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Evaluation of possible hepatoprotective potentials of *Parinari kerstingii* methanol stem bark extract and molecular docking of its compounds against CYP2E1 enzyme

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Liver diseases are becoming rampant due to the increasing number of hepatotoxicants, including drug abuse. Medicinal plants are known for their hepatoprotective properties. This study evaluated possible *in vivo* and *in silico* hepatoprotective potentials of *Parinar kestingii* extract. Thirty Wistar rats assigned to six groups (A-F) of five rats per group were used to investigate *in vivo* hepatoprotective effects. Rats in groups A and B were pretreated with distilled water, while groups C, D, E and F rats received 200, 400, and 600 mg/kg of the extract and 100 mg/kg of silymarin, respectively, as pretreatments. All pretreatments lasted for 14 days, and on day 15, acetaminophen (2000 mg/kg) was administered to all the rats except those in group A. Forty-eight hours after, sera samples for assay of biochemical parameters (ALT, AST, ALT Bilirubin) were obtained, and the liver was harvested for histopathology studies. Gas chromatography-mass spectrometry was used to separate and identify the compounds present. Molecular docking on Butylated hydroxytoluene, Indazole, 4-methyl pyrazole and CYP2E1 was performed using Autodock Vina. The mean ALT, AST, and ALP activities of the extract-treated rats were significantly lower than that of group B but were comparable with those of groups A and F. Hepatocytes of the extract-treated showed less severe lesions compared to those of the negative control. Gas chromatography-mass spectrometry results indicated various compounds in the extract. The binding affinities (kcal/mol) between CYP2E1 and the ligands butylated hydroxytoluene, indazole, and 4-methyl pyrazole were -6.7, -5.1, and -3.8, respectively. It was concluded that the extract possesses both *in vivo* and *in silico* hepatoprotective abilities.

Keywords: CYP2E1, Hepatotoxicity, Histopathology, Liver-damage markers, Molecular docking, *Parinari kerstingii*

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Introduction

The liver is one of the vital organs in the body saddled with crucial roles ranging from detoxification and biotransformation to synthetic functions, amongst others. Due to its vast biological roles, the liver encounters a lot of insults that tend to compromise its status. Liver ailments are among the most killer diseases worldwide[1]. The causes of injuries to the hepatocytes include viruses, alcohol poisoning, drug abuse, chemicals and other toxic substances[2,3]. Medicinal plants from ancient times are known to cure various ailments, including liver diseases.

Acetaminophen-induced hepatotoxicity is occasioned by N-acetyl-p-benzoquinone imine (NAPQI), an active and toxic metabolite produced during acetaminophen metabolism. The CYP2E1 is a phenotype of cytochrome p450 enzyme, which is responsible for metabolizing acetaminophen into NAPQI, which usually will be conjugated by glutathione and thus made harmless[4]. However, in acetaminophen abuse or intoxication, the level of NAPQI generated overwhelms the conjugating ability of glutathione and thus precipitates injury to the hepatocytes due to the formation of adducts to cellular proteins[5].

Liver diseases can be diagnosed by assaying a panel of liver-damage markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities[6]. Alanine aminotransferase and aspartate amino-transferase activities assess the hepatocellular status of the liver, while alkaline phosphatase evaluates the integrity of the hepatobiliary system[7]. Other parameters that can be used to assess the liver include bilirubin, cholesterol, total protein, albumin levels, and gamma-glutamyl activities[6].

Medicinal plants have been in use for the management of ailments since ancient times. *Parinari*

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kerstingii Engl belongs to the family Rosaceaae and has fruits that are ovoid in shape. The plant is a native of West Africa. It is an evergreen plant that can grow as high as 20 metres[8]. Locally, the plant is known as "kaikayi" by the Hausa people of northern Nigeria, "Okpe" by Yorubas in western Nigeria, "Kakyiki" in Ghana, "Ningelia" in Togo and "Aramon" by Ivorians[9]. It is known as "Koko" by igbos, southeastern Nigerians. In ethnomedicine, the plant is used in treating pains and bronchopneumonia and as a purgative and emetic agent[9]. Researchers have confirmed the anti-inflammatory, analgesic and antioxidant activities of *P. kerstingii* extract[10].

Gas chromatography-mass spectrometry (GC-MS) separates compounds present in an extract by using gas as a mobile phase, and then the mass-charge (m/z) ratio to attempt identification of the compounds eluted, taking cognizance of the retention time. It is a hyphenated system that has been shown to be sensitive and accurate[11]. Molecular docking is a computer-aided artificial intelligence technique that is used in drug design and discovery. Molecular docking tries to select the best-fit orientation between the ligands and the target protein/receptor. It, therefore, gives the binding affinities between the ligand and the protein in the form of binding energies in kcal/mol. The lower the binding energy, the more activity will be elicited[12].

There is a dearth of information regarding the GC-MS profile and hepatoprotective abilities of *P. kerstingii*. It is a medicinal plant that has been evaluated for its anti-inflammatory properties. The main objective of this study was to profile the methanol extract of this plant and evaluate its possible beneficial effects on liver disorders. In the present study, we evaluated the *in vivo* and *in silico* hepatoprotective potentials of *P. kerstingii* and determined the compounds present in its methanol stem bark extract.

Materials and Methods

Plant materials

A Botanist (Mr. A.O. Ozioko) from the Department of Plant Science and Biotechnology, University of Nigeria, collected the stem barks of *P. kerstingii* in March 2023 from the Nsukka, Enugu state, Nigeria and correctly identified them with the sample voucher specimen number: InterCEDD/16285 and thereafter deposited in the herbarium of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The stem barks were dried under shade.

Preparation of plant extract

The stem barks were gathered, cleaned to remove dust, air dried, and then pulverized with a mill and pestle. The powdered components (500 g) were extracted with 80% methanol over 48 hours using a cold maceration process and vigorous shaking at 2-hour intervals. The mixture was then filtered, and the methanol extract was concentrated using a rotary evaporator under decreased pressure.

Animals

Female Wistar strain rats weighing 130-135 g were procured from a rat breeder. The animals were assigned into groups and housed in stainless wire mesh cages for two weeks to acclimate to 28-32°C climatic conditions. During the stabilization period and throughout the trial, a commercial pellet diet (Chikun feed®) and clean water were fed *ad libitum*.

This study was approved by the Faculty of Veterinary Medicine, University of Nigeria Nsukka Institutional Animal Care and Use Committee (IACUC) with the approval number FVM-UNN-IACUC-2023-09/116a.

Acute toxicity study

Thirty albino Wistar rats weighing between 180 and 200 g were assigned to six groups (A-F) of five rats per group. Group A received distilled water to serve as normal control while groups B-F were administered 50, 200, 800, 3200, and 5000 mg/kg of the extract, respectively. The rats were watched for 48 h for signs of toxicity such as sedation, excitement, writhing or death[13].

Experimental design

A total of 30 rats were assigned into six groups (A-F), each with five rats. The extracts were given to the rats orally by gastric gavage. Groups A and B served as normal and negative controls, receiving simply distilled water (10 mL/kg), respectively. Groups C-E were given 200, 400, and 600 mg/kg of a methanol extract of *P. kerstingii*, respectively, whereas group F rats were given Silymarin (100 mg/kg), a conventional medication for liver disorders. All therapies were administered once a day for 14 days. On day 15, rats in groups B-F were given a single dosage of 2000 mg/kg acetaminophen orally. Blood samples were collected from the retrobulbar plexus in the medial canthus of the eye 48 hours after acetaminophen treatment, and sera were retrieved for analysis of some biochemical parameters (ALT, AST, ALP, total Bilirubin). Thereafter, the rats were

humanly sacrificed. The liver was harvested for histological examination.

Determination of some serum biochemical parameters

The activities of serum alanine aminotransferase and aspartate aminotransferase were determined using the Reitman-Frankel colourimetric method developed by Reitman and Frankel[14], while serum alkaline phosphatase activity was determined using the phenolphthalein monophosphate method[15,16]. The Jendrassik-Grof method[17] was used to determine serum bilirubin.

Histopathological examination

Histopathological examination was done following Drury *et al.*[18].

Gas Chromatography-Mass Spectrometry Analysis

The samples' clear extracts were analyzed using a Trace 1310 gas chromatograph coupled to an ISQTM 7000 single quadrupole mass spectrometer equipped with an auto-sampler and controlled by a computer running the ChromeleonDionex Version 7.2.10.23925 software (ThermoScientificTM, Italy). A DB-5 fused silica capillary column (27 27 17.7 cm; 12.9 L) coated with 5% phenylmethyl polysiloxane was utilized during the analysis. The carrier gas was helium, which had a constant linear velocity of 1 mL/min. In a split mode of a 10:1 ratio, a 1 L sample volume was injected. The ramp temperature schedule for the GC oven was 40°C (held for 5 minutes), 80°C at 50 C/mins (held for 5 minutes), and lastly 250°C at 100 C/min (held for 10 minutes). The injector, transfer line, and ion source temperatures were set at 280, 250, and 200°C, respectively, and the analysis ran for 48 minutes. The input temperature was 150°C, with a 3 mL/min front inlet purge flow. Using the electron impact (EI) mode, the mass spectrometer was operated in the full scan mode with an electron ionization voltage of 70 eV. The MS scan range was set to 45-500 atomic mass units (amu), with a dwell period of 0.2 seconds. The start time for the MS was set to 2.46 minutes, while the front inlet pressure was set to 100 kPa. The resulting spectra were compared to the NIST (National Institute of Standards and Technology) database version 2.3 (2017). Prior to sample injection, the syringe was prewashed with the matrix (methanol) and sample to eliminate contamination.

Molecular docking analysis

The enzyme CYP2E1 (3gph) and the ligands butylated hydroxytoluene, indazole, and 4-methyl pyrazole are docked using molecular docking to discover the best-fit orientation. BIOVIA, Discovery Studio (version 2021), and AutoDock Vina software were used to bind the protein (3gph) with the ligands. The binding energies were determined. The ligands and target proteins were produced according to normal protein and ligand preparation procedures, and the files were uploaded to AutoDock Vina. The docked data and the resultant binding energies and binding contacts of each ligand were evaluated using Discovery Studio Visualizer[19].

Preparation of the ligands

Butylated hydroxytoluene, indazole, and 4-methyl pyrazole structures were obtained from the PubChem database (http:// pubchem. ncbi. nlm. nih. gov/).

CYP2E1 (3gph) retrieval

The protein was chosen because of its interactions with the ligands. A Protein Data Bank (PDB) was used to obtain the proteins' 3D X-ray crystal structures.

Preparation of CYP2E1 (3gph)

The CYP2E1 was created by translating the original PDB structure into premade protein models with the Discovery Studio software. By eliminating the water molecules from the structures, an X-ray crystal structure of CYP2E1 was created. The Discovery Studio software was also utilized to examine ligand structure, hydrogen bond interactions, and non-bond interactions with active site residues, as well as to generate high-quality photographs.

Docking proper

AutoDock Vina was used to examine the prepared ligands as well as the CYP2E1. In the docking process, the numerous conformations of the ligands were created, and the final energy refinements of the ligands' posture were conducted. The docking scores of the various poses into the cyclooxygenases were determined for all of the tested ligands.

Data analysis

The study's data were investigated using One-way Analysis of Variance (ANOVA). The Duncans Multiple Range post hoc test was used to discern between distinct means. P (probability) values less than 0.05 were considered significant. The results were presented in tables and charts as mean ± standard error of the mean (SEM). Discovery Studio version 2021 was used for post-docking analysis.

Results

Acute toxicity studies

The results of the acute toxicity studies showed that there were no signs of toxicity, even at the highest dose of 5000 mg/kg.

Liver-damage markers

The results of the liver-damage markers indicate that the ALT, AST and ALP activities of the rats in group B (pretreated with distilled water for 14 days, then administered acetaminophen on the 15th day) were significantly (p<0.05) higher than those of the rats in other groups (Table 1). The ALT activities of the rats in groups A, D, and F were statistically comparable (p>0.05) but significantly (p<0.05) lower than those of groups B, C, and E. Similarly, the AST activities of the rats in groups D and F were statistically similar (p>0.05) but significantly (p<0.05) lower than those of the groups B, C and E. The activities of the ALP of rats in groups A, E and F were not different statistically (p>0.05) but were significantly (p<0.05) lower than those of groups B, C and D. The total bilirubin values of group B rats were significantly higher than those of the other groups while those of groups D and F were significantly (p<0.05) lower when compared to those of the other groups. The total bilirubin values of group A rats were compared very well (p>0.05) with that of the group F rats (Table 1).

Liver histophotomicrograph (H&E x400)

The hepatic histomorphology of normal rats showed a normal central vein and hepatocytes arranged in cords, while the liver of rats intoxicated with acetaminophen presented centrilobular necrosis and cytoplasmic vacuolation. Rats pretreated with 200 mg/kg of *P. kerstingii* extract and intoxicated with acetaminophen showed mild degenerative lesions of the hepatocytes, whereas rats pretreated with 400 mg/kg of *P. kerstingii* extract and intoxicated with acetaminophen expressed normal liver histoarchitecture with central vein. The group E rats, which were pretreated with 600 mg/kg of *P. kerstingii* extract and intoxicated with acetaminophen, showed mild degeneration and hyperaemia, while the rats pretreated with 100 mg/kg of Silymarin and intoxicated with acetaminophen presented normal histoarchitecture and hepatic bile duct (Fig. 1).

GC-MS Chromatogram of *P. kerstingii* methanol stem bark extract

The GC-MS chromatogram of the *P. kerstingii* methanol stem bark extract showed the presence of various compounds as indicated by peaks (Fig. 2). Each peak indicates a compound eluting at a particular time (retention time). The compounds are present in various amounts, as shown by their relative abundance or percentage area. The compound (Tetratriacontyl heptafluorobutyrate) that eluted at 48.328 mins has the highest relative abundance of 6.0 x 10^6.

Compounds in the methanol stem bark extract of *P. kerstingii*

The results show compounds present in the methanol stem bark extract of *P. kerstingii* with their retention times and percentage areas (which indicates the relative abundance). The table shows over 70 compounds, with about 16 having a percentage area above one (Table 2).

Most abundant compounds in the methanol stem bark extract of *P. kerstingi*i

The results are shown in Table 3. Tetratriacontyl heptafluorobutyrate, Tetratriacontyl pentafluoropropionate and Dotriacontylpentafluoropropionate are the most abundant with percentage area of 24.01 (Table 3). 1-Hexacosene. Tetradecanaland 13-Methyltetradecanal show a percentage area of 20.93 and are the second most abundant. Octatriacontyl pentafluoropropionate, Octatriacontyl trifluoroacetate, 2- Chloropropionic acid, octadecyl ester, Hexatriacontyl pentafluoropropionate, Butylated Hydroxytoluene are all found to be abundantly

Table 1 — The liver damage marker parameters of rats pretreated with methanol stem bark extract of *P. kerstingii* and intoxicated with acetaminophen

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total bilirubin (mg/dL)
A	26.35±0.51^{a}	43.33±0.33^{a}	100.35±0.91^{a}	0.91±0.00^{b}
B	45.78±0.64^{c}	82.00±0.57^{e}	199.15±0.62^{d}	1.11±0.01^{d}
C	30.35±1.25^{b}	50.00±1.00^{c}	123.96±2.03^{c}	1.01±0.02^{c}
D	26.90±0.47^{a}	48.00±0.00^{b}	108.81±4.24^{b}	0.85±0.03^{a}
E	31.06±1.04^{b}	54.66±0.67^{d}	96.53±2.84^{a}	1.03±0.01^{c}
F	27.78±0.63^{a}	47.66±0.33^{b}	98.81±0.42^{a}	0.87±0.03^{ab}

The different letter superscripts, a, b, c, d and e, across the groups (in the same column) indicate significant differences at P <0.05.

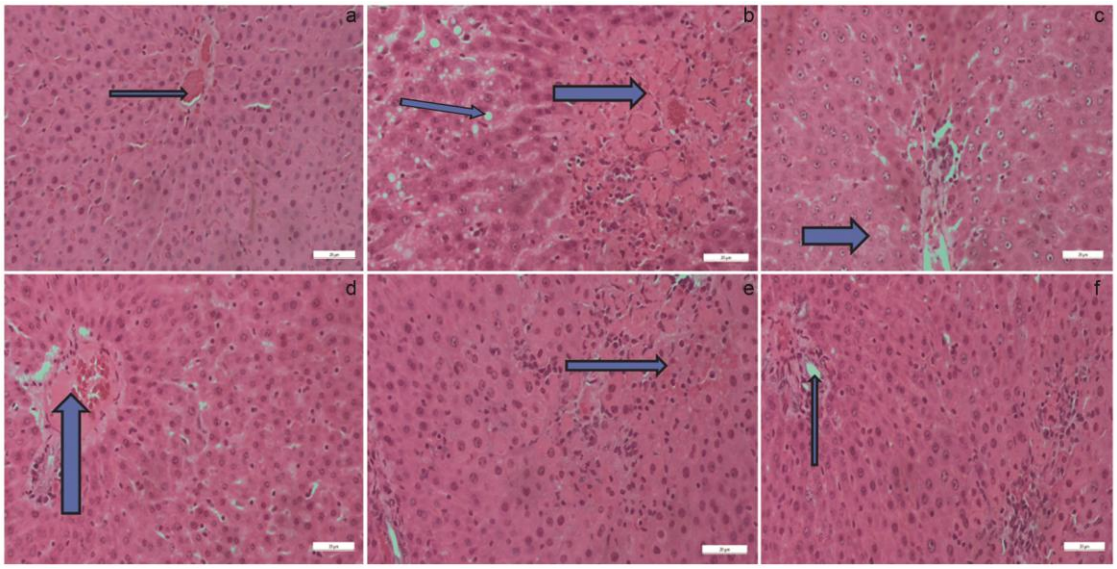

Fig. 1 — The liver histophotomicrograph (H&E x 400). a) The hepatic histomorphology of normal rats with the central vein (arrow) and the hepatocytes arranged in cords; b) The liver of rats intoxicated with acetaminophen with centrilobular necrosis (large blue arrow) and cytoplasmic vacuolation (narrow sky-blue arrow); c) rats pretreated with 200 mg/kg of *P. kerstingii* extract and intoxicated with acetaminophen showing mild degenerative lesions of the hepatocytes (arrow); d) rats pretreated with 400 mg/kg of *P. kerstingii* extract and intoxicated with acetaminophen showing normal liver histoarchitecture with central vein that has red blood cells inside (arrow); e) rats pretreated with 600 mg/kg of *P. kerstingii* extract and intoxicated with acetaminophen showing mild degeneration and hyperaemia (arrow); and f) rats pretreated with 100 mg/kg of Silymarin and intoxicated with acetaminophen showing normal histoarchitecture and hepatic bile duct (arrow).

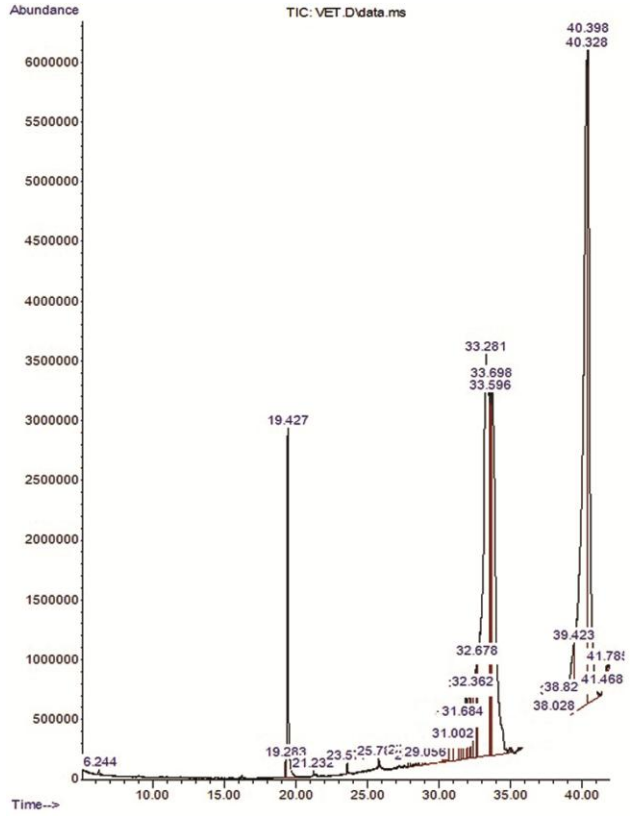

Fig. 2 — GC-MS Chromatogram of *P. kerstingii* methanol stem bark extract.

present in the methanol stem bark extract of *P. kerstingii*.

The binding energies (kcal/mol) between CYP2E1 and the various ligands

The results of the binding energies show that Butylated hydroxytoluene had the least binding energies ranging from -6.7 to -5.8 followed by indole (-5.1 to -4.5) and lastly by 4-methylpyrazole which recorded binding energies that ranged between -3.8 to -3.2 (Table 4).

Active site view of CYP2E1 (3gph) amino acid residues interacting with ligands; A: Butylated hydroxytoluene, B: Indole, C: 4-methyl pyrazole

The results of Fig. 3 showed that butylated hydroxytoluene made van der Waals interactions and alkyl bond associations mainly with the amino acid residues of CYP2E1 enzymes, while both the indole and 4-methyl pyrazole ligands, which are standard drugs, contacted the active site amino acid residues via hydrogen bonds in additions to other few interactions such as van der Waals, alkyl, pi-sigma and carbon-hydrogen bonds. Indole contacted LYS486 via a hydrogen bond, while 4-methyl pyrazole approached the enzyme through a hydrogen bond with MET200, GLU302, and ASN204 (Fig. 3).

Table 2 — Compounds present in the methanol stem bark extract of *P. kerstingii* following Gas Chromatography-Mass Spectrometry analysis

S.No.	Retention time	% Area	Compound name
1	6.244	0.05	Trichloromethane
2	19.427	3.34	Butylated Hydroxytoluene
3	21.232	0.04	9-Octadecene, (E)-
4	23.577	0.07	Dodecanoic acid, propyl ester
5	25.784	0.06	Trichloroacetic acid, tetradecyl ester
6	27.901	0.11	Tetradecanoic acid, propyl ester
7	28.401	0.06	Tricosyl heptafluorobutyrate
8	28.658	0.14	Hexadecanoic acid, methyl ester
9	28.931	0.08	Hexacosyl propyl ether
10	29.056	0.03	Octatriacontyl pentafluoropropionate
11	29.476	0.51	Diethyl Phthalate
12	29.906	1.28	Tetratriacontyl heptafluorobutyrate
13	30.218	0.09	Dotriacontylpentafluoropropionate
14	30.282	0.11	1-Hexacosene
15	30.395	0.08	Octacosyl trifluoroacetate
16	30.432	0.08	Tetracosyl heptafluorobutyrate
17	30.571	0.07	Hexacosyl heptafluorobutyrate
18	30.632	0.08	Tetratriacontyl heptafluorobutyrate
19	30.666	0.10	Dotriacontyl heptafluorobutyrate
20	31.002	0.55	Hexatriacontyl pentafluoropropionate
21	31.380	0.99	1-Hexadecanol, 2-methyl-
22	31.492	0.99	Octadecane, 1-iodo-
23	31.684	0.77	2-Piperidinone, N-[4-bromo-n-butyl
24	31.856	1.07	Propyl triacontyl ether
25	31.928	0.48	Silane, trichlorooctadecyl-
26	32.053	0.48	Tetrapentacontane, 1,54-dibromo-
27	32.166	0.38	Nonadecane
28	32.362	1.12	2- Chloropropionic acid, octadecyl ester
29	32.594	2.00	5-Methyl-Z-5-docosene
30	32.678	0.77	Silane, trichlorooctadecyl-
31	33.281	20.93	13-Methyltetradecanal
32	33.525	2.02	Octacosyl trifluoroacetate
33	33.576	1.19	Tetratriacontyl heptafluorobutyrate
34	33.596	1.99	Dotriacontyl heptafluorobutyrate
35	33.698	13.47	2- Chloropropionic acid, octadecyl ester
36	34.910	0.04	Octacosyl trifluoroacetate
37	35.003	0.07	1-Hentetracontanol
38	35.531	0.07	17-Pentatriacontene
39	35.888	0.02	Triacontyl pentafluoropropionate
40	36.035	0.04	Tetratriacontyl trifluoroacetate
41	36.162	0.02	Hexadecane, 1-chloro-
42	36.233	0.01	Triacontyl heptafluorobutyrate
43	36.423	0.15	Tetracosyl heptafluorobutyrate
44	36.630	0.03	Dotriacontyl heptafluorobutyrate
45	36.727	0.01	Tetratriacontyl pentafluoropropionate
46	36.870	0.04	Hexatriacontylpentafluoropropionate
47	36.954	0.04	Octacosyl trifluoroacetate
48	37.009	0.04	Dotriacontyl heptafluorobutyrate
49	37.081	0.04	Silane, trichlorooctadecyl-
50	37.208	0.04	Octatriacontyl trifluoroacetate
51	37.305	0.05	Octacosyl trifluoroacetate

(*Contd*.)

Table 2 — Compounds present in the methanol stem bark extract of *P. kerstingii* following Gas Chromatography-Mass Spectrometry analysis (*Contd*.)

S.No.	Retention time	% Area	Compound name
52	37.349	0.02	Hexatriacontyl pentafluoropropionate
53	37.516	0.11	Tetratriacontyl trifluoroacetate
54	37.582	0.08	Dotriacontyl heptafluorobutyrate
55	37.864	0.36	Octatriacontyl pentafluoropropionate
56	37.959	0.04	Hexatriacontyl pentafluoropropionate
57	38.000	0.06	Dotriacontyl trifluoroacetate
58	38.028	0.07	Dotriacontyl heptafluorobutyrate
59	38.396	0.74	Heptacosane
60	38.462	0.09	Dodecane, 2,6,11-trimethyl
61	38.490	0.05	Hexadecane, 1-iodo-
62	38.530	0.27	Tetrapentacontane, 1,54-dibromo
63	38.829	0.51	Tetratriacontyl trifluoroacetate
64	39.423	2.56	Hexatriacontyl pentafluoropropionate
65	40.328	24.01	Tetratriacontyl heptafluorobutyrate
66	40.398	14.41	Octatriacontyl pentafluoropropionate
67	41.351	0.00	Heptacosane
68	41.403	0.01	Tetratriacontyl pentafluoropropionate
69	41.468	0.01	1-Hexacosanol
70	41.736	0.14	Hentriacontane
71	41.785	0.06	Silane, trichlorooctadecyl-

Table 3 — Most abundant compounds of methanol stem bark extract of *P. kerstingii* following Gas Chromatography-Mass Spectrometry analysis

S.No.	Retention time	Area (%)	Name of Compound	Mol. Formula	Mol. Weight (g/mol)
1	19.427	3.34	Butylated Hydroxytoluene	$C_{15}H_{24}O$	220.35
3			Tetratriacontylpentafluoropropionate	$C_{38}H_{69}F_7O$	690.9421
4			Octatriacontylpentafluoropropionate	$C_{41}H_{77}F_5O_2$	697.041
5	33.698	13.43	2- Chloropropionic acid, octadecyl ester	$C_{21}H_{41}ClO$	361.002
6			Hexatriacontylpentafluoropropionate	$C_{39}H_{73}F_5O_2$	668.9877
7	33.281	20.93	1-Hexacosene	$C_{26}H_{52}$	364.6911
8			Tetradecanal	$C_{14}H_{28}O$	212.377
9			13-Methyltetradecanal	$C_{15}H_{30}O$	226.398
10	40.328	24.01	Dotriacontylpentafluoropropionate	$C_{35}H_{65}F_5O_2$	612.8814
11	40.398	14.41	Octatriacontyl trifluoroacetate	$C_{40}H_{77}F_3O_2$	646.587585

Table 4 — The binding energies (kcal/mol) between CYP2E1 and the various ligands

Conformations	Butylated hydroxytoluene	Indole	4-methylpyrazole
One	-6.7	-5.1	-3.8
Two	-6.7	-5.0	-3.7
Three	-6.7	-5.0	-3.4
Four	-6.1	-4.9	-3.4
Five	-5.8	-4.9	-3.4
Six	-5.8	-4.6	-3.3
Seven	-5.8	-4.5	-3.3
Eight	-5.8	-4.5	-3.3
Nine	-5.8	-4.5	-3.2

Discussion

The absence of signs of toxicity observed in the acute toxicity tests indicate that the extract is safe at the tested dose. Natural products such as plant extracts are known to have a wider safety margin compared to their synthetic counterpart. Researchers in their previous studies reported relative safety of the aqueous extract of *P. kerstingii* after 14 days[10].

The increases in the serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the group B rats (administered with only acetaminophen) are indications of liver damage. Acetaminophen is an over-the-counter analgesic drug that has been incriminated in causing injury to the liver when abused or taken in overdose[20]. The destruction or the degeneration and necrosis of the hepatocytes by the overdose of acetaminophen is occasioned by the metabolism of the drug into N-acetyl-p-benzoquinone imine (NAPQI), a toxic metabolite fingered as the main cause of hepatic injury[21]. The rats that were

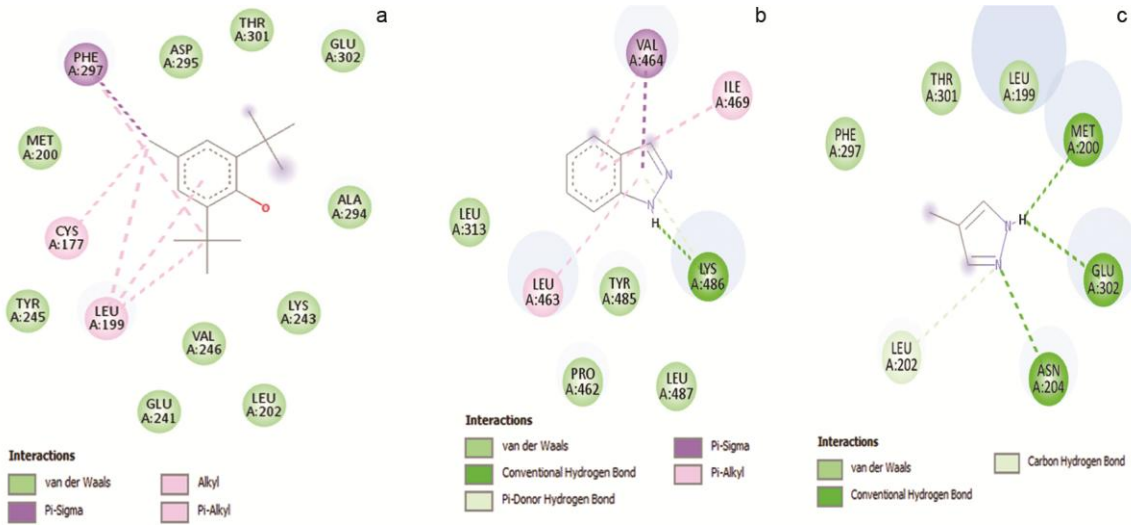

Fig. 3 — Active site view of CYP2E1 (3gph) amino acid residues interacting with ligands, a) Butylated hydroxytoluene; b) Indole; and c) 4-methyl pyrazole.

treated with the extract at varying doses showed lower serum enzyme activity when compared with those of group B rats. Their ALT, AST and ALP activities were comparable with that of the normal control rats, especially at the dose of 400 mg/kg. The implication of the results is that the extract ameliorated hepatocyte injuries in those rats that received them as pretreatment. The ALT and AST are enzymes that catalyze transamination reactions during amino acid metabolism. They are ubiquitously present in the liver. Injuries to the liver cells lead to their elaboration into the general circulation and thus act as markers of hepatocellular injury[5].

On the other hand, alkaline phosphatase is a marker of hepatobiliary injury. The enzymes are present in the biliary epithelium and are expressed when there is an injury to the hepatobiliary system[6]. Bilirubin is a byproduct of red blood cell metabolism, particularly the catabolism of haemoglobin. They are transported by albumin to the liver for their metabolism. Increases in the serum levels of unconjugated bilirubin are indications of liver damage since the liver is responsible for conjugating bilirubin into bilirubin diglucuronide[22]. In the present study, the total bilirubin values of rats in group B were significantly higher than those of the other groups, while that of the normal control rats (group A) compares very well with that of the group F rats.

The liver histopathology results showed massive degeneration and necrosis of the hepatocytes of the rats that received only acetaminophen (group B). Some researchers had earlier shown that an overdose of acetaminophen can cause death of hepatocytes[23]. The histoarchitecture of the rats treated with the extract compares well with that of the normal control rats, especially the group D (Fig. 1).

The results of gas chromatography-mass spectrometry are presented in Fig. 2, Tables 2 and 3. Gas chromatography hyphenated to mass spectrometry is capable of separating and identifying mixtures of compounds such as plant extracts[24]. In the present study, the *P. kerstingii* extract was subjected to GC-MS analysis and the different compounds with their retention times and percentage areas were obtained (Fig. 2, Tables 2 and 3). Several researchers have used GC-MS techniques to obtain various compounds in the past [25].

A literature search of biological activities on the most abundant compounds revealed that butylated hydroxy toluene has antioxidant activities[26], a property that is closer to *in vivo* hepatoprotective activity shown by the extract. Researchers have also reported the hepatoprotective properties of antioxidants[27]. Oxidative stress and production of free radicals have also been incriminated in the pathogenesis of liver injury[28]. This prompted *an in silico* investigation of its possible hepatoprotective effects. The binding energies of butylated hydroxytoluene (a compound from the *P. kerstingii* methanol stem bark extract), indole and 4-methylpyrazole (standard drugs), and the target enzyme (CYP2E1) are -6.7, -5.1 and -3.8 kcal/mol respectively. Molecular docking of a receptor or protein and its ligand usually gives the best-fit orientation of the ligand in the active site of the

protein[12]. This complex is represented as binding energies that help estimate the possible biological efficacy that will be elicited. The lower the binding energy, the more efficacy the compound has in suppressing the enzyme[29]. The binding energies obtained from the present study indicated that the butylated hydroxytoluene has a better interaction compared to the two standard drugs.

The active site view of CYP2E1 shows the interactions between the ligands and the CYP2E1 enzyme. The results showed that the butylated hydroxytoluene interacted with the active site amino acid of CYP2E1 via van der Waals forces, pi-sigma, pi-alkyl and alkyl bonds, while indole, in addition to the above forces, also interacted with the enzymes' amino acid residues via hydrogen bond made between LYS 486 and hydrogen atom of indole. The 4-methyl pyrazole, the second standard drug, also contacted the active site via a hydrogen bond made with ASN 204, GLU 302 and MET 200. Hydrogen bonds are formed between the electronegative elements such as nitrogen, oxygen and hydrogen atoms. Hydrogen bonds are crucial in bonding at the active site as they position ligands for maximum activity[30].

Conclusion

The results of both *in vivo* and *in silico* hepatoprotective abilities of the methanol stem bark extract of *P. kerstingii* indicated that the extract possesses *in vivo* hepatoprotective potentials, which were corroborated by the results of molecular docking between CYP2E1 and butylated hydroxytoluene, one of the abundant compounds found in the extract via the instrumentality of GC-MS.

Ethical approval

This study was approved by the Faculty of Veterinary Medicine, University of Nigeria Nsukka Institutional Animal Care and Use Committee (IACUC) with the approval number: FVM-UNN-IACUC-2023-09/116a.

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Conflict of interest

Authors declare no conflict of interest.

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