being more potent. The study indicated that the various parts of *Tetrapleura tetraptera* were rich in phytochemical, possessed antioxidant and antimicrobial activities, supporting ethnomedicinal uses and could be evaluated further for other pharmacological activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DATA AVAILABILITY

All data generated or analysed during this study are included in this published article.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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