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Lupeol Isolation and Hepatoprotective Activity of the Methanol Aerial Parts Extract of *Indigofera Stenophylla* (Fabaceae)

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Abstract

Introduction: *Indigofera stenophylla* Guil. and Perr. (Fabaceae) is a medicinal plant used in traditional medicine for the treatment of diseases. This study aimed to isolate phytoconstituent(s) and establish the hepatoprotective activity of the methanol aerial parts extract of *Indigofera stenophylla* (MAEIS). **Methods**: The plant material of 1500 g was extracted with 70% methanol using a maceration method, and the extract was partitioned respectively into hexane and chloroform fractions (HF and CF). The HF was subjected to column chromatographic techniques to afford 5 mg of compound AR1 characterized using ^{1}H and ^{13}C-NMR. A single dose of 7,12 Dimethylbenz(a)anthracene was administered orally at 30 mg/kg body weight, followed by 28 days treatment with graded doses of MAEIS and Silymarin. Blood samples were collected and analyzed for Aspartate Amino Transaminase (ASP), Alanine Amino Transaminase (ALT) and Total Protein (TP) assays. The livers were harvested for histopathological examination. **Results**: The isolated compound AR1 was elucidated as lup-20(29)-en-3-ol (lupeol). The MAEIS group at dose of 600 mg/kg and silymarin 200 mg/kg body weight respectively lowered the levels of AST and ALT significantly ($p<0.05$) indicating a reduction in liver damage. The MAEIS increased the total protein levels and normal histopathological profile of the liver tissue. **Conclusion**: This is the first isolation of lupeol from MAEIS. MAEIS provides a scientific basis for its ethnomedicinal uses and demonstrated significant hepatoprotective activity.

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Introduction

Medicinal plants are utilized in the treatment and prevention of specific diseases that affect human beings. They are largely unexploited source for the development of potentially new drugs (Sanusi *et al*., 2018). *Indigofera stenophylla* is a medicinal plant that belongs to the Indigofera genus, which has over 750 species in the Fabaceae family, known for producing indigo dye. Common species in Nigeria include *I. hirsute, I. paniculata, and I. arrecta,* the latter mainly found in the north. The Fabaceae family is the third-largest among angiosperms, with over 19,325 species (Sanusi *et al*., 2024). In West Africa, there are approximately 78 Indigofera species, with Nigeria having around 60, predominantly in the northern region (Sanusi *et al*., 2024).

The genus *Indigofera* has a rich history of ethnomedicinal use across various cultures, particularly in regions of Asia and Africa. Traditional healers have utilized various species for treating ailments such as gastrointestinal disorders, abdominal pain, and respiratory issues. *Indigofera tinctoria* is noted for its applications in managing diabetes, while *Indigofera oblongifolia* has been used for its hepatoprotective properties. *Indigofera pulchra* is recognized for its snake-venom neutralizing capabilities (Rahman *et al*., 2018). *Indigofera oblongifolia*, commonly known as hasr, is use for its analgesic and anti-inflammatory properties, treating conditions such as pain, hepatitis, and respiratory ailments (Murshed *et al*., 2025a). *Indigofera tinctoria*, commonly known as true indigo, has been employed for treating a variety of ailments, including nervous disorders, epilepsy, bronchitis, liver diseases, and antimicrobial properties, particularly against pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Murshed *et al*., 2025b). *Indigofera suffruticosa*, commonly known as anil, has been utilized for treating ailments such as nervous disorders, epilepsy, bronchitis, and liver diseases. Its leaves and roots are often prepared as infusions or decoctions to leverage their febrifuge, antispasmodic, diuretic, and analgesic properties (Campos *et al*., 2018). *Indigofera heterantha var. gerardiana*, commonly known as Himalayan Indigo, has been utilized to treat a range of health issues, including abdominal pain, skin disorders, infectious diseases, and spastic pain (Ahmed and Sadaf, 2025). *Indigofera ammoxylum* is utilized in Reunion Island for diabetes and hypercholesterolemia, prepared as an infusion, while *Indigofera tinctoria* serves as a remedy for toothache and gingivitis, typically administered as a decoction or mouthwash. *Indigofera arrecta* is employed for ringworm and skin mycosis, applied topically after crushing, whereas *Indigofera aspalathoides* is noted for treating dermatological diseases and psoriasis, often mixed with other plants in polyherbal formulations. In Ethiopia, *Indigofera spicata* is used for diarrhea, prepared by crushing and macerating the roots, and *Indigofera suffruticosa* is recognized for its anti-inflammatory properties, typically extracted as a decoction. Other species, including *Indigofera hirsuta* and *Indigofera caerulea*, are utilized for ailments like diabetes and infections, with preparations varying from infusions to topical applications (Gerometta *et al*., 2019).

The pharmacological activities of *Indigofera* species that have been validated are numerous through various studies and their therapeutic potential. This effect includes significant antimicrobial activity against pathogens like *Staphylococcus aureus* and *Escherichia coli*, alongside anti-inflammatory properties seen in *Indigofera aspalathoides* and *Indigofera hirsuta*, which reduce edema. Additionally, *Indigofera tinctoria* exhibits strong antioxidant activity and anticancer potential, particularly from compounds like indirubin. Antidiabetic effects are noted in extracts from *Indigofera tinctoria* and *Indigofera cordifolia*, while *Indigofera suffruticosa* shows promising anticonvulsant effects. Moreover, *Indigofera caerulea* and *Indigofera oblongifolia* provide hepatoprotective benefits, and *Indigofera linnaei* is recognized for wound healing. *Indigofera spicata* demonstrates

significant antidiarrheal activity (Gerometta *et al*., 2019). Pharmacological investigations have revealed significant bioactivities associated with *Indigofera* species, attributed to their rich profile of secondary metabolites. The crude extracts and isolated compounds exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, antidiabetic, and cytotoxic effects. *Indigofera suffruticosa* has shown promising results in inhibiting carcinogenic cell lines, while *Indigofera heterantha* has demonstrated in vitro lipoxygenase inhibitory activity. Additionally, *Indigofera tinctoria* has been reported to possess antidiabetic and antioxidant properties (Rahman *et al*., 2018). *Indigofera oblongifolia* exhibits significant antimicrobial, hepatoprotective, and antimalarial properties, making it a candidate for further pharmaceutical exploration (Murshed *et al*., 2025a). *Indigofera tinctoria*, particularly noted for its antimicrobial, antioxidant, and hepatoprotective activities (Murshed *et al*., 2025b), further exemplifies the therapeutic potential of this genus. *Indigofera suffruticosa* shows significant antimicrobial properties against several pathogenic strains, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Studies have confirmed its anti-inflammatory, antioxidant, and hepatoprotective activities. The plant also displays cytotoxic effects against tumor cell lines, indicating its potential application in cancer therapy (Campos *et al*., 2018). *Indigofera heterantha var. gerardiana* exhibits significant antimicrobial properties against various pathogenic strains, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Studies have demonstrated its antifungal, anti-inflammatory, antioxidant, and hepatoprotective effects, with extracts showing potential in inhibiting advanced glycation end products, thus offering protective benefits in diabetic conditions. Furthermore, the plant's extracts have shown cytotoxic effects against tumor cell lines, indicating its potential application in cancer therapy (Ahmed and Sadaf, 2025). Extensive literature search has shown that there is limited pharmacological studies on the root, shoot, stem, leaves, flower, and fruit of *Indigofera stenophylla* reported, though antiproliferative and antioxidant activity reported (Sanusi *et al*., 2025).

Extensive literature review on the phytochemicals studies have led to the isolation of 65 distinct compounds from various species within the *Indigofera* genus. These include flavonoids, terpenoids, nitro compounds, and steroids. Compounds such as lupeol from *Indigofera kirilowi* and indirubin from *Indigofera suffruticosa* have been isolated and characterized for their bioactive properties (Rahman *et al*., 2018). *Indigofera suffruticosa* has resulted in the identification of multiple bioactive compounds, such as quercetin derivatives, indigo, and indirubin. (Campos *et al*., 2018). *Indigofera heterantha var. gerardiana* has also identified various bioactive compounds, including quercetin derivatives, indigo, and indirubin (Ahmed and Sadaf, 2025). *Indigofera stenophylla* the isolation and characterization of 5-Hydroxy-Rutin-6,7-Dimethyl Ether and cilicicone β flavonoids also reported (Sanusi *et al*., 2024). The diverse chemical constituents isolated from various species emphasize the genus's potential in drug discovery and development, as they exhibit various therapeutic activities that warrant further exploration. Extensive literature search has shown that there is limited phytochemical study on the root, shoot, stem, leaves, flower, and fruit of *Indigofera stenophylla.*

Liver is a vital organ with a remarkable capacity for repair and regeneration, essential for survival after various forms of damage. It functions as a metabolic hub, regulating glucose, lipid, and amino acid metabolism, and is involved in detoxification and immune responses. The liver's regenerative processes are complex, orchestrated through intricate cellular and molecular networks, including various signaling pathways and cellular interactions. These mechanisms enable the liver to restore its function and structure after injury, making it a focal point for understanding both physiological

functions and pathological conditions (Ma *et al*., 2025). The liver contains parenchymal and non-parenchymal cells such as hepatocytes, Kupffer cells, stellate cells, sinusoidal endothelial cells, and cholangiocytes that coordinate detoxification, metabolism, and immune regulation. Due to its high metabolic activity, the liver is a major site for reactive oxygen species (ROS) generation from mitochondria, cytochrome P450 enzymes, peroxisomes, and NADPH oxidases. When ROS exceed antioxidant defenses such as glutathione, catalase, and superoxide dismutase, oxidative stress develops and damages proteins, lipids, and nucleic acids. This imbalance causes mitochondrial dysfunction, lipid peroxidation, and calcium dysregulation, resulting in hepatocyte apoptosis or necrosis and the release of pro-inflammatory signals that activate Kupffer and stellate cells. Chronic oxidative stress underlies the progression of many liver diseases, linking cellular injury to fibrosis and cancer. In drug-induced liver injury, such as acetaminophen toxicity, glutathione depletion leads to oxidative cell death and acute liver failure. In chronic diseases including viral hepatitis (HBV and HCV), non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease, and genetic conditions like hemochromatosis and Wilson's disease sustained ROS production promotes inflammation, stellate cell activation, and excessive collagen deposition, leading to fibrosis and cirrhosis (Allameh *et al*., 2023). Extensive literature search has shown that there is limited pharmacological and no hepatotoxic study on the root, shoot, stem, leaves, flower, and fruit of *Indigofera stenophylla.*

7, 12 dimethylbenz(a)anthracene (DMBA) is commonly used in hepatotoxicity research. It's highly toxic polycyclic aromatic hydrocarbon (PAH) that can injure the liver through well-established biological mechanisms. It is a genotoxic and a reactive metabolism, forming metabolites capable of damaging the liver cells and other cellular structures including lipids, proteins through DNA adduct and overexpression of reactive oxygen species. The subsequent alteration in antioxidant enzyme system (superoxide dismutase, catalase enzymes), changes in serum liver damage markers (ALT, AST, ALP and TP) and histological necro-inflammatory changes are assessment indicators for DMBA induced structural and functional hepatic damage (Arora *et al*., 2014).

Therefore, the present study was designed to isolate, characterize phyotochemical(s), and investigate the hepatoprotective activity of the methanol aerial parts extract of *Indigofera stenophylla* against 7,12-dimethylbenzaanthracene-induced liver damage in Wistar rats.

Materials and methods

Plant material collection and identification

The aerial parts of the plant *Indigofera stenophylla* was collected in September, 2023 at Gadau village, Itas Gadau Local Government Area of Bauchi State. The aerial parts of the plant were identified and authenticated by Namadi Sanusi at the Haberium Unit, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria by comparing with the herbarium reference voucher specimen (No. 0763). The plant was air dried under shade, pulverized to powder, labelled and stored at room temperature.

Preparation and extraction of Indigofera stenophylla plant materials

The powdered plant material of *Indigofera stenophylla* weighed and measured 1500 g was extracted with 70% w/v methanol using maceration method for three days. The liquid methanol extract was air-dried, leaving the dried extract referred to as methanol aerial parts extract of *Indigofera stenophylla* (MAEIS) (Sanusi *et al*., 2024).

Chemicals, reagents, drugs, and apparatus

The apparatus used include the following; TLC tank, column (3.5×75 cm), burette and pre-coarted alumium thin-layer chromatographic plate 60 GF_{257} 20×20 cm in dimension (Sigma Aldrich, Germany). The solvents include methanol, acetone, ethyl acetate (2.5 L), chloroform (2,5 L), n-hexane (2.5 L), acetic acid (Qualikems) and Silica gel of mesh size 60-120 µm (Qualikems). Silymarin, and 7,12-dimethylbenzanthracene which were analytical grade purchased from Cardinal Scientific.

Equipment

The equipment used include; Analytical balance, and Electro-thermal melting point apparatus (Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria), ABB-MB 3000 UV spectrophotometer, Bruker AVANCE III (400 MHz) NMR spectrometer (Scottland) and IR and UV spectrophotometer (NARICT, Zaria).

Experimental animals

The study was undertaken at Ahmadu Bello University, Animal House, Pharmacology Department, in accordance with the Animal care and use's guidelines. Female Wistar rats (200-250g) fed with laboratory diet and water under standard conditions (12-hour light-dark cycle) in a propylene cage at room temperature (25±2°C).

Isolation, purification and characterization of compounds AR1

The initial column (3.5x75 cm) was packed with silica gel (60-120 µm) by wet slurry method. Hexane sample HF weighing 12.7 g was subjected to extensive column chromatography by adsorbing the sample on silica gel using Hexane. The elution was carried out using gradient elution starting with hexane (100 %), followed by hexane: ethyl acetate (95:5) and the polarity was gradually increased by 10 unit, until ethyl acetate (100 %). The elution was monitored using TLC to afford a total of 62 column fractions of 5 ml each which were collected and pooled together based on their TLC profiles to give 11 pooled fractions. The pooled fraction numbered 2 was subjected to a 1.2x50 cm column chromatography with silica gel and using isocratic solvent elution of hexane: ethyl acetate (90:10) which afforded 36 column collections of 2 ml each led to the isolation of compound AR1. (Sanusi *et al*., 2024). The characterization of the isolated compound AR1 was determined using solubility test, chemical test, melting point, TLC, UV, IR, and NMR experiments (Sanusi *et al.,* 2025b).

Induction of hepatotoxicity

A single dose of 30 mg/kg of 7,12-dimethylbenzanthracene (DMBA) was administered to the experimental rats to induce liver injury intraperitoneally, and they were observed for 24 hours prior to the administration of MAEIS for the signs and symptoms of hepatotoxicity

Experimental design

The research work was grouped into six groups each containing six rats; group 1 (normal control) received normal saline 10 ml/kg body weight. Group II: Negative control, received DMBA 30 mg/kg body weight on the first day only followed by normal saline 10 ml/kg body weight for 28 days. Groups III, IV and V groups received DMBA 30 mg/kg body weight on the first day, followed by single daily dose of MAEIS for 28 days at 150, 300, and 600 mg/kg body weight. Group IV: Positive control, received DMBA 30 mg/kg body weight on the first day, followed by silymarin 200 mg/kg for 28 days.

Blood samples collections

On the 29^{th} day, the rats were humanely euthanized under chloroform anesthesia. Blood samples were collected for biochemical analysis using non heparinized container and livers were harvested for histopathological examination. Parameters such as aspartate aminotransferase

(AST), alanine aminotransferase (ALT), and total protein (TP) were analysed.

Histopathological examination of the liver

The harvested livers of the sacrificed rats were rinsed off of blood with normal saline. Subsequently, sections were taken from the livers of the sacrificed rats. These liver organs were fixed in 10% formosaline before dehydration with 100% ethanol and subsequently embedded in paraffin. The processed 4–5 µm thick sections were of each liver was stained with haematoxylin-eosin and observed under a microscope (Agbaje *et al*., 2019).

Statistical analysis

Data were presented as Mean ± SEM. The effect of the MAEIS was analyzed using one-way Analysis of Variance (ANOVA) followed by Dunett post-hoc test for multiple comparisons. Differences were considered significant at $p<0.05$.

Results

Extraction and fractionation yield of the aerial parts of *Indigofera stenophylla*

The extraction of the aerial parts of *Indigofera stenophylla* afforded 12.78% yield. The fractionation of methanol aerial parts extract of *Indigofera stenophylla* with hexane and chloroform successively afforded 19.88% of HF, 8.49% of CF and a residual fraction (RF) of 69.12% as presented in **Table I**

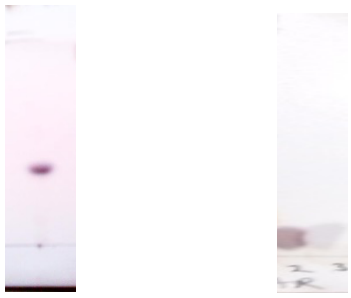
TLC profiles of isolated compound AR1

The TLC analysis of the isolated compound AR1 showed R_f value of 0.38 and 0.10 using Hexane/Ethyl acetate (8:2) and Hexane/Ethyl acetate (20:1) with single pink spot as observed in **Plate I**.

Table I: Percentage Yield of Fractionation Yield of Methanol Aerial Parts Extract of *Indigofera stenophylla*

Solvent	Weight (g)	Yield (%)
HF	29.82	19.88
CF	12.73	8.49
RF	103.33	69.12
Total	145.88	97.49

Key: HF (Hexane fraction), CF (Chloroform fraction), and RF (Residual fraction).



(A) **(B)**

Plate I: TLC Profiles of Isolated Compound AR1; (A) Hexane: Ethyl acetate (8:2), (B) Hexane: Ethyl acetate (20:1)

Physico-chemical properties of compound AR1

The isolated compound AR1 afforded 5 mg which exhibited a melting point range of 213-221°C, insoluble in ethyl acetate but completely soluble in chloroform. The formations of a purple colour to Liebermann-Burchard test, a reddish-brown color at the interface of the two layers for Salkowski as seen in **Table 2**.

Table 2: Physico-Chemical Properties of Compound AR1

Physico-chemical properties	Compound AR1
Melting point	213 - 221^{0}C
Solubility in Ethyl acetate	Insoluble
Solubility in Chloroform	Completely soluble
(a). Liebermann-Burchard test	Purple color on TLC
(b). Salkowski test	Reddish brown colour

Spectra of compound AR1

The UV spectrum of the isolated compound AR1 showed absorption maxima at 245 nm in **Fig. 1.** The IR spectrum exhibited absorption bands at 3354.6 cm^{-1}, 2929.7 cm^{-1} and 2862.6 cm^{-1}, 1703.4 cm^{-1}, 1457.4 cm^{-1} and 1379.1 cm^{-1} as in **Fig. 2**. The 1H NMR spectrum showed signals at δ 4.56, 4.69 (1H, dd, 2.2, 1.3, 2H), δ 3.18, (1H, dd, 6.25, 6.20, 1H), δ 1.01, 0.94, 0.76, 0.82, 0.99, 0.78 and 1.68 in **Fig. 3 and 4.** The ^{13}C-NMR spectrum displayed 30 carbon signals as seen in **Fig. 5 and Table 3**.

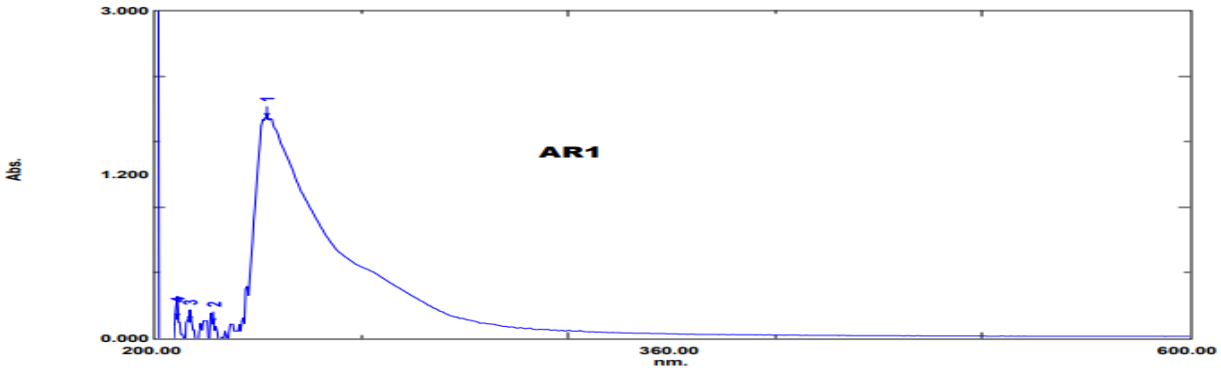


Fig. 1: UV Spectrum of Compound AR1

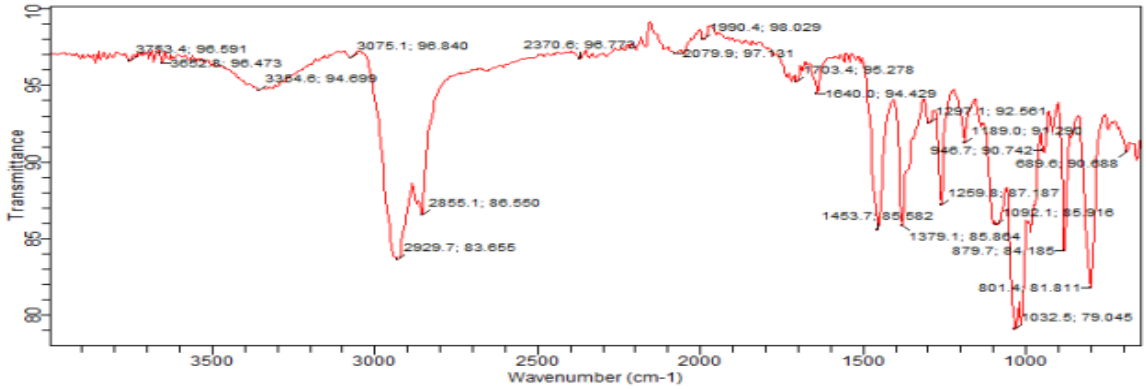


Fig. 2: IR Spectrum of Compound AR1

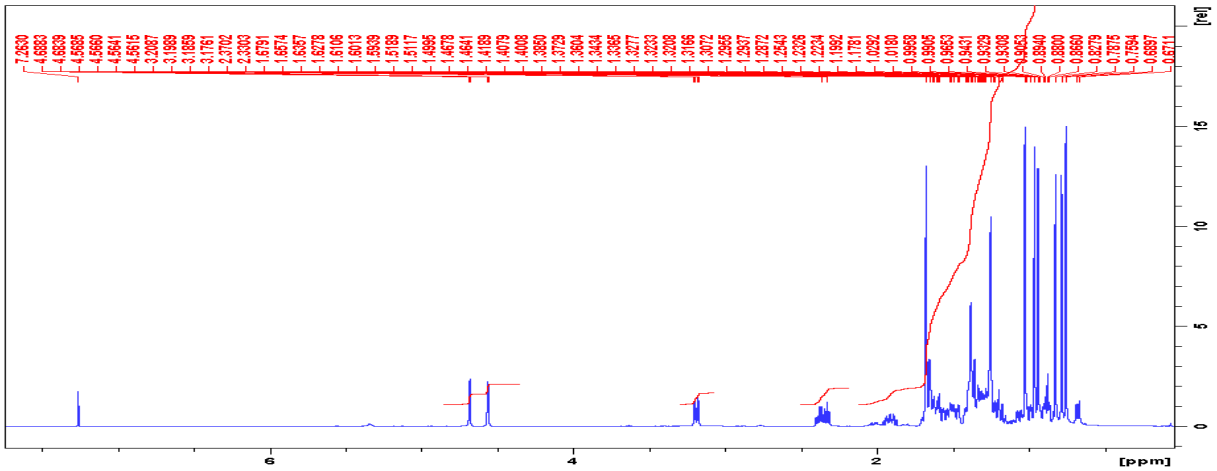


Fig. 3: [1]H-NMR Spectrum of Compound AR1

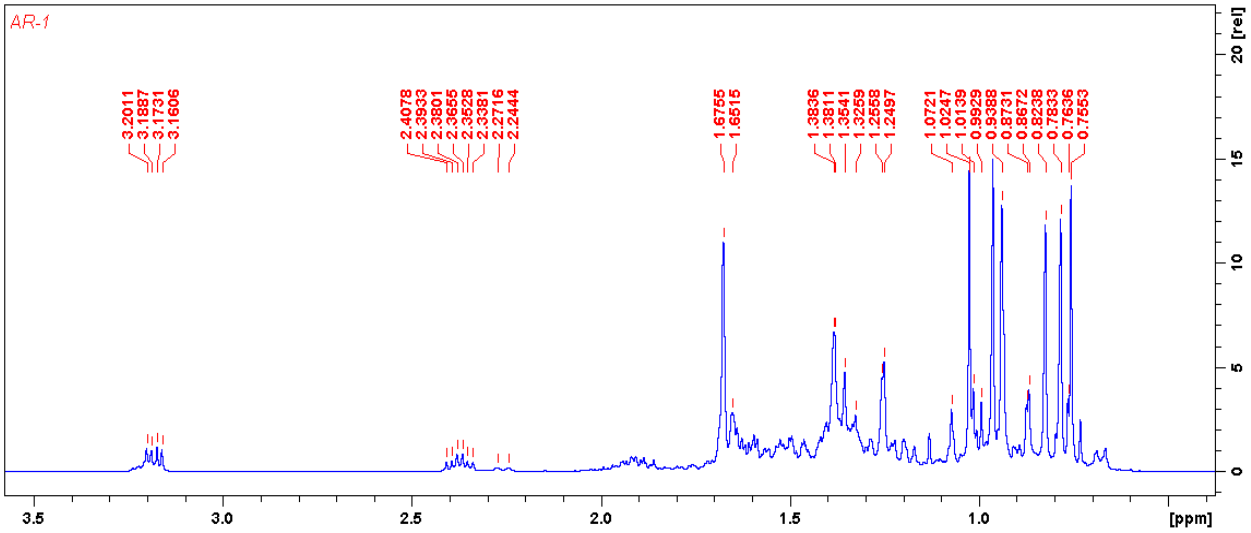


Fig. 4: [1]H-NMR Spectrum of Compound AR1 Expanded

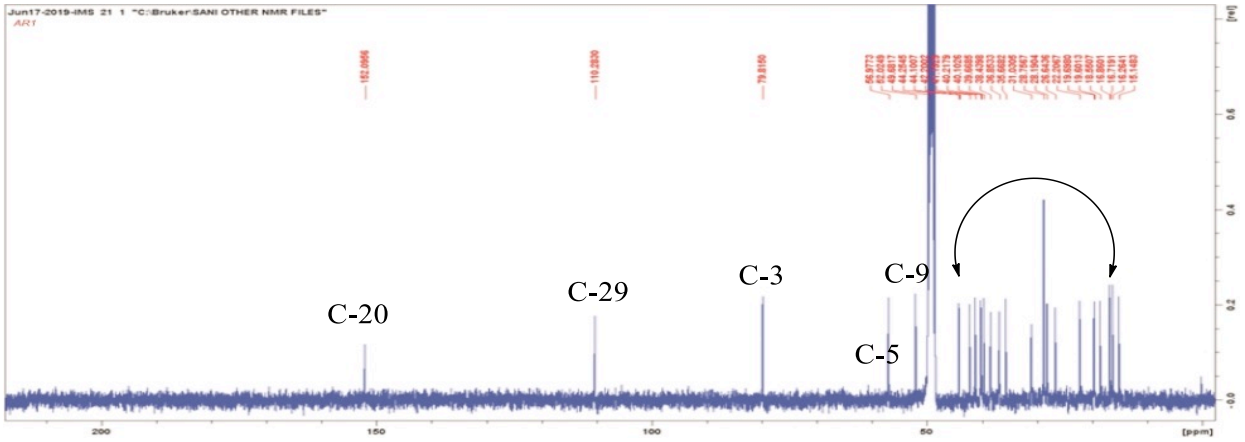


Fig. 5: ^{13}C-NMR Spectrum of Compound AR1 in $CDCl_3$

Table 3:^{1}H *and* ^{13}C-NMR Spectral Data of Compound AR1 Compared with Reported Literature

Position	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
	Compound AR1		[21]	
1	39.67	------	38.7	-------
2	28.19	1.01, s	27.4	1.01, s, 3H
3	79.82	3.18, (1H, dd, 10. 5, 6.20)	78.9	3.17 (1H, dd, 10.8, 5.2)
4	40.10	-----	38.9	-------
5	56.98	-----	55.3	-------
6	19.60	-----	18.3	-------
7	35.67	-----	34.2	-------
8	41.13	-----	40.8	-------
9	52.04	-----	50.4	-------
10	36.85	-----	37.2	-------
11	22.21	-----	20.9	-------
12	26.64	-----	25.1	-------
13	38.44	-----	38.0	-------
14	42.20	-----	42.8	-------
15	28.19	-----	27.4	-------
16	35.67	-----	35.5	-------
17	44.10	-----	43.0	-------
18	49.65	-----	48.3	-------
19	44.25	2.37 sextet	48.0	
20	152.10	2.38, (1H, dt, 10.0, 7.3)	151.0	2.36 (1H, dt. 10.7, 6.0)
21	31.03	-----	29.8	-------
22	40.22	-----	40.0	-------
23	28.76	0.94, s	27.9	0.92, S, 3H
24	16.72	0.76, s	16.0	0.74, s 3H
25	18.56	0.82, s	18.0	0.81, s 3H
26	16.86	-----	16.1	-------
27	15.15	0.99, s	14.6	0.96, s, H
28	16.25	0.78, s	15.4	0.77, s 3H
29	110.28	4.56, 4.69 (1H, dd, 2.2, 1.3)	109.3	4.67, 4.55 (1H, dd, 2.4, 1.5)
30	19.70	1.68, s	19.3	1.71, s, 3H

Liver biomarker

The activity of ALT and AST enzymes in the negative control group with DMBA increased to 143.3 (IU/L), and 156.7 (IU/L) respectively, compared to normal control group, and a decrease to 6.1 (g/dl) was observed in serum level of TP in **Table 4**. In all test groups 3, 4, and 5 that received the MAEIS and DMBA simultaneously, the activity of ALT, and AST enzymes decreased significantly ($p< 0.05$), with group 5 showed a better decrease compared to other test groups as seen in **Table 4**. In groups 4 and 5 that received the MAEIS and DMBA simultaneously, the serum proteins results showed increased in the TP concentration significantly ($p< 0.05$), with group 5 showed a better activity compared to other test groups as seen in **Table 4**. The histopathological results of liver tissue of rats showed that in all test groups 3, 4, and 5, the intravenous congestion, glycogen reduction, and the perturbation of the classical lobule structure of liver were observed in **Plate II.**

Table 4: Effect of Methanol Aerial Part Extract of *Indigofera Stenophylla* on Biochemical Parameters using 7,12-Dimethylbenzanthracene Induced Liver Damage in Wister Rats.

Group	Dose mg/kg	ALT (IU/L) ±SEM	AST (IU/L) ±SEM	TP (g/dL) ±SEM
Normal saline	10	23.33±0.85	13.3±0.14	7.9±0.18
DMBA	30	143.3±0.68	156.7±008	6.1±0.49
DMBA±MAEIS	150	66.7±0.97*	93.3±0.11*	6.9±0.73
DMBA±MAEIS	300	40.0±79*	63.3±0.11*	7.2±0.93*
DMBA±MAEIS	600	43.3±0.68*	26.7±008*	7.3±0.49*
DMBA±SYL	200	10.6±0.001*	15.1±0.094*	7.6±0.003*

Keys: One-way analyses of variance, post hoc test: Dunett, ALT: Alanine amino transaminase, AST: Aspartate amino transaminase: TP:Total protein: MAEIS: Methanol Aerial part extract of *Indigofera stenophylla,* DMBA: 7,12 Dimethylbenzanthracene, SYL: Silymarin,*: Showing significant at $p<0.05$ SEM: Standard error of mean

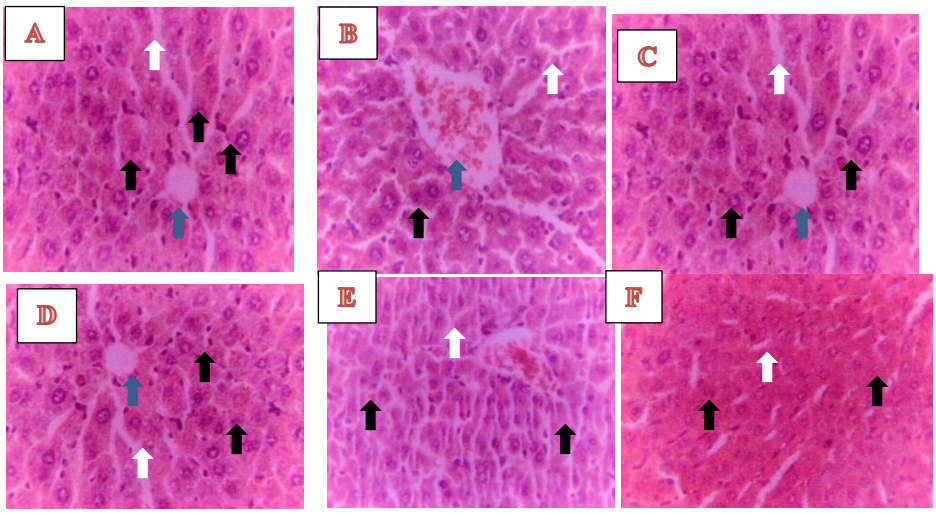


Plate II: Photomicrograph of a Section of Liver following 28 Days Oral Administration of Methanol Aerial Parts Extract of *Indigofera stenophylla* on DMBA Induced Liver Damage in Rats (H and E, 400X Magnification)

Key: Key: A (Normal control), B (Negative control), C (150 mg/kg MAEIS treated group), D (300 mg/kg MAEIS treated group), E (600 mg/kg MAEIS treated group), F (Positive control), Black (hepatocytes), blue (Central vein) and white colour (sinusoids).

Discussion

The presence of single spot using two different solvent systems confirms the purity of the isolated compound AR1. The isolated compound AR1 exhibited sharp melting point range, insoluble in ethyl acetate but completely soluble in chloroform, positive to Liebermann-Burchard and Salkowski test confirmed the presence of triterpene skeleton. The UV spectrum of the isolated compound AR1 absorption maxima at 245 nm is due to conjugated exocyclic double bond which is characteristic of the unsaturated lupene skeleton. The IR spectrum of compound AR1 absorption bands at 3354.6 cm^{-1} (hydroxyl group), 2929.7 cm^{-1} and 2862.6 cm^{-1} (aliphatic C-H stretching), 1703.4 cm^{-1} signal could be due to impurity considering the signal intensity, absence of carbonyl signal from NMR spectral, and 1457.4 cm^{-1} and 1379.1 cm^{-1} (gem-dimethyl groups). The TLC, physicochemical profiles, UV, IR signals of compound AR1 are consistent with the structural features of the triterpene nucleus as reported (Sawale *et al*., 2017; Anas, 2018; Muktar *et al*., 2018; Mailafiya *et al*., 2020; Adeshina *et al* 2021; Ipav *et al*., 2022; Sanusi *et al*., 2025). The 1H NMR spectrum of compound AR1 signals at δ 4.56, 4.69 (1H, dd, 2.2, 1.3, 2H), is a characteristic of the exocyclic methylene protons at C-29. The signal at δ 3.18, (1H, dd, 6.25, 6.20, 1H) is a diagnostic characteristic of H-3 proton. A sextet, integrated for one proton at δ 2.37 assigned to 19β- H is characteristic of lupeol. The presence of seven tertiary methyl signals at δ 1.01, 0.94, 0.76, 0.82, 0.99, 0.78 and 1.68, these signals further confirmed the presence of triterpene skeleton, suggestive of lupeol as reported**.** The ^{13}C-NMR spectrum of compound AR1 signals at δ 110.3 assigned to C-29 and 152.1 to C-20 were attributed to the exocyclic methylene and the olefinic carbon, respectively, signal at δ 79.8 assigned to the C-3 carbon bearing the hydroxyl group with a total of 30 carbon signals displayed is in conformity with the molecular formula of lupeol as reported. The TLC analysis, melting point, positive test to Liebermann-Burchard and Salkowski, combined spectroscopic data (UV, IR, 1H NMR, and 13C NMR) data of compound AR1 corroborate literature data, which confirmed the identification of the isolated compound AR1 as lupeol, a pentacyclic triterpene alcohol. These assignments are in conformity with the structure of lupeol reported (Sawale *et al*., 2017; Anas, 2018; Muktar *et al*., 2018; Mailafiya *et al*., 2020; Adeshina *et al* 2021; Ipav *et al*., 2022; Sanusi *et al*., 2025).

Lupeol is a pentacyclic triterpenoid, a member of the triterpenoid class characterized by six isoprene units, specifically derived from lupane with a hydroxyl group substituting the hydrogen at the 3β position. Naturally occurring in the skin of lupin seeds, as well as in the latex of fig trees and rubber plants, lupeol is also found in various edible fruits and vegetables, including green pepper, mangoes, strawberries, grapes, white cabbage, and olives. This compound is recognized for its preventive and therapeutic effects against numerous health issues, exhibiting biological activities such as anti-inflammatory, antioxidant, antimicrobial, antiprotozoal, nephroprotective, and anticancer properties, making it a significant lead compound for drug development (Ipav *et al*., 2022).

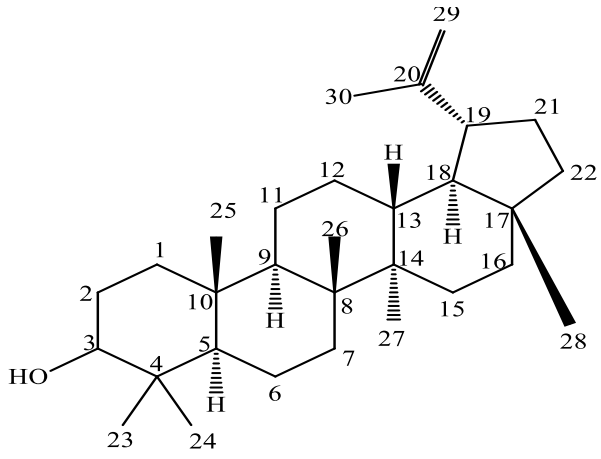


Fig. 5: Chemical Structure of Lupeol

Liver is involved in metabolism, detoxification, and secretion within the body, and its disorders

are numerous with no effective remedies. However, the search for new medications is ongoing. Many folk remedies of plants origin have long been used for the treatment of liver diseases. (Fernando and Soysa, 2014). Hepatotoxic effects of (DMBA) are primarily caused by activating the production of reactive oxygen species (ROS), leading to the generation of peroxidation products and modulating phase I and II liver enzymes (Sanusi *et al*., 2025). The active (ROS) could employ inceptive cell stress by a broad span of mechanisms counting exhaustion of GSH or binding to lipids, nucleic acids, enzymes, and other cells (Sanusi *et al*., 2025). The results of this study also revealed a significant increase in serum Aspartate Transaminase (AST), Alanine Transaminase (ALT), and decreased levels of Total Protein (TP) in rats treated with DMBA compared to control. The metabolic changes observed in the liver may be attributed to the cytotoxic effects of DMBA, causing leakage of these liver markers into the bloodstream. MAEIS pretreatment significantly reversed these alterations in the liver ($p<0.05$), offering protection against oxidative stress by mitigating AST, ALT, and TP activities (Premalatha *et al*., 2015; Lakshmana *et al*., 2018). Alanine Transaminase (ALT) is abundant in the cytosol of hepatic parenchymal cells, whereas Aspartate Transaminase (AST) is found in both the cytosol and mitochondria of hepatocytes. AST is also distributed in cardiac muscle, skeletal muscle, pancreas, and kidneys. Hence, ALT measurement is more specific for determining liver damage. The increased ALT levels in DMBA-induced liver toxicity in the experimental group were due to the loss of structural integrity in the liver. As the enzyme is localized in the cytoplasm, it is released into the blood circulation following cellular damage, resulting in its elevation. On the other hand, increased AST levels indicate damage to both the plasma membrane and mitochondrial membrane. (Chatterjee and Parames, 2006; Bhagavan *et al*., 2013; Omprakash *et al*., 2013) MAEIS could significantly ameliorate acute liver damage, as demonstrated by the reduction of serum ALT and AST levels and the improvement of histopathological changes. Apart from mild hydropic degeneration of hepatocytes, the liver had a nearly normal appearance in DMBA-treated rats simultaneously treated with MAEIS at doses of 300 and 600 mg/kg, which is testimony to the increased level of total protein (TP), restoring liver functions.

The administration of DMBA produced a hepatotoxic effect like other toxic chemicals that resulted in the alteration of ALT and AST enzymes activities of the hepatocytes and TP concentration. The mean values of the serum concentration of AST and ALT were found to be lowest in group in the treated with graded doses at significant ($p>0.05$). This result shows that the hepatoprotective or healing effect of the MAEIS on liver function enzyme assay are in concordance with the results of the gross and histopathological examinations, showing that the best hepatoprotective effect of the MAEIS was observed in the group of rats administered 600 mg/kg.

The effects of methanol aerial part extract of *Indigofera stenophylla* on serum marker enzymes are presented and total protein. The levels of serum ALT and AST were markedly increased, and total protein decreased in DMBA-treated animals, indicating liver damage. Administration of MAEIS at doses of 150, 300, and 600 mg/kg, as well as silymarin at a dose of 200 mg/kg, significantly prevented DMBA induced hepatotoxicity.

Histopathological studies showed that the induced hepatotoxicity group exhibited severe hepatocellular degeneration, necrosis, inflammatory infiltration, and steatosis with the highest score of 6/18. In contrast, the treated groups that received 150, 300 and 600 mg of MAEIS showed substantially reduced histopathological alterations with only mild to minimal changes, characterized by minimal presence of degeneration, necrosis, inflammatory infiltration and steanosis scored 2/18, 2/18 and 3/18 respectively. The group that

received silymarin at the dose of 200 mg displayed minimal histological abnormalities with no degeneration, necrosis, inflammatory infiltration and steanosis scored a minimal of 1/18 compared with the DMBA hepatotoxic-induced group and the normal control group scored 0/18 as seen in **Plate II**. (Arora *et al*., 2014). This semi quantitative scoring system of liver injury is in accordance with Arora *et al*. (2014), significantly prevented DMBA induced hepatotoxicity.

Conclusion

This is the first isolation of lupeol and hepatoprotection activity of the MAEIS against DMBA-induced hepatotoxicity.

Authors' contributions

The research was conceptualized, designed, and drafted by Abdulrazaq Sanusi. Hepatoprotective studies were conducted by Lateef Abiola Akinpelu, Ibrahim Yunusa, Oyeronke Medinat Aiyelero, Jesutoroti Ajayi Oyeniyi, Abubakar Bello Ahmad, Umaima Muhammad Musa, Ahmad Musa, Abubakar Umar, Saifullahi Aminu, and Abdulrazaq Sanusi. Software and data analysis were handled by Ibrahim Yunusa and Abdulrazaq Sanusi, while isolation and structural elucidation were performed by Abdulrazaq Sanusi, Ngaitad Stsnislaus Njinga, and Sa'ad Toyin Abdullahi. The research was reviewed and supervised by Abdulrazaq Sanusi, Halimatu Sadiya Hassan, and Moji Taibat Bakare-Odunola. The study was funded by the authors, who have all read and approved the final manuscript.

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Ethical approval statement

The study was approved at Ahmadu Bello University, in accordance with the Animal Care and Use's guidelines with the **approval number of ABUCAUC/2023162** issued for the study.

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of Generative AI use

I declare that generative AI tools were used in the writing process of this manuscript.

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