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Original Research Article

Chemical Elucidation, Microbial Growth and Free Radical Inhibitory Effects of *Dennettia tripetala* Fruit: *In vitro* and *In vivo* Model Experiments

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ABSTRACT

Natural products are being exploited in the treatment of diverse ailments stemming from microbial and free radical incursion in the African native societies. The current study was designed to elucidate the bioactive constituents, microbial growth and free radical inhibitory effects of *Dennettia tripetala*fruit, employing *in vitro* and *in vivo* models. Briefly, phytochemical analysis was qualitatively investigated. The free radical scavenging assays using *2,2-diphenyl-1-picrylhdrazyl* (DPPH) and *2,2'-azino-bis (3-ethylenzoline-6-sulfonic acid* (ABTS) radical solutions using vitamin C as reference antioxidant. Antibacterial activity was carried out using agar diffusion well. Bioactive elucidation was conducted using Gas chromatography Mass Spectroscopy (GC-MS)**.** Liver protective effect of the extract was investigated on rats' model. Phytochemical analysis of the crude extracts (hexane and ethanol) revealed saponins, tannin, flavonoid, steroids, phenols and terpenoids. GC-MS analysis revealed various bioactive compounds. DPPH inhibitory effect of the hexane and ethanol extracts, displayed IC_{50} values, 3.86 and 4.01 µg/mL against a corresponding vitamin C (IC_{50} value, 0.007 µg/mL). Both extracts solution scavenged ABTS radical (IC_{50} values, 4.53 and 4.79 µg/mL) against vitamin C (IC_{50} values, 0.005 µg/mL). The extracts demonstrated promising antibacterial activities against eight selected multidrug-resistant strains, comprising Gram positive and negative strains. The crude extract protects liver induced carbontetrachloride toxic onslaught through significant ($p < 0.05$) increased in the catalase, superoxide dismutase activities, reduced glutathione and depleted malonedialdehyde levels in the rats' liver co-treatment groups. Overall, the findings suggest *D. tripetela's* leaf extract a promising antidote for the treatment of free radical and microbial , infectious diseases.

***Keywords*:**Antibacterial, Inhibition, Antioxidant, Phytochemicals.

Introduction

Plant-based products with both antioxidant and bacteria growth inhibitory potential play an important role in the curative medicine.[1,2] A plant is designated as medicinal, when it is useful as a therapeutic agent or an active ingredient of any medicinal preparation.[3,4]Treatment of infectious diseases, as a result of resistant bacterial strains, constitutes a major challenge due to inefficacy of a specific antibiotics.[5,6]Multidrug resistance by the invading microorganism to the conventional antibiotics, has been shown to constitute a global health issue of concern. In the recent times, plant-based antibacterial agents have been considered safe to combat resistant opportunistic microorganism.[7–11]

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Antioxidant compounds from natural products have been shown to have significant inhibitory effects against microbial colony, growth and including those of multidrug resistance variations[12–14]. Antibacterial compounds have been shown to elicit antimicrobial actions in other to inhibit major cellular activities central to their survival through different mechanism of actions.[15–18]

Antioxidant compounds from natural products on other hand, have been documented to have significant inhibitory effects on the deleterious activities of pro-hepatotoxins both in *vitro* and in *vivo*[19–21]. The mediatory effects of these agents have always been through the scavenging ability of free radicals released as xenobiotic products or in its activated form or to a large extent inhibition of lipid peroxidation chain formation by the activation of antioxidant enzymes *in vivo.*[22]

Acute liver disease in any case is the result of injury to the hepatocytes with the loss of about 80 to 90% biological function leading to several symptoms, such as impaired *de novo* protein synthesis and liver encephalopathy.[23,24] Liver injury in "acute term" is the appearance of liver cell damage that occurs within a short period of time.[23] It is a disease condition characterised by gradual destruction of the regenerative ability of the hepatic parenchyma and a compromised function of liver organ.[25] In most cases, in contrast to acute liver disease, chronic liver disease occurrence is due to viral infections, parasitic, alcoholic liver disease, and fibrosis caused by biliary and non-alcoholic fatty liver disease.[26]Carbon tetrachloride is a common

chemical toxin used in experimental animal model to induce acute liver injury.[27,28]Biotransformation of this toxicant (CCl_4) results in the production of trichloromethyl free radical CCl_3* and trichloromethyl peroxyl radical ($CClO_2$*) influenced by the cytochrome P450 enzymes action (mainly P450 2E1), occurring within the endoplasmic reticulum region with the cellular activation of kupffer.[29,30] Hence, the release of tumor necrosis after kupffer cells activation have been shown to trigger tumor necrosis factor-alpha (TNF-α), nitric oxide (NO), interleukin-1beta (IL-1β), IL-6 and IL-10 production. They are the well-known cellular agents responsible for inflammatory processes in the liver.[31]

A considerate part of the research conducted in the recent times focused on the development of novel drugs from plants or their isolated products to treat microbial infections and other free radical related diseases.[32,33]However, to the best of our knowledge, the chemical elucidation, microbial growth and free radical inhibitory effects of *Dennettia tripetala* fruit, comprising *in vitro* and *in vivo* model in any experimental investigation or in a comparative status, are yet to be exhaustively elucidated. The current study has been optimized impart, to elucidate bioactive compounds, investigate the microbial growth and free radical inhibitory effects of *Dennettia tripetala* fruit, in both *in vitro* and *in vivo* model, using carbontetrachloride as a toxic inducer of hepatotoxicity in rat model experiment.

D. tripatela (DNT), at any rate is described a home-grown peppery fruit, a therapeutic plant belonging to the family *Annonaceae*, commonly grown in the Southern part of Nigeria where it is normally consumed due to its spicy taste. It has been documented that both the root, fruit and leaves of this plant are employed by indigenous trado-medical practitioner in combination with other curative plant to treat different diseased conditions such as typhoids, convulsion, stomach upset, vomiting, cancer, cough, toothache, diabetes, sore throat, nausea during pregnancy, asthma etc.[34,35] Literature search on *Dennettiatripetala*revealedseparately, antimicrobial, antioxidant, anti-inflammatory, insecticidal, anti-hyperglycemic, analgesic, anti-inflammatory, antiulcer properties.[36–39]

Materials and Methods

Collection of the plant material

The fully matured fruits of *D. tripetala* were collected in May, 2021 from a farm in Ofante, Olamaboro L.G.A., Kogi State, Nigeria. Sample identification and attestation were performed by a qualified Taxonomist (Mr. SuleAyegba) at the Prince Abubakar University, Anyigba, Nigeria. The fruit was given a voucher number (Larayetan: CHM-003) and kept in the Plant Science and Biotechnology Department's herbarium Unit.

Extraction of D. tripatela extracts

Matured fruits were air-dried at room temperature before being ground into powder using a mechanical grinder (Polymix, PX-MFC 90D). The extraction was done using the cold maceration method. 400 g of powdered *D. tripatela* fruit was soaked in 800 mL of hexane and ethanol, respectively, then swirled for 48 hours on an orbital shaker. A Whatman No. 1 filter paper was used to filter the resulting mixtures. The filtered filtrates were concentrated at 40°Cin a rotatory evaporator before being slowly evaporated to dryness at room temperature. The resulting concentrations were well-preserved in centrifuge tubes with a tight seal and kept refrigerated at 4 °C until analysis time.

Phytochemical screening

Using Yadav and Agarwala's conventional techniques, the hexane and ethanol extracts of *D. tripatela* were subjected to qualitative phytochemical screening to determine the presence of tannins, saponins, flavonoids, phenols, tepenoids and alkaloids.[40]

GC-MS analysis

The compound identification and separation were carried out using a mass selective detector (5973) connected to an Agilent gas chromatograph (6890N) with HP-5MS column (30 m × 250 m x 0.25 m). The carrier gas (Helium) was adjusted to a flow rate of 1 mL/min, with a typical velocity and nominal starting pressure of 26 cm/sec and 13 psi, respectively, and the ion source and chamber temperatures of 230°C and 150°C The acquisition scan mass varied between 50 and 500 amu. The initial temperature was set to 70°C with a 2-minute hold, and the temperature was then conditioned to range from 30 °C/min to 300 °C, yielding a total run-time of 49.67 minutes. The hexane and ethanol fruit extracts were re-suspended in hexane (1 uL) and then introduced in a splitless mode at 250ºC with a 50 mL/min purge flow.

The GC-MS chromatogram was used to identify the crude extract components of *D. tripatela* fruit extracts. Comparison of the extracts' retention times (RT) with homologous series of n-alkanes in the NIST library 2014 was used to investigate them. The identity of each compound's mass fragmentation patterns and computed retention durations were validated and compared to those in databases.[34,41]

Free radical scavenging (In vitro Assay)

DPPH assay

D. tripatela extracts were tested for antioxidant properties and radical scavenging potential against the free radical DPPH. For around 40 minutes at room temperature in the dark, five different doses (25-125 μgmL^{-1}) of *D. tripatela* extracts and synthetic antioxidant Vitamin C were incubated with a DMSO solution of DPPH. A vortex machine meticulously mixed the separate solution, and the sample's absorbance was measured at 517 nm. The ability of *D. tripatela* extract to scavenge DPPH free radicals in the solution was assessed using the equation below:

$$\% \text{ Inhibition} = [(A_{control} - A_{vo}) / A_{control})] \times 100$$

Where $A_{control}$is the absorbance of DPPH+DMSO; $A_{ext}$is the absorbance of DPPH *D. tripatela* extracts or the synthetic antioxidant agent. Dose-response curve was plotted and IC_{50} value of the synthetic antioxidant, hexane and ethanol extracts were calculated.[42]

ABTS assay.

The ABTS efficiency of the hexane and ethanol extracts of *D. tripatela* was evaluated using the Nantitanon procedure with minor modifications.[43] The working solution was made by combining ABTS stock solution (7 mM) with 2.4 mM potassium persulfate in equal volumes and allowing the mixture to react at room temperature for nine hours. An aliquot of the resulting solution (0.4 mL) was combined with 40 mL of ethanol and an absorbance reading at 734 nm (0.706 0.001) was acquired within 8 minutes by means of a UV-spectrophotometer. In a nutshell, several doses of extracts (range from 0.025 to 0.4 mg/mL) were combined with an ABTS methanol solution for 8 minutes at room temperature in the dark. After that, the absorbance was measured spectrophotometrically at 760 nm, and the ABTS percent inhibition by the extracts and commercial antioxidant (vitamin C) was calculated using the equation shown above for the DPPH assay.

Antibacterial studies

Sensitivity of bacteria to the hexane and methanol fruit extracts of *D. tripatela* was evaluated using Collins',[44] of agar well diffusion technique. Microbial cultures were injected with Oxoid nutrition broth and incubated for 24 hours at 37 ± 0.1 °C. Perforating holes of 6 mm in diameter in the uniformly distributed solidified Mueller Hilton agar (Oxoid) in Petri plates was done with a sterilized cork borer. Test bacteria cultures were standardized to the McFarland standard of 0.5 and distributed on solid media using a sterilized swab stick soaked with bacterial suspension. The decorticated hexane and ethanol extracts were re-diluted in 5 % Dimethyl Sulfoxide (DMSO) to a stock concentration of 30 mg/mL. Each of the wells received around 45 µL of *D. tripatela* extract, which was labelled suitably. For comparison, a 5% DMSO solution was used as a negative control. The disk diffusion technique was used to determine the sensitivity of test microorganisms to streptomycin. Streptomycin (10 µg) disk (MAST-Group United Kingdom) was used as a positive control. The plates were incubated for 24 h at 37°C, and the inhibitory zone was measured in millimetres via a vernier caliper.

Hexane and ethanol determination of MIC and MBC of D. tripatela extracts

The extracts' MICs were determined using the agar dilution method described by Ouedrhiri *et al*[45]and Akinpelu *et al*[46] Extracts and controls were serially diluted twice, and the different concentrations of extracts were integrated into a sterile molten nutrient agar in McCartney bottles to achieve a final required concentration. The final values in this investigation fluctuated between 30 mg/mL and 0.03 mg/mL. The contents of the McCartney bottles were put into separate Petri dishes that were clearly labelled and left to set. The plates were inoculated by streaking test organisms adjusted to 0.5 McFarland standard over the extract-seeded plates once, and the set-up was incubated at 37°C for 24 h. A positive control of streptomycin was employed, while a negative control of 5 % DMSO was used. The MIC endpoint was defined as the lowest concentration of extract that completely stops the growth of the bacteria being tested. Samples were obtained from MIC plates with no obvious growth and sub-cultured onto freshly prepared nutrition agar plates. The bacteria-inoculated plates were incubated at 37°C for up to 72 hours. The MBC was defined as the concentration of extract that did not show any signs of bacterial growth on the surface.

In vivo studies

Experimental animals and Ethical procedure

Thirty (35) male of *wistar* strain of experimental rats weighing between 190-200 g, were procured from the Department of Biochemistry, Prince Abubakar Audu University Anyigba, Nigeria. Guidelines of the Animal Research Ethics Committee of the University Anyigba with reference protocol number, CHSREC/2022/0007, were strictly adhered to throughout the experimental period. The animals were maintained on standard animal feeds and clean tap water *ad libitum*, kept for one week to acclimatize before various administrations.

Acute toxicity studies (determination of LD_{50})

After acclimatization period, the acute oral toxicity studies was conducted in *Wistar* rats using Up and Down Procedure of Organization for Economic and Cultural Development (OECD, 2001) as outlined by Erhirhie *et al*.[47] Briefly, five animals were randomly selected (by weight range and health status) for limit dose test. In each group, 500, 1000, 1500, 2000, 3000 mg/kg body weight of *D. tripatela*ethanolextractswere given in a single dose to the animal. The observation time were at 3 hr, 5 hr, 8hrs, 24 hrs and to a period of 48 hrs for possible signs of adversity tremors, aching, depression, weakness, food and water refusal, salivation and possibly death, was observed.

Induction of toxicity in animals

The protocols outlined by Farombi[48] and Rao *et al*,[49] with minor modification were adopted. In preparation, liquid parafin (vehicle) was mixed with 30% carbon tetrachloride (CCl_4) (in a ratio, 3:7 (v/v), a model hepatotoxicity inducer product of Merck (Germany). *Dennettia tripetala* and Silymarin (a known hepatoprotective drug, Sigma-Aldrich, South Africa), were administered orally, while CCl_4 induction was done *intraperitoneally*. A total of thirty rats were used. The animals were divided into six (6) groups of five (5) rats in each group.

Animal grouping

Normal control animals were administered daily dose of olive oil, 1mL/kg body weight *per os*, for fourteen days. Induction group were injected intraperitoneally (*i.p.*) with 1 mL/kg body weight of 30% CCl_4, three (3) times a week for fourteen (14) days. Co-treatment animals (received silymarin at 100 mg/kg bw; extract at 100 mg/kg bw; 200 mg/kg bw & 300 mg/kg bw, concurrently with 30% CCl_4, three (3) times a week for fourteen (14) days.

After an overnight fast, all animals were sacrificed using halothane anaesthesia. Blood wascollected and processed to serum at 4 ºC using centrifugation technique for the estimation of biochemical parameters. Liver samples were harvested for the antioxidant parameters analysis.

Preparation of serum tissue homogenate

A freshly cut of approximately 0.5 g were excised from the whole liver tissue. The fraction was homogenised in 5 mL of phosphate buffer (pH 7.5) containing 50 mM sodium phosphate and 10% triton X-100. The resultant composition (containing the homogenate and buffer components) was centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant was stored at −20°C for further bioassay analysis.

Assessment of serum liver markers

Serum assay procedure of quantification of biochemical parameters was analysed using Automated Chemistry Analyzer (LabmaxPlenno, Labtest Co. Ltd., Lagoa Santa, Brazil) with commercial assay kits in accordance with manufacturer's protocol.

Determination of oxidative stress markers

The oxidative stress biomarkers were analysed in the various reaction mixtures by determining superoxide dismutase (SOD) activities,[50] Catalase (CAT) activities,[51] Reduced glutathione levels [52] and lipid peroxidation levels (MDA),[53]

Statistical analysis

Data were analysed using GraphPad Prism version 8.10 (244) for Windows, GraphPad, Software, La Jolla California USA for animal studies analysis only. Mean and standard error of mean of five replicates (n=5) were graphed. One-way analysis of variance (ANOVA) was used to assess the statistical significance, followed by a Dunnett's Multiple Comparison Test with the toxicity-induced carbon tetrachloride as the comparison control. A *p*-value lower than 0.05 was considered to be statistically significant.

Results and Discussion

Elucidation of the chemical profiles of the plant's extracts (hexane and ethanolic) leaf extract of *D. tripetala* in the current investigation showed diverse chemical family and classes of natural products. The qualitative chemical screening conducted on both extracts in this study, showed prominent phyto-compoounds such as saponins, tannin, flavonoids, steroids, phenols and terpenoids (Table 1). Analysis of the GC-MS results showed thirty-one and thirty-five bioactive components identified in the hexane and ethanolic extracts (Table 2), under the condition of our investigation. The major constituents found in the hexane extract are 1-nitro-2-phenylethane (15.98%), nerolidol (9.15%), Palmitic acid (7.10%), 1-octadecene (6.01 %), cis-vaccenic acid (5.81%), vitamin E (4.20%) and squalene (3.60 %). While the key constituents in the ethanol extract are elaidic acid (15.34%), amitriptyline (12.94%), Palmitic acid (9.33%) and γ-sitosterol and others.Palmitic acid has been shown to exhibit antioxidant potentials by preventing atherosclerosis in rats model through its anti-lipidemic activities *in vivo*.[54] Similarly, the1-nitro-2-phenylethane, present in both plant extract has been earlier investigated for the insecticidal properties of *D. tripatela*and was shown to display hypnotic, anticonvulsant, and anxiolytic effects in a mice model experiment.[55,56] The 1-nitro-2-phenylethane and nerolidol composites are documented inhibitors of the urease, enzyme.

Table 1: Qualitative Biochemical analysis of hexane and ethanol extracts of *D. tripatela* fruit

Phytochemicals components	Hexane extract	Ethanol extract
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	+
Phenols	+	+
Terpenoids	+	+

Keys: (+) = Present, (-) = Absent

The inhibitory effect of urease by both components are thought to be facilitated by both hydrophobic and hydrogen bond interactions between the amino acid residues of the enzyme's active site,[57] Aside, the nutritional significance affiliated to these active compounds, their synergestic system *in vivo*, have been shown to mediate decrease in serum lipoprotein (LDL) cholesterol contents and boost high-density lipoprotein (HDL) cholesterol contents in animal studies.[58]

In vitro investigation of the free radicals potencies of hexane and methanol fruit extracts of *D. tripatela* using two known radical models (DPPH and ABTS) (Table 3), displayed noteworthy antioxidant abilities with IC_{50} values; 3.86 and 4.01 µg/mL against DPPH assay and 4.53 and 4.79 µg/mL as against ABTS assay, respectively. The IC_{50} value of the hexane extract (3.86 µg/mL) was found to be lower than the ethanol extract (4.01 µg/mL) in the DPPH assay and same for the ABTS assay. A reference control, vitamin C displayed a high activity than the two extracts with IC_{50} values, 0.007 µg/mL for DPPH and 0.005 µg/mL for ABTS, respectively. Free radicals when produced in an uncontrolled amount have been shown with unusual alteration and specifically targeted at the DNA base sequence and important biomolecules in human[59] Inhibition of DPPH and ABTS radical solution by *D. tripetela* crude extract are thought to be mediated by certain endogenous chemicals such as phenolics and flavonoids,[60] known for the scavenging activities which has earlier been reported by.[61,62]It is most likely that the plant act basically as an *in vitro* electron donating agent aimed at averting the proliferation of the radicals emanating from the synthetic free radicals (DPPH and ABTS) employed.

The antibacterial inhibitory growth effect of the hexane and methanol extract of *D. tripatela* were assessed on six Gram-positive and two Gram-positive bacteria strains of medical importance (Table 4). The zone of inhibition (ZI) attained by streptomycin, hexane and ethanol extracts are represented in figures; 0.0 ± 0.0 to 21 ± 0.3 mm, 0.0 ± 0.0 to 23.5 ± 0.7 mm and 0.0 ± 0.0 to 20.0 ± 0.7 mm, respectively. The negative control (5% DMSO) did not show any activity against any of the tested isolates. The ZI values exhibited by the hexane fruit extract were experimentally higher than that exhibited by the methanol fruit extract. The highest inhibitory activity, which is a function of ZI values were exhibited by both hexane and ethanol fruit extract on *Klebsiella pneumonia* (NCIB 418) (23.5 ± 0.7 and 20.0 ± 0.7) and *Bacillus cereus* (NCIB 6349) (20.5 ± 0.7 and 17.0 ± 1.5), during our investigation. Determination of the minimum inhibitory concentrations (MICs) alongside with the minimum bactericidal concentrations (MBCs) evaluated are depicted in Table 4. The MIC displayed by the hexane extracts against test bacteria were in the range between 1.38 ± 0.00 and 30.00 ± 0.01 mg/mL, while that of the ethanol extract ranged between 1.38 ± 0.01 mg/mL and 15.00 ± 0.02 mg/mL and the corresponding streptomycin values (1.25 ± 0.00 and 10.02 ± 0.02 µg/mL) (Table 5). On the other hand, the MBC observed against the test bacterial isolates were in the range of 1.38 ± 0.02 and 30.00 ± 0.02 mg/mL for hexane extract and 7.50 ± 0.03 and 30.00 ± 0.03 mg/mL for theethanol extracts. The MBC values for streptomycin, a control drug are in the range between 5.00 ± 0.00 and >10 mg/mL. Both extracts from *D. tripatela* fruit displayed significant antibacterial activities against all the panels of bacteria tested in this study comparatively with the positive control (streptomycin). Phenolic acid extract of *D. tripatela* have been recorded for its inhibitory effects against growth of food-borne microorganisms such as *Staphyloccocus aureus*, *Salmonella sp*, *Escherichia coli*, and a host of others.[63] Tannins, saponins, and flavonoids, for example, are shown to be effective against diabetes, antimicrobial infections and other anti-inflammatory responses.[64,65] The antimicrobial agent(s) of plants origin in any case are known to demonstrate either bactericidal or bacteriostatic potential or both on multidrug resistance pathogenic bacteria. The preliminary screening on activity-based can are exploited as precursor for antibiotics preparation in the treatment and management of infectious diseases, primarily, urinary tract infections instigating bacteria.[66–68]

Table 2: Bioactive constituents in hexane and ethanol fruit extracts of *D. tripatela*

S/N	R.T	Name of compounds	Hexane Extract % Area (Comp)	Ethanol Extract % Area (Comp)	MF	MW
1	4.688	N-Methyl-3-piperidinecarboxamide	-	1.34	$C_7H_{14}N_2O$	142
2	4.693	Methylene Chloride	1.32	-	CH_2Cl_2	84
3	4.991	Cyclohexane	-	1.19	C_6H_{12}	84
4	5.443	1-Dodecene	4.19	1.47	$C_{12}H_{24}$	168
5	5.722	1, 2, 3, 6-Tetrahydropyridine	-	0.82	C_5H_9N	83
6	5.884	1-nitro-2-phenylethane	15.98	3.69	$C_8H_9NO_2$	151
7	6.067	1-Tetradecene	-	2.19	$C_{14}H_{28}$	196
8	6.313	Oxirane, (2, 2-dimethylpropyl)	-	2.05	$C_7H_{14}O$	114
9	6.387	*α*-Farnesene	2.27	-	$C_{15}H_{24}$	204
10	6.410	2, 4-tert-butylphenol	1.27	1.28	$C_{14}H_{22}O$	206
11	6.547	Nerolidol	9.15	1.20	$C_{15}H_{26}O$	222
12	6.599	2-Tetradecene	3.50	3.75	$C_{14}H_{28}$	196
13	6.782	Isopulegol	0.43	-	$C_{10}H_{18}O$	154
14	6.885	*α*-Eudesmol	0.63	0.70	$C_{15}H_{26}O$	222
15	7.022	Benzene, (3-nitropropyl)-	0.57	-	$C_9H_{11}NO_2$	165
16	7.074	1-Octadecene	6.01	3.76	$C_{18}H_{36}$	252
17	7.286	Bicyclo[3.3.1]nonane-2, 8-dione	-	3.27	$C_9H_{12}O_2$	152
18	7.348	Methyl veratrol		3.15	$C_9H_{12}O_2$	152
19	7.371	6, 11-Dimethyl-2, 6, 10-dodecatrien-1-ol	0.92	-	$C_{14}H_{24}O$	208
20	7.423	Palmitoleic acid	-	0.72	$C_{16}H_{30}O_2$	254

21	7.463	Palmitic acid	7.10	9.33	$C_{16}H_{32}O_2$	256
22	7.526	5-Eicosene, (E)	3.75	3.37	$C_{20}H_{40}$	280
23	7.812	Phytol	1.66	-	$C_{15}H_{24}O$	296
24	7.881	cis-Vaccenic acid	5.81	0.95	$C_{18}H_{34}O_2$	282
25	7.886	Elaidicacidpolmj' O	-	15.34	$C_{18}H_{34}O_2$	282
26	7.989	1-Docosene	2.90	3.05	$C_{22}H_{44}$	380
27	8.407	9,10-Anthracenedione, 2-[4-(acetyloxy)tetrahydro-2H-pyran-2-yl]-1, 3, 6, 8-tetramethoxy-, cis	2.99	3.09	$C_{25}H_{26}O_9$	470
28	8.521	Cyclotetracosane	1.73	1.69	$C_{24}H_{48}$	336
29	8.911	1, 2-Propanediol, 3-benzyloxy-1, 2-diacetyl	2.43	-	$C_{14}H_{18}O_5$	266
30	9.031	Bis(2-ethylhexyl) phthalate	-	0.88	$C_{24}H_{38}O_4$	390
31	9.197	Z-5-Nonadecene	-	1.06	$C_{19}H_{38}$	266
32	9.305	1H-Indole, 5-methyl-2-phenyl	3.13	-	$C_{15}H_{13}N$	207
33	9.351	5-Methyl-2-phenylindolizine	5.17	-	$C_{15}H_{13}N$	207
34	9.414	(2, 3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans	4.12	-	$C_{22}H_{20}OS$	332
35	9.637	Tricosene	0.64	-	$C_{23}H_{48}$	324
36	10.169	Dioctyl sebacate	0.87	1.87	$C_{25}H_{50}O_4$	426
37	10.295	Squalene	3.60	0.86	$C_{30}H_{50}$	410
38	10.759	Heptadecane	1.33	-	$C_{17}H_{36}$	240
39	12.012	Dimethyl palmitamine	-	1.62	$C_{13}H_{39}N$	209
40	12.144	Amitriptyline	-	12.94	$C_{20}H_{23}N$	277
41	12.390	Eicosane	0.60	-	$C_{20}H_{42}$	282
42	12.950	Vitamin E	4.29	-	$C_{29}H_{50}O_2$	430
43	14.398	Campesterol	-	0.86	$C_{28}H_{48}O$	400
44	14.724	Stigmasterol	0.55	2.10	$C_{29}H_{48}O$	412
45	15.222	Furazan, 3-(dimethylaminomethylenamino)-4-(1, 2, 4-triazol-3-yl)	-	2.00	$C_7H_9N_7O$	207
46	15.617	γ-sitosterol	1.06	3.79	$C_{29}H_{50}O$	414
47	22.866	3-Penten-2-one, 4-(2, 2, 6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-, (E)	-	0.71	$C_{14}H_{22}O_2$	222
48	23.370	1,5-Bis[4-fluorophenyl]-2, 5-dihydro-2, 2-dimethyl-1H-imidazo[4, 5-b] phenaine 4	-	1.11	$C_{27}H_{20}F_2N_4$	438
49	31.638	2-(Pentafluoropropionyl) oxybenzylidene acetophenone	-	2.78	$C_{18}H_{11}F_3O_3$	370
Total			99.97%	99.98%		

Keys: S/N = Serial Number, R.T = Retention Time, M.F = Molecular Formla, M.W = Molecular Weight.

A comparative display of antibacterial activity between hexane (a non polar solvent) and ethanol (being a polar solvent) is partly due to the presence of phenolic, nerolidol, α-eudesmol, stigmasterol, γ-sitosterol in both extracts. This observation is somewhat different from Dhawan and Gupta [69] account on the view that polar solvent display high antibacterial effect than the non polar counterpart. Previous researches report on the basic characteristic and selective toxicity of antibacterial agent(s).[70–73]Moreso, a specific interference with the metabolic process of the infectious bacteria due to varying concentration tolerance with respect to the host cells have been shown a determinant for a potent antibacterial efficacy. [74,75] Hence, antibacterial compounds are thought to elicit antimicrobial actions through four major cellular mechanism.[15] This includes inhibition of cell wall synthesis,[16] alteration in the permeability of cell membrane or active transport across the cell membrane, [17] inhibition of protein synthesis that include blockage of the cellular translational and transcriptional process of genetic material [18] and the inhibition of nucleic acid synthesis.[76]

In the same vein, acute toxicity test experiment of the crude extract on experimental animals were devoid of adverse effects and any sign of toxicity. The median lethal dose (LD_{50}) of the crude extract was greater than 3000 mg/kg body weight. Oral administration of the crude extract of plant has no influenced on the rats weights (results not shown). However, induction of hepatotoxicity challenged by CCl_4 on the liver in this study, significantly ($p < 0.05$) lead to increase in the serum levels of AST, ALT, ALP, total bilirubin and a concomitant decreased in the total protein content. The plant extract and reference anti-hepatotoxic drug, silymarin suppressed serum oxidative markers indices (Figure 1).

CCl_4-induced acute liver damaged, has been shown to affect directly, levels of liver biomarkers in animal studies.Liver injury rooting from hepatotoxins are known to cause a range of pathologies with increased deleterious.[77,78] The mode of hepatotoxicity of CCl_4 is mediated through its reactive intermediates, which serve as the precursors of oxidative damage of the liver tissues, protein conjugate, membranes layer and ultimately liver failure. [79,80] Therefore, increased level of activities and concentration of these oxidative parameters; ALT (alanine aminotransaminase), AST (aspartate aminotransaminase), ALP (alkaline phosphatase), total bilirubin, and a decreased in the

protein moiety (albumin and total protein) contents in the serum instigated by exogenous toxins are effective indicators of liver injury .[81] In the current study, elevated serum concentration of total bilirubin content, being one of the indicators of oxidative stress, observed in an increasing generation of free radicals *in vivo*,[82] depicts extent of liver damage. A concomitant oral administration of the extract at varying doses (100 to 300 mg/kg bw) and CCl_4 mitigate the previously elevated concentrations of ALT, AST, ALP, total bilirubin in all the intervention rats groups comparatively with the reference standard hepatoprotective agent (silymarin).

Furthermore, as a direct effect on the intervention study, the co-treatment rats' groups treated with either extract or drug increased liver homogenate contents of catalase, superoxide dismutase activity and reduced glutathione levels, respectively. Decreased levels of lipid peroxidation products (malondialdehyde value or MDA) significantly ($p < 0.05$) different from the inducer control rats liver homogenate content (Figure 2). Assessment of *in vivo*antioxidants in a toxic challenge is in part a pharmacological pattern in view of the. intracellular protective offer against toxic chemical inducers and other opportunistic invaders [83] *In vivo* defence system is a family of enzymatic and non-enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GSH) and peroxidises (GPX) and other antioxidant vitamins. They are constitutive in their biological configuration, whose functions are in the line of scavenging free radical invaders of bodily architecture, detoxification of harmful substance to a predestined harmless moiety, distribution, recycling and incorporation of vital exogenous substance into the biological stream.[84,85] In this study, estimation of catalase, reduced glutathione and contents of the serum liver homogenate in the crude leaf extract of *D. tripetela*co-treatment animal groups with CCl_4, revealed an impressive increase in the concentration of the liver antioxidant parameters, significantly ($p < 0.05$), compared with the CCl_4 animal group. The results of the antioxidant contents in the serum liver homogenate of the co-treatment animal groups showed no significant different when compared to the standard liver protective drug (silymarin), under the condition of our investigation. Increased levels of these liver antioxidants molecules are important markers of homeostasis in a healthy liver state.[86] However, reduction in the activity and levels of CAT, GSH and SOD in the liver indicate liver state of compromised functions, occasioned by the induction of hepatotoxicity. [87]

The pharmacological consequences of CCl_4, is the frequent acute liver injury, due to a diminished synthesis of cyclooxygenase-1 (COX-1) (being a constituted form of COX enzyme family responsible for the production of bioactive lipids termed, prostanoids; prostaglandins, thromboxane and leucotriene).[23] Other effects include, alteration in a normal liver histology, increased serum levels of amino transaminases in the face of increasing production of oxidative stress arising from reactive metabolites of CCl_4 formed via cytochrome-P450 2E1-dependent pathway. And ultimately, increased inflammatory responses and activated macrophages, apoptosis through the intrinsic and extrinsic apoptic pathways.[23,88]

Table 3: Antioxidant capacity of hexane and ethanol fruit extracts of *D. tripatela*

Extracts	Hexane Extract	Ethanol Extract	Vitamin C
DPPH IC_{50} (µg/mL)	3.86	4.01	0.007
ABTS IC_{50} (µg/mL)	4.53	4.79	0.005

DPPH = 2, 2-diphenyl-1-picrylhydrazyl (DPPH). ABTS = 2, 2-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid) diammonium,

Table 4: Antibacterial sensitivity test of hexane and ethanol extract of *D. tripatela*against the test organisms

Test Organisms	Positive Control Streptomycin (10 µg) mm	Zone of Inhibition (mm)	
		Hexane Fruit Extract (30mg/mL) mm	Ethanol Fruit Extract (30mg/mL) mm
Gram-Positive			
Bacillus stearothermophilus(NCIB 8222)	21.0 ± 0.3 (S)	13.0 ± 1.4	15.0 ± 0.5
Clostridium sporogenes (NCIB 532)	20.0 ± 0.7 (S)	18.0 ± 0.0	14.0 ± 0.7
Bacillus subtilis(NCIB 3610)	0.0 ± 0.0 (R)	19.0 ± 1.4	17.0 ± 0.9
Bacillus cereus(NCIB 6349)	18.0 ± 0.3 (S)	20.5 ± 0.7	17.0 ± 1.5
Staphylococcus aureus (NCIB 8588)	20.0 ± 0.8 (S)	13.5 ± 0.7	17 ± 0.3
Micrococcus luteus (NCIB 196)	19.0 ± 0.3 (S)	13.0 ± 1.4	15.0 ± 0.7
Gram-Negative			
Klebsiella pneumonia (NCIB 418)	0.0 ± 0.0 (R)	23.5 ± 0.7	20.0 ± 0.7
Pseudomonas aeruginosa (NCIB 950)	0.0 ± 0.0 (R)	17.0 ± 1.4	19.0 ± 0.9

NCIB= National Collection of Industrial Bacteria, R: Resistance, I: Intermediate

Evaluation of the extent of liver damage by the CCl_4 and repair ability of the extract, were assessed through lipid peroxidation product (malondialdehyde, MDA) otherwise known as lipid peroxide value. Serum liver homogenate has been regarded as an important parameter in the evaluation of liver status.[53] Evaluation of the lipid peroxidation products in this study, showed significant ($p < 0.05$) depleted MDA level in the serum liver homogenate contents of animals concurrently administered crude leaf extract and CCl_4, compared to the non-intervention (CCl_4-intoxicated animals') group. A depleted amount of MDA in the co-treatment animals (animal groups with extract intervention bias) serum liver homogenate, suggest an increased synthesis and replenishment of the damaged liver tissues and endogenous antioxidants.[83,89] The observed trends in these findings corroborate with previous researches. [61,90,91]In view of the data generated, the crude extract of *D. tripetela* leaf's extract, to a large extent, apparently excertsphyto-antioxidant-dependent mechanism, through donation of electrons(s), chelating, reduction of metallic ion [92,93]*in vitro*. And possibly, elicit inductive initiation of the *de novo* synthesis template for endogenous antioxidative molecules.[61,91]

Conclusion

D. tripetela's leaf demonstrated potent *in vitro* and *in vivo* antioxidant activity. The plant extract exhibited promising inhibitory effects on both negative and positive microbial references strains. The ethanol crude extract of the plant was effective in normalizing and mitigating toxic effects of carbon tetrachloride on rats' liver. Phytochemicals detected are thought to elicit the biological actions. Hence, isolation, purification and characterization of lead compound (s) responsible for the bioactivities are recommended for a further studies.

Table 5: Minimum inhibitory concentrations (MICs) and Minimum bactericidal concentrations of antibacterial agents

Bacteria Strains	Hexane Extract		Ethanol Extract		Streptomycin		DMSO
	MIC (mg mL^{-1})	MBC (mg mL^{-1})	MIC (mg mL^{-1})	MBC (mg mL^{-1})	MIC (µg mL^{-1})	MBC (µg mL^{-1})	0.4 mL
B. stearothermophilus (NCIB 8222)	1.38 ± 0.01	30.00 ± 0.02	15.00 ± 0.02	30.00 ± 0.03	5.02 ± 0.01	10.00 ± 0.02	VG
C. sporogenes (NCIB 532)	1.38 ± 0.03	15.0 ± 0.01	3.75 ± 0.02	7.50 ± 0.03	10.01 ± 0.02	>10	VG
B. subtilis (NCIB 3610)	1.38 ± 0.00	3.75 ± 0.01	3.75 ± 0.00	7.50 ± 0.02	ND	ND	VG
B. cereus (NCIB 6349)	1.38 ± 0.01	3.75 ± 0.02	3.75 ± 0.02	15.00 ± 0.02	5.00 ± 0.00	5.00 ± 0.00	VG
S. aureus (NCIB 8588)	1.38 ± 0.02	3.75 ± 0.02	1.38 ± 0.01	15.00 ± 0.01	5.00 ± 0.01	10.02± 0.01	VG
K. pneumonia (NCIB 418)	30.00 ± 0.01	30.00 ± 0.02	2.76 ± 0.01	15.00 ± 0.01	5.00 ± 0.00	>10	VG
M. luteus **(**NCIB 196)	1.38 ± 0.00	1.38 ± 0.02	1.38 ± 0.00	30.00 ± 0.01	1.25 ± 0.00	5.00 ± 0.02	VG
P. aeruginosa (NCIB 950)	7.50 ± 0.00	1.38 ± 0.02	3.75 ± 0.00	15.00 ± 0.01	ND	ND	VG

Key: ND = Not determined, VG = Visible growth, NVG = No Visible Growth

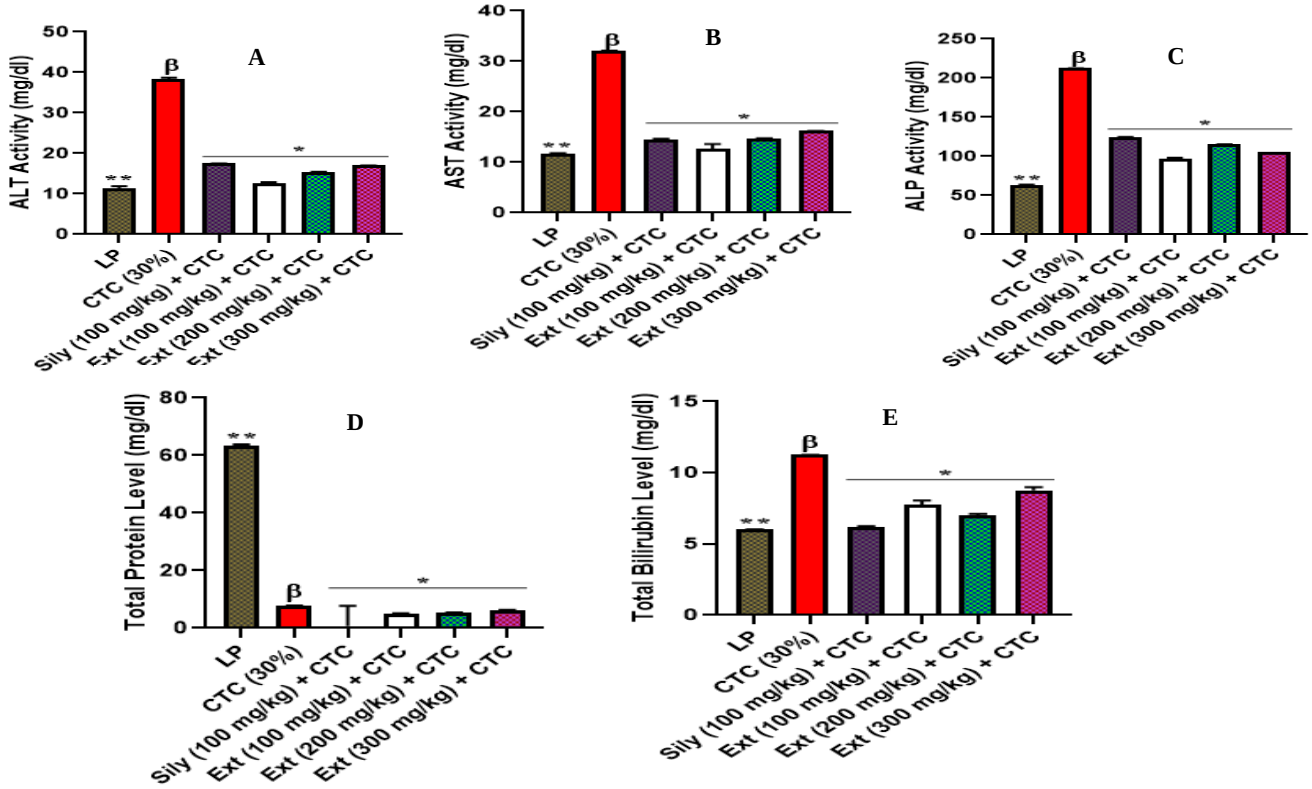

Figure 1: The protective activity of *D. tripetala* against carbon tetrachloride-induced toxicity, tested at different doses. Data were represented as mean ± standard error of mean of five replicates (n=5). One-way analysis of variance, p-value < 0.05 using Dunnett's Multiple Comparison Test, β = comparing column, *= *p*-value < 0.05, ** = *p*-value < 0.01. LP = liquid paraffin; Sily= Silymarin; CTC = Carbon tetrachloride; AST =aspartate aminotransferases;ALT = alanine aminotransferases & ALP = Alkaline phosphatise.

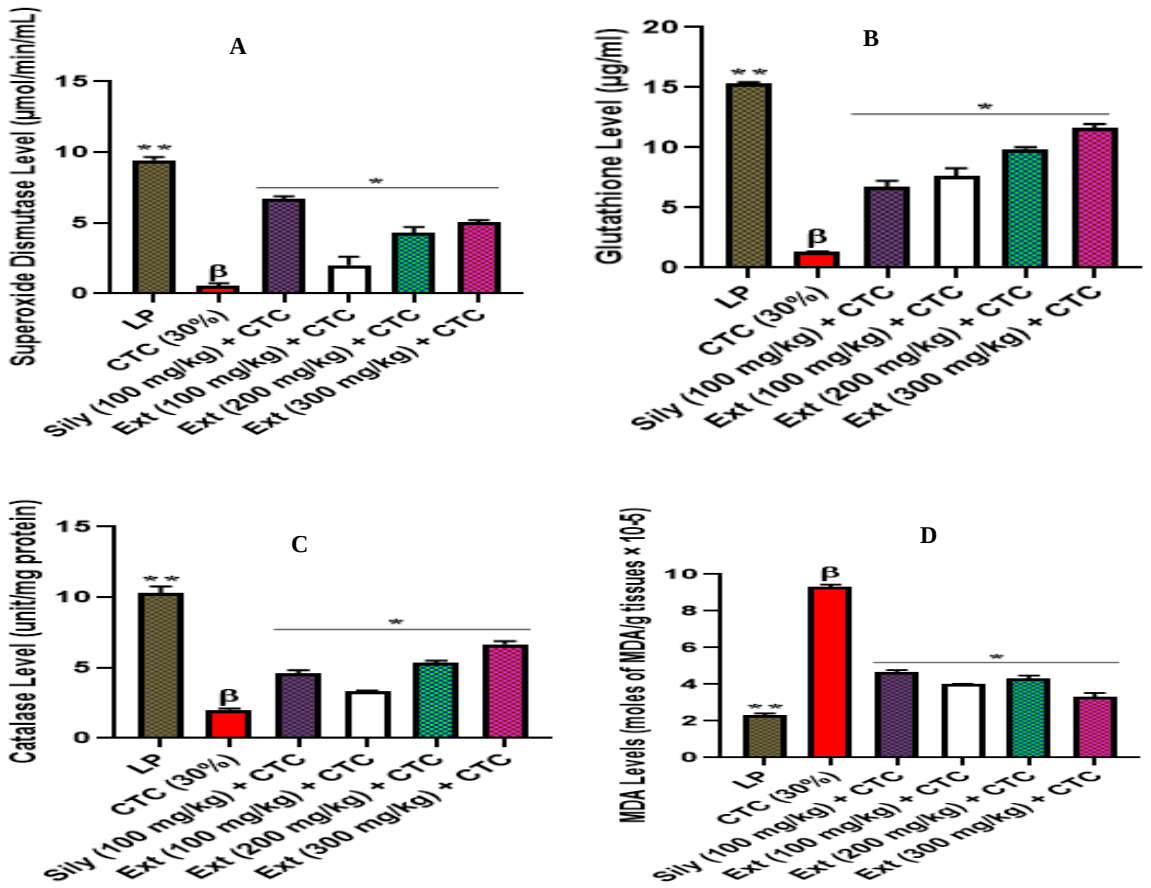

Figure 2: The protective activity of *D. tripetala* against carbon tetrachloride-induced toxicity, tested at different doses. Data were represented as mean ± standard error of mean of five replicates (n=5). One-way analysis of variance, p-value < 0.05 using Dunnett's Multiple Comparison Test, β = comparing column, *= *p*-value < 0.05, ** = *p*-value < 0.01. LP = liquid paraffin; Sily = silymarin; CTC = carbon tetrachloride and MDA = malondialdehyde.

Conflict of Interest

The authors declare no conflict of interest.

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Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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