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Phytochemical Composition of *Alstonia boonei* De Wild Stem Bark and Its *In Vivo* Acute Toxicity Index in Adult Male Mice

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ABSTRACT: *Alstonia boonei* De Wild has shown promising ethnopharmacological relevance in trado-medical settings. However, its safety like other herbs remains a source of concern. Consequently, the objective of this paper was to investigate the phytochemical composition of *Alstonia boonei* De Wild Stem bark and its *in vivo* acute toxicity index in Adult male mice using appropriate standard procedures. Results indicated no visible signs of toxicity, death or significant alterations in haematological parameters and liver architecture in treated mice after 24 h and up to 14 days of observation. The oral lethal dose (LD_{50}) is greater than 1600 mg/kg body weight (bw). Forty compounds comprising hydrocarbons, fatty acids, alcohols, steroids, nitrogen-containing compounds, terpenes and esters were identified. This suggests that the crude extract of *A. boonei* De Wild stem bark is safe for oral administration at doses not exceeding 1600 mg/kg bw and contains rich phytochemical constituents with potent pharmacologic effects.

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With increasing levels of poverty and decline in purchasing power of average house-holds in low to middle income countries like Nigeria (NBS, 2025; WBR, 2025), access to medicare remains a challenge. Worse still, the dilapidated health system, increased burden of diseases, illiteracy, poor hygiene, poor access to orthodox medication, etc., have further compounded the already moribund health care delivery system. In the light of this, many people have embraced traditional medicine as an alternative to the conventional orthodox medication for their health needs (WHO, 2013). This system relies heavily on natural options such as herbs for the management and treatment of diseases. However, one major challenge associated with herbal products is their safety for human consumption. The chemical compositions of these herbs which are responsible for the observed biological activities, can also influence their safety index. Hence, toxicity studies and chemical profiling are essential in determining the safety or otherwise of herbal remedies in line with the guidelines from stake holders like the Food and Drug Administration- FAD, whose guidelines stipulates a comprehensive toxicological profile of herbal products based on frequency and duration of usage (FDA, 2016).

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Alstonia boonei De Wild is a medicinal plant that is native to Africa, the Americas and Asia. The large deciduous tree is an Apocynaceae family member with over forty species that include *A. macrophylla, A. congensis, A. scholaris*, *A. longifolia,* etc., (Orumwensodia and Uadia, 2023). Some of its common names are stoolwood, cheesewood and patternwood (Palla, 2008). Locally, it is called Awun in Yoruba, Egbe, Egun, Egbu-ora in Igbo, Ukpukunu in Urhobo, Egbu in Ijaw and Ukhu, Uhu, Ogiegbukhu in Edo (Aigbokhan, 2014). Okoye *et al*. (2021) reported that several parts of *A. boonei* including the root, stem bark, leaves, and stem bark latex have been used for healing purposes, however, the frequency of use suggests that the thick stem bark of the matured plant is most commonly used. For instance, the stem bark is claimed to possess analgesic, antipyretic, insecticidal anti-inflammatory and antidiabetic properties (Olajide *et al*., 2000; Osadebe, 2002; Oigiangbe *et al*., 2007; Ashafa, 2015; Klue *et al*., 2016). Orumwensodia and Uadia, (2023) found that partially purified stem bark extract and fractions possessed antimalarial, antioxidant activities and a good nutritional content. However, despite the inherent gains ascribed to herbal products, the criticism over usage fueled by suspicion of toxicity has remained and necessitates toxicity determination. Therefore, the objective of this paper was to investigate the phytochemical composition of *Alstonia boonei* De Wild Stem bark and its *in vivo* acute toxicity index in Adult male mice.

MATERIALS AND METHODS

Chemicals: All solvents (petroleum ether and ethanol) were of analytical grade, products of Sigma-Aldrich, Germany, and purchased from accredited dealers.

Plant material: The Stem bark of *A. boonei* was obtained from its trees in the forest area of Ogun State, Southwest Nigeria within the month of November. The plant material was assigned voucher No. UBHa210 after authentication in the Department of Plant Biology and Biotechnology, University of Benin, where herbarium sample was deposited. The samples were washed to remove earthy materials, air dried under shade and pulverized (< 1 mm) to obtain the crude powdered sample. The pulverized samples were stored in air-tight containers.

Extraction and fractionation: Pulverized stem bark sample of *A. boonei* (1200 g) was subjected to successive maceration under repeated stirring (4 days × 3) (Cannell, 2006) using 70% ethanol/water (3 L: v/v) at room temperature to obtain the hydro-ethanol extract. Portions (18 g) of the extract was defatted in petroleum ether to obtain the oily petroleum ether fraction. This soluble fraction was evaporated with a rotavapor (Buchi, Germany) at 45 °C under reduced pressure (20–200 mbar).

Animal handling: Adult male MRL mice weighing 20 ± 2 g were used for this study and housed in disinfected plastic cages in a temperature-controlled room of 22 to 25ºC, relative humidity of 44 to 56%, and maintained under a 12 h light-dark photoperiod. They were fed *ad libitum* standard pellet diets and water, and handled in accordance with the Institute for Laboratory Animal Research (ILAR, 2011). A one-week period of acclimatization was allowed before commencement of treatment.

Toxicity study: The study was carried out according to the modified method of Lorke (1983). In phase I of the study, nine adult male mice were divided into three groups (I, II and III) of three animals each. The crude *A. boonei* stem bark extract was administered orally as a one-time dose through an appropriate gastric cannula at doses of 10, 100, and 1000 mg/kg bw to groups I, II, and III, respectively. The animals were individually observed for the first 30 min after administration with special attention given during the first 4 h and periodically during the next 24 h. General symptoms of toxicity and mortality within 24 h were observed. Observations included monitoring changes in eyes, skin, nose, mucous membranes, and in the behavioral, circulatory, respiratory, nervous and somatic motor systems.

Notice was taken of any convulsions, tremors, coma, salivation, diarrhea and/or exhaustion. Subsequently, another nine mice were divided into three groups of three animals each, marking phase II of the study. The extract was administered at doses of 400, 800, and 1600 mg/kg bw. Again, general symptoms of toxicity (as earlier mentioned) and mortality in each group were observed within 24 h and for the next 14 days. The mortality ration was estimated using equation (1). Thereafter, haematology and histology were performed on the blood and liver samples, respectively, at the end of the experiment. The LD_{50} was determined according to equation (2).

$$Mortality = \frac{Number\ of\ death}{Number\ of\ survival} \quad (1)$$

$$LD_{50} = \frac{\sqrt{D_0 * D_{100}}}{2} \quad (2)$$

Where D_0 = Highest dose that gave no mortality, D_{100} = Lowest dose that produced mortality

Collection of samples: Blood samples were collected in EDTA tubes from overnight fasted mice after they were euthanized using an intraperitoneal injection of a cocktail of anesthesia (xylazine, ketamine and normal saline) at a dose of 10 mg/kg bw (Flecknell, 2015). Standard hematological protocol was employed for the measurement of blood parameters. The liver was excised and weighed portions processed, and observed under photomicroscope for histopathological changes.

Haematological Analysis: A known volume (1 mL) of the blood samples was aspirated and diluted with isotonic saline solution in the human auto haematology analyzer (Sysmex XP100, Sysmex Corporation, Singapore). The following blood parameters were analyzed; haemoglobin concentration (HGB), red blood cells count (RBCs), haematocrit (HCT), white blood cells count (WBCs), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Histological Procedure: The liver was excised immediately from the euthanized mice and fixed in 10% formaldehyde for gross necropsy, and histopathological examinations. After about 72 h, the liver was transferred into an automatic processor where it went through a process of dehydration in ascending grades of alcohol (ethanol) 70%, 80%, 95% and absolute alcohol for two changes each. It was then cleared in xylene and embedded in paraffin wax and about 5 μm thick sections were cut on a rotary microtome. The liver tissue was deparaffinized, hydrated and stained using hematoxylin-eosin staining (Drury, 1980). Thereafter, slides of tissue sections were examined under microscope and photomicrographs of the tissues were generated for further analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis: The GC-MS analysis of the petroleum ether fraction (viscous oil) of *A. boonei* De Wild stem bark was performed in a Gas Chromatography-Triple Quadrupole Mass Spectroscopy instrument equipped with Agilent USB39375HHP-5MS column and capillary dimensions 30 m x 250 µm x 0.25 µm using helium as the carrier gas at a flow rate of 1.2 mL/min.

The pressure was maintained at 10.97 psi, while the injection volume was 1 µL. The oven was equilibrated for 30 min and temperature was pre-set at 70 ^{o}C for 5 min, then 10 ^{o}C/min to 180 ^{o}C for 5 min, then 10 ^{o}C/min to 280 ^{o}C for 10 min, thereafter, 5 ^{o}C/min to 290 ^{o}C for 30 min. The MS transfer line was sustained at a temperature of 325 ^{o}C, and the total run time was 73 min. Electron ionization at 70 eV was used for ionization and at a source temperature of 250 ^{o}C. Compound identification was by total ion count (TIC) at start mass of 20 amu and end mass of 650 amu for scan time of 200 min. The Spectra of the separated compounds were compared with the database of the National Institute of Standards and Technology (NIST) Reference Spectra Library using Automated mass spectral deconvolution and identification software (AMDIS V 2.69). Match factor (MF) ≥ 700 was taken as satisfactory.

Data analysis: Data were expressed as mean ± SME. The data were subjected to one-way analysis of variance (ANOVA), where applicable, and differences between means were determined by Duncan's multiple range test, using the Statistical Analysis System (SPSS Statistics 20.0). Bars were plotted using GraphPad Prism v10.0. Significance was set at p values ≤ 0.05.

RESULTS AND DISCUSSION

The safety of herbal medicine remains a major concern owing to issues around toxicity, dosage, administration, etc. Our previous study (Orumwensodia and Uadia, 2023) on *A. boonei*, found that its stem bark crude extract and partially purified fractions had good nutritional composition, and possessed antioxidant as well as antimalarial properties. This study therefore, examined the acute toxicity profile of *A. boonei* stem bark extract in Murphy Roths Large (MRL) adult mice and also, characterised the chemical composition of its oily petroleum ether fraction.

Acute Toxicity of Alstonia boonei Crude Extract: Oral administration of *A. boonei* stem bark extract at doses up to 1600 mg/kg bw did not result in any mortality in treated mice (Table 1) for the 14-day period of observation. There were no observable signs of toxicity during the experimental period.

The acute toxicity study revealed that the plant extract falls under category 5 of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), indicating a low-level toxicity, with LD_{50} greater than 1600 mg/kg bw (GHS, 2021a). At this maximum dose, there was no mortality in MRL mice for the first 24 hours and up to 14 days of observation. Also, there were no visible signs of toxicity, as no significant changes in breathing, central nervous systems, sense of touch or sound, behaviour pattern and locomotor activity were observed. Furthermore, convulsion, tremor, excessive salivation, diarrhea and edema were not noticed, suggesting that it is relatively safe for use

Table 1: Acute Toxicity Profile of Crude Extract of *Alstonia boonei* Stem bark in MRL Mice

PHASE I				
Dose (mg/kg bw)	**No. of Mice**	**No. of deaths**	**No. of survival**	**Mortality Ratio**
10	3	0	3	0/3
100	3	0	3	0/3
1000	3	0	3	0/3
PHASE II				
400	3	0	3	0/3
800	3	0	3	0/3
1600	3	0	3	0/3

Data are number of death and survival of mice. No. of deaths recorded = Nil.; No. of survival = All. Oral LD_{50} > 1600 mg/kg bw.

Effect of Alstonia boonei Crude Extract on Haematology: The results of haematology (Figs. 1 – 7) conducted post-administration of *A. boonei* stem bark extract revealed no significant ($p > 0.05$) changes in the values of HGB, RBCs, HCT, MCV, MCH, MCHC, WBCs and PLT in the treated groups compared with the control mice. Although, there were slight decreases in the levels of HGB in the group administered 400 mg/kg bw, as well as RBCs and HCT in the group treated with 800 mg/kg bw. Also, WBCs increased non-significantly ($p > 0.05$) in the group administered 400 mg/kg bw, while PLT non-significantly ($p > 0.05$) increased in all the treated groups. The blood is a connective tissue which serves as the body's logistic hub and contains several haematological components such as erythrocytes, leukocytes, platelets, etc., that are likely to be perturbed during disease outbreak or adverse conditions, including toxicity. Thus, making these components important markers of physiological and pathological conditions (Alexy *et al*., 2022; Ali *et al*., 2025; Shah *et al*., 2025), hence hematological investigation is necessary in toxicity studies. The results of haematology obtained, revealed mild perturbations in the values of some of the parameters determined when compared with the control group. For RBCs and HCT, the group administered 800 mg/kg body weight had a non-significant ($p > 0.05$) reduction compared to the control. Similarly, there was a non-significant ($p > 0.05$) reduction in haemoglobin levels in the group administered 400 mg/kg body weight. Red blood cell indices such as MCV, MCH and MCHC, which are estimates and used to mathematically define the concentration of HGB within the cell also varied accordingly. However, all values were within normal range of these parameters as proposed by (Stahl *et al*., 2018). This suggests possible erythropoietic potentials for the plant extract coupled with an enhanced blood function. Other researchers have shown that some herbs could be used as hematinic agents thereby protecting against anemic conditions (Adias *et al*., 2013; Okochi *et al*., 2013; Sree *et al*., 2021). The presence of phytochemicals in *A. boonei* with antioxidant property (Orumwensodia and Uadia, 2023) could play a role in red cell membrane stabilization and protection against anemic conditions in treated mice. Furthermore, the values of WBCs in the treated groups were non-significantly ($p > 0.05$) altered by different doses of the plant extracts. On the other hand, PLT count significantly ($p < 0.05$) increased in all groups when compared with the control group. This slight variations in the WBCs and PLT counts suggest a possible modulatory effect linked to an inflammatory response which could be adversarial in the long run, hence, an extended toxicity study is required. Similar results were obtained for the plant by (Gabriel *et al*., 2007; Olanlokun and Olorunsogo, 2018), who noticed a beneficial effect of *A. boonei* after testing them in experimental rats. However, Olanlokun and Olorunsogo (2018) advised its usage with caution.

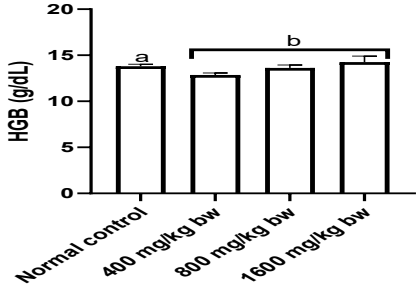


Fig 1: effect of *A. boonei* crude stem bark extract on haemoglobin concentration. a = non-significantly different compared to the test groups. b = non-significantly different compared to the control group.

The result of histopathological examination showed no deleterious damage to the liver architecture, except for mild vascular congestion in the groups administered 800 and 1600 mg/kg body weight. This again reinforces the need for an extended toxicity study and cautious use of the plant in ethno-medicinal practice, especially at high dosage. The findings from our toxicity study support the continued development of *A. boonei* stem bark extract as a potential herbal remedy.

Effect of Alstonia boonei Crude Extract on Histology of the Liver: Normal hepatocytes architecture, sinusoids, bile ducts and portal tracts were observed in the histopathological section of the liver in the control group at x40 magnification. Also, the *A. boonei* stem

bark-treated mice showed normal lobular architecture, central veins and sinusoids. Meanwhile, aside mild vascular congestion noticed at doses of 800 and 1600 mg/kg, there were no adverse signs of toxicity such as inflammation, fatty change or fibrosis observed (Plate 1 – 4).

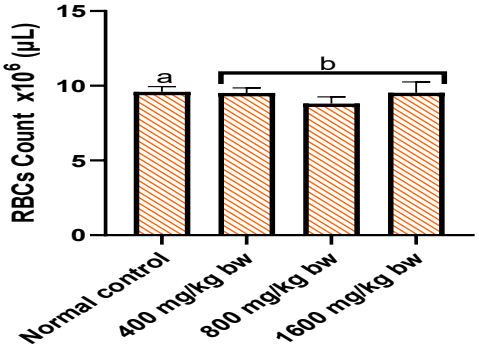


Fig 2: effect of *A. boonei* crude stem bark extract on red blood cells count. a = non-significantly different compared to the test groups. b = non-significantly different compared to the control group.

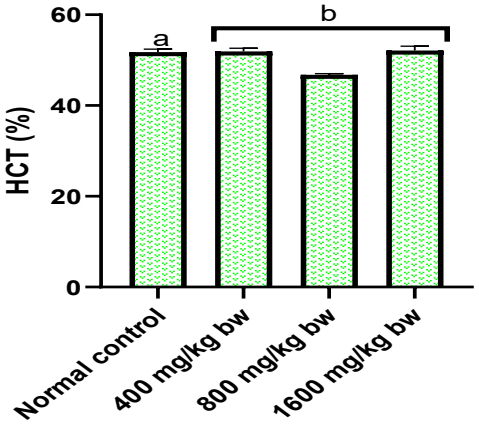


Fig 3: effect of *A. boonei* crude stem bark extract on haematocrit concentration. a = non-significantly different compared to the test groups. b = non-significantly different compared to the control group.

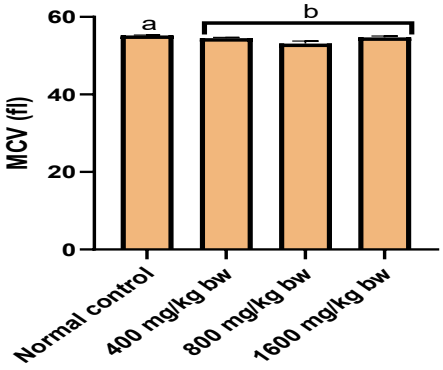


Fig 4: effect of *A. boonei* crude stem bark extract on mean corpuscular volume. a = non-significantly different compared to the test groups. b = non-significantly different compared to the control group.

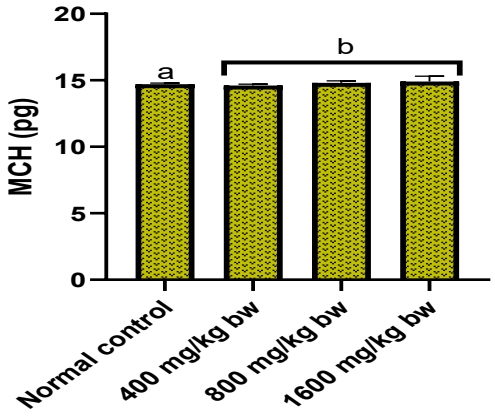


Fig 5: effect of *A. boonei* crude stem bark extract on mean corpuscular haemoglobin. a = non-significantly different compared to the test groups. b = non-significantly different compared to the control group.

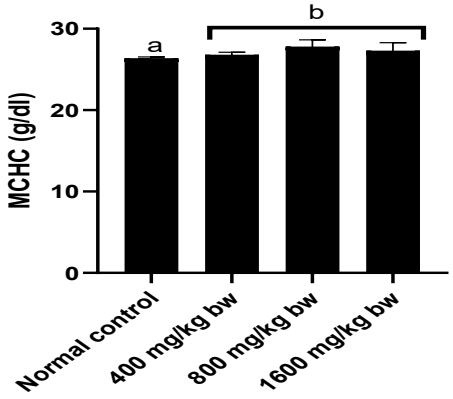


Fig 6: effect of *A. boonei* crude stem bark extract on mean corpuscular haemoglobin count. a = non-significantly different compared to the test groups. b = non-significantly different compared to the control group.

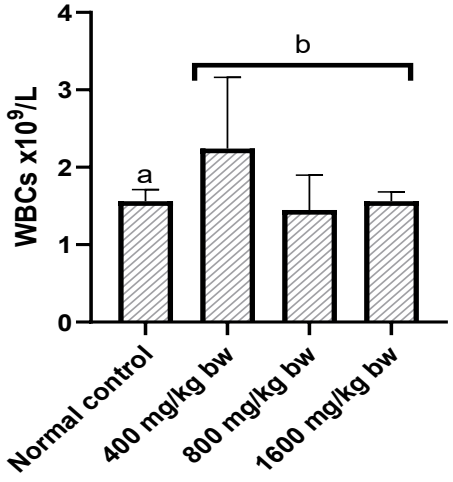


Fig 7: effect of *A. boonei* crude stem bark extract on white blood cells count. a = non-significantly different compared to the test groups. b = non-significantly different compared to the control group.

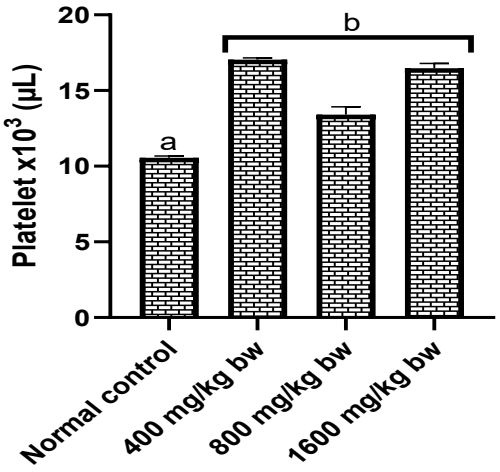


Fig 7: effect of *A. boonei* crude stem bark extract on platelet concentration. a = non-significantly different compared to the test groups. b = non-significantly different compared to the control group.

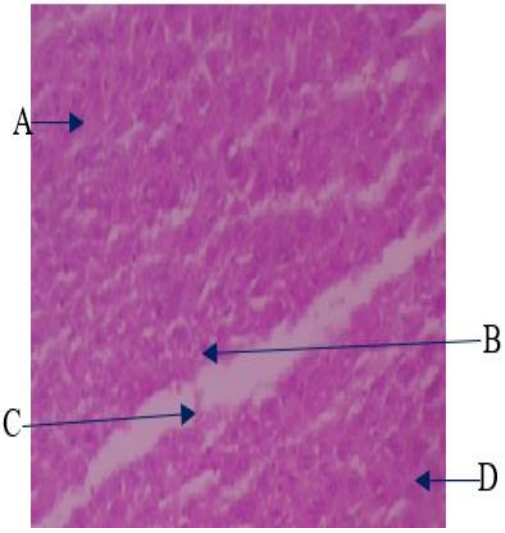


Plate 1: Section of mouse liver from the untreated control group administered normal saline only (H&E x 40).
Key: A: hepatocytes, B: sinusoids, C: portal vein, D: bile duct.

Chemical Composition of Alstonia boonei Stem bark oil: The GC-MS chromatograms in Fig. 8 revealed forty (40) peaks matching the chemical finger print of compounds in the classes of hydrocarbons, fatty acids, alcohols, steroids, nitrogen-containing compounds, terpenes and esters. Their molecular formula, molecular weight, retention time, and reverse match factor are contained in Table 2, while their structures are presented in Figs 9. The nitrogen containing compound, 3-ethylpyridine, eluted the fastest with retention time of 4.16 min and a molecular weight of 107, while stigmastane-3,6-dione, (5α)- had the longest retention time of 62.83 min with a molecular weight of 428. The GC-MS profile of the oily petroleum ether fraction of *A. boonei* stem bark showed a rich fingerprint of diverse phytochemicals. Hydrocarbons, fatty acids, alcohols, steroids, nitrogen-containing compounds, terpenes and esters, totaling forty compounds, were characterized.

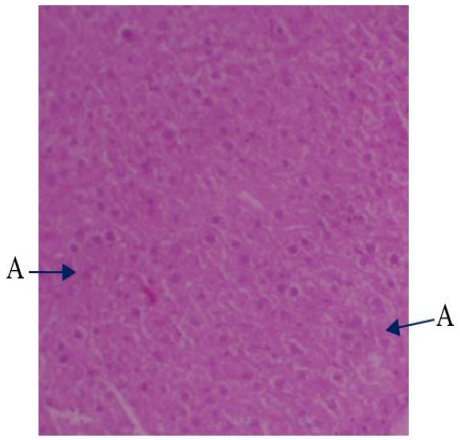


Plate 2: Section of mouse liver from Group II administered 400 mg/kg bw (H&E x 40).
Key: A: hepatocytes.

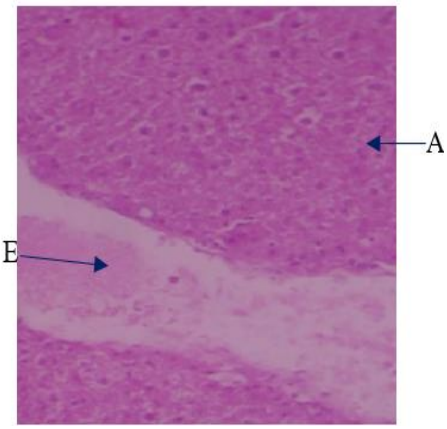


Plate 3: Section of mouse liver from Group III administered 800 mg/kg bw (H&E x 40).
Key: A: hepatocytes, E: mild vascular congestion.

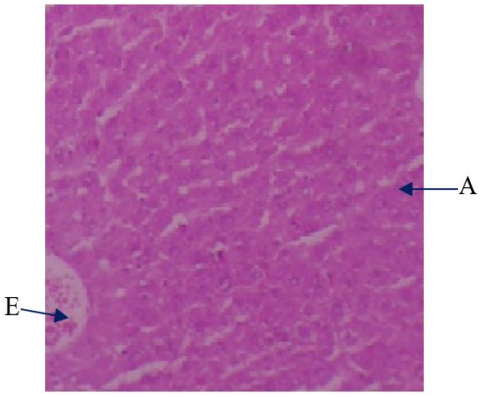


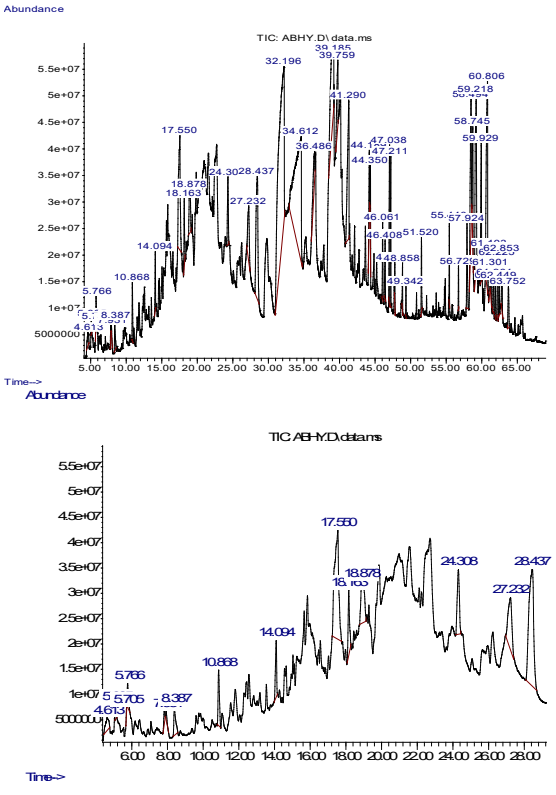
Plate 4: Section of mouse liver from Group IV administered 1600 mg/kg bw (H&E x 40).
Key: A: hepatocytes, E: mild vascular congestion.

Table 2: Compounds identified in *Alstonia boonei* stem bark oil

Compound name	Molecular formula	MW	RT (min)	RMF (DB)
3-Ethylpyridine	C_7H_9N	107	4.61	952
1,2,3-Trimethylbenzene	C_9H_{12}	120	5.08	901
2-Ethyl-1-hexanol	$C_8H_{18}O$	130	5.76	970
(-)-trans-Pinocarvyl acetate	$C_{12}H_{18}O_2$	194	7.88	790
Methyl nicotinate	$C_7H_7NO_2$	137	8.39	939
Bicyclo[3.1.0]hex-3-en-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2α,5α)- (5-Isopropyl-2-methylbicyclo[3.1.0]hex-3-en-2-ol)	$C_{10}H_{16}O$	152	7.82	764
2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)- (Carveol)	$C_{10}H_{16}O$	152	7.92	757
Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate	$C_{12}H_{18}O_2$	194	10.87	801
3-Allyl-6-methoxyphenol	$C_{10}H_{12}O_2$	164	14.08	829
3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-	$C_{12}H_{16}O_5$	240	17.53	826
Undecanoic acid, 10-methyl-, methyl ester	$C_{13}H_{26}O_2$	214	18.15	857
1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl-, (R)-	$C_{10}H_{10}O_3$	178	18.89	887
Hexadecanoic acid, ethyl ester (Palmitic acid, ethyl ester)	$C_{18}H_{36}O_2$	284	24.30	809
Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester)	$C_{17}H_{34}O_2$	270	28.43	898
n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256	34.61	921
Ethyl 14-methyl-hexadecanoate	$C_{19}H_{38}O_2$	298	36.47	833
10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	36.66	843
(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	39.03	879
cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	40.16	887
9,12-Octadecadienoic acid (Z, Z)-, 2,3-dihydroxypropyl ester (Linolein, 1-mono-)	$C_{21}H_{38}O_4$	354	41.28	706
Ethyl hexadecanedioate	$C_{20}H_{38}O_4$	342	44.19	849
Hexanedioic acid, dioctyl ester (Adipic acid, dioctyl ester)	$C_{22}H_{42}O_4$	370	44.36	839
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	46.06	758
10,13-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	46.42	759
1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	47.02	949
Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	562	47.72	752
3-Ethyl-5-(2'-ethylbutyl) octadecane	$C_{26}H_{54}$	366	47.78	753
17-Pentatriacontene	$C_{35}H_{70}$	490	49.36	783
Ibogamine-18-carboxylic acid, 3,4-didehydro-, methyl ester, (2α,5β,6α,18β)-	$C_{21}H_{24}N_2O_2$	336	51.53	761
Olean-13(18)-ene	$C_{30}H_{50}$	410	55.45	822
Ursa-9(11),12-dien-3-one	$C_{30}H_{46}O$	422	56.72	848
4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	424	57.93	880
α-Amyrin	$C_{30}H_{50}O$	426	58.55	848
β-Amyrin	$C_{30}H_{50}O$	426	58.50	884
Lup-20(29)-en-3-one (Lupenone)	$C_{30}H_{48}O$	424	58.73	894
Lupeol	$C_{30}H_{50}O$	426	59.24	906
Lup-20(29)-en-3-ol, acetate, (3β)- (Lupeol acetate)	$C_{32}H_{52}O_2$	468	60.80	912
7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	502	61.07	759
9,19-Cyclolanostan-3-ol, 24,24-epoxymethano-, acetate	$C_{33}H_{54}O_3$	498	61.93	787
Stigmastane-3,6-dione, (5α)-	$C_{29}H_{48}O_2$	428	62.83	882

GC-MS was performed by 'Agilent GC-MS triple quad USB39375HHP-5MS. The identification of compounds was based on a mass spectral survey performed using NIST library for spectral comparison and identification

They include 3-Ethylpyridine (1); 1,2,3-Trimethylbenzene (2); 2-Ethyl-1-hexanol (3); (-)-trans-Pinocarvyl acetate (4); Methyl nicotinate (5); Bicyclo[3.1.0]hex-3-en-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2α,5α)- (6); Carveol (7); Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate (8); 3-Allyl-6-methoxyphenol (9); 3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo- (10); Undecanoic acid, 10-methyl-, methyl ester (11); 1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl-, (R)- (12); Palmitic acid, ethyl ester (13); Palmitic acid, methyl ester (14); Palmitic acid (15); Ethyl 14-methyl-hexadecanoate (16); 10-Octadecenoic acid, methyl ester (17); (E)-9-Octadecenoic acid ethyl ester (18); cis-Vaccenic acid (19); 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester (20); Ethyl hexadecanedioate (21); Adipic acid, dioctyl ester (22); Ethyl iso-allocholate (23); 10,13-Eicosadienoic acid, methyl ester (24); 1,2-Benzenedicarboxylic acid, diisooctyl ester (25); Oleic acid, eicosyl ester (26); 3-Ethyl-5-(2'-ethylbutyl) octadecane (27); 17-Pentatriacontene (28); Ibogamine-18-carboxylic acid, 3,4-didehydro-, methyl ester, (2α,5β,6α,18β)- (29); Olean-13(18)-ene (30); Ursa-9(11),12-dien-3-one (31); 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one (32); α-Amyrin (33); β-Amyrin (34); Lupenone (35); Lupeol (376; Lupeol acetate (37); 7,8-Epoxylanostan-11-ol, 3-acetoxy- (38); 9,19-Cyclolanostan-3-ol, 24,24-epoxymethano-, acetate (37) and Stigmastane-3,6-dione, (5α)- (40). Available literature suggests that these compounds, either acting alone or in a network effect, play vital biological and structural functions. For example, palmitic acid, ethyl ester and palmitic acid, methyl ester, both naturally occurring fatty acid derivatives, have demonstrated potent emollient and lubricant, antimicrobial/antifungal, anti-inflammatory, neuroprotective and cardioprotective properties (Saeed *et al*., 2012; Hamed *et al*., 2020; Enearepuadoh and Tamunonengiofori, 2025; Ghfil *et al*., 2025). The natural monoterpene, carveol, has been associated with cytoprotective, antioxidant, immunoregulatory, anti-inflammatory, neuroprotective and relaxant effects (Hritcu *et al*., 2020; Alvi *et al*., 2021; da Silva *et al*., 2020; Serafim *et al*., 2021; Badshah *et al*., 2024). α and β-Amyrin on the other hand, are linked to antioxidant, anti-inflammatory, apoptotic, antineoplastic, antiviral, immunostimulant, anti-bone loss and antilipemic activities, where they function by reducing oxidative stress, inhibiting key enzymes (like cyclooxygenase- COX-2, xanthine oxidase), modulating signaling pathways (Nuclear Factor Kappq-B- NF-κB, Mitogen Activated Protein Kinase-MAPK), etc., (Singh *et al*., 2015; Dimmito *et al*., 2021; Bokelmann, 2021; Viet *et al*., 2025). Lupenone, a lupine-type triterpenoid with diverse biological activities, exerts various pharmacological activities including anti-inflammatory, anti-viral (functioning through pathways such as NF-κB and Phosphoinositide-3-Kinase- PI3K/Protein Kinase B-Akt/ mammalian Target of Rapamycin- mTOR), anti-diabetes (by improving insulin sensitivity, inhibiting Protein Tyrosine Phosphatase 1B- PTP1B, and reducing inflammation) and anticancer (by affecting cell signaling and reestablishing immune responses) (Xu *et al*., 2018; Xu *et al*., 2022). While lupeol, a multi-target agent and natural pentacyclic triterpene, function as an anti-inflammatory (through pathways like NFκB, cellular FLICE Inhibitory Protein (cFLIP), Fas, Kirsten rat sarcoma viral oncogene homolog (Kras), PI3K/Akt and Wnt/β-catenin), antioxidant (radical scavenging) and anticancer (by mechanisms such as apoptosis, inhibiting the Akt/PKB pathway, migration and invasion of cancer cells, and suppressing cell proliferation) agent; it also lowers cholesterol (hypocholesterolemic), improves HDL cholesterol, and reduces activity of certain ATPase enzymes (Saleem, 2009; Sharma *et al*., 2020; Dias *et al*., 2021; Liu *et al*., 2021). Therefore, the presence of these compounds could play a vital role in the healing potentials of *A. boonei* stem bark and influence its use in ethnopharmacological practice.



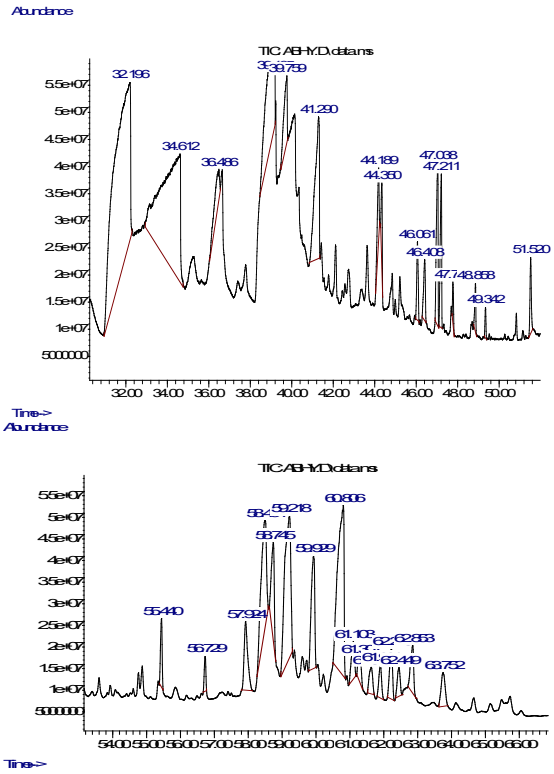
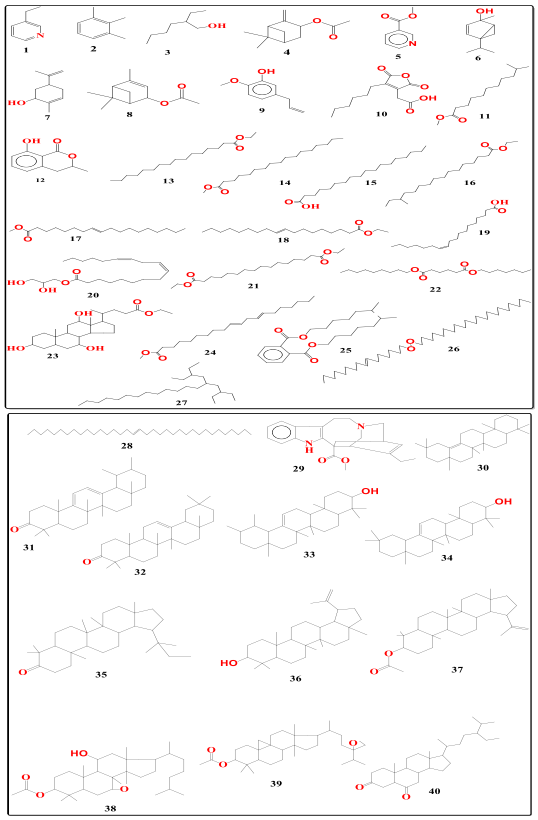


Fig 8: Chromatograms of chemical constituents in *Alstonia boonei* stem bark oil**.**



Figs 9: Structures of compounds identified in *Alstonia boonei* stem bark oil.

Conclusion: The phytochemical composition of *A. boonei* stem bark and its *in vivo* acute toxicity index in adult MRL male rats have been determined in this study. Our findings reveal a rich phytochemical presence and a relatively safe toxicity index. This strengthens the basis for the use of the plant in ethnopharmacological practice. However, further extended studies are recommended to amply evaluate its safety level. Nevertheless, these results pave the way for future studies meant to explore the potential clinical applications of this plant, which contains significant amounts of biologically active phytochemicals that can be cautiously harnessed for therapeutic gains.

DECLARATIONS

Ethical Approval: This study involved animal experimentation and therefore ethical approval was obtained from the the Animal Care and Use Committee of the International Centre for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan with Animal Handling Licence No. 20160034. All procedures involving data handling and analysis were conducted responsibly and in accordance with standard academic and institutional guidelines.

Disclaimer on Artificial Intelligence usage: Authors hereby declare that no generative Artificial Intelligence (AI) technologies such as Large Language Models (ChatGPT, COPILOT, XLNet, Ernie 3.0 Titan etc) and text-to-image generators have been used during writing or editing of this manuscript.

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Declaration of Conflict of Interest: The authors declare that no known conflict of interest or personal relationships have influenced the work reported in this paper

Declaration of Data Availability: All data relating to this study have been included in this article.

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