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Research Article

Protective Effect of Aqueous Extract of Root Bark of *Salacia nitida* Against Alcohol-Induced Hepatic Injury in Wistar Rats

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ABSTRACT

The root bark of *Salacia nitida* are used traditionally to treat malaria, typhoid fever, heart, and liver problems by the people of Ogoni in Nigeria. Based on this traditional application, the study aimed to evaluate the protective effect of aqueous extract of root bark of *S. nitida* against alcohol-induced hepatic injury in Wistar rats. The phytochemical constituents of the aqueous extract of root bark were analyzed using gas chromatography-flame ionization detector technique. Rats induced with ethanol were treated with oral doses (700, 900, 1100 mg/kg body weight) of aqueous extract of root bark of *S. nitida* (ARBS) for eighteen days. The levels of total protein, albumin, globulin, total bilirubin, total cholesterol, triacylglycerol and malondialdehyde as well as activities of liver function (aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase and gamma-glutamyl transferase) and antioxidant (superoxide dismutase, catalase, glutathione peroxidase) enzymes, were measured. The gas chromatography-flame ionization detector (GC-FID) analysis of the extract revealed the presence of tannins, alkaloids, glycosides, flavonoids, steroids, phytates, and oxalates. Treatment with the extract showed significant ($p < 0.05$) positive modulations of biochemical indices, improvement in body weights and attenuations of liver tissue distortions. These data supports that aqueous extract of root bark of *S. nitida* exhibit hepatoprotective effect and is very rich in bioactive phytocompounds with hepatoprotective property.

Keywords: *Hepatoprotection, Hepatotoxicity, Phytocompounds, Salacia nitida*

INTRODUCTION

The use of indigenous plants in African folk medicines, especially among the people of the Southern part of Nigeria, still play a crucial role in the management and treatment of various diseases like typhoid fever, liver and heart-related problems. *Salacia nitida* (Benth.) N.E.Br (Celastraceae) is a climbing shrub commonly found in the tropical rainforest zone of West Africa. Plant species of the Salacia genus are rich in various phytochemicals and bioactive compounds, which make them suitable candidate for the treatment of different human diseases (Chavan *et al*., 2024). Salacia species are emerging food source for human nutrition with so many health promoting benefits and are used as functional bioactive food ingredient because their rich bioactive phytonutrients (Chavan *et al*., 2025). The hepatoprotective, antioxidative, anti-inflammatory, antilipidemic, and free radical scavenging activities of some Salacia species including *S. chinensis, S. nitida, S. reticulata, S. lehmbachii, S. oblonga*, and *S. pallescens* have been reported (Asuti, 2010; Essiet *et al*., 2016; Deepak, *et al*., 2020; Tamboli and Namdeo, 2020; Nwankwo *et al*., 2021; Obiodun *et al*., 2021; Premakumara and Abeysekera, 2023; Bhat *et al*., 2023; Chavan et al., 2024; Chavan *et al*., 2025). *Salacia nitida* root bark has been shown to ameliorates lipid peroxidation and hepatosplenomegaly in *Plasmodium berghei* malaria-infected mice (Nwiloh *et al*., 2021) and its antimalarial properties has also been reported (Nwiloh *et al*., 2017). Dooka and Ezejiofor, (2017) reported that *S. nitida* root possessed cytoprotective property. The decoctions of *S. nitida* root bark are used traditionally to treat malaria and typhoid fever in Nigeria.

In the Southern part of Nigeria, alcohol is used as a vehicle for the root bark of *S. nitida* in treating diseases. Alcohol, a psychoactive substance, is associated with biochemical abnormalities, including an increased risk of liver injury as a result of excess reactive oxygen species (ROS) generation when consumed in excess (Tan *et al*., 2020).

Presently, treatment options for hepatic injuries are very limited. Consequently, medicinal plants are used as alternatives by people with low socio-economic status for the treatment of diseases. This might be due to the medicinal potential of the phytochemical compounds present in plants. The presence of secondary metabolites in plants with pharmacological activities has been established (Kaushik *et al*., 2021; Floares *et al*., 2023). Therefore, this study is aimed at evaluating the phytochemical compounds and hepatoprotective effects of aqueous extract of root bark of *S. nitida* (ARBS) on ethanol-induced hepatotoxicity in Wistar rats.

MATERIALS AND METHODS

Chemicals and reagents

All assay kits, ethanol, and physiological saline used in this study were of analytical grades. The assay kits for total protein, TP, albumin, total bilirubin, TB, total cholesterol, TC, triacylglycerol, TAG, malondialdehyde, MDA (Teco Diagnostics, USA); superoxide dismutase, SOD, glutathione peroxidase, GPx, catalase, CAT, aspartate aminotransferase, AST, alanine aminotransferase, ALT, alkaline phosphatase, ALP, lactate dehydrogenase, LDH, and gamma glutamyltransferase, GGT (Randox Laboratory Ltd, USA); potassium hydroxide, hexane, anhydrous sodium sulphate, pyridine, and ethanol (BDH, England), and physiological saline were all purchased from Austin Laboratory, Alakahia. The silymarin, (Silybon-140, Micro Laboratories Limited, India) was purchased from Dooka pharmacy, Alakahia, Port Harcourt, Rivers, Nigeria.

Plant materials and extract preparation

The plant *S. nitida* was harvested in February, 2017 from Nyogor-Beeri, Khana Local Government Area, Rivers State, Nigeria, and identified at the University of Port Harcourt Herbarium, Choba, Rivers State, Nigeria (Accession Number: UPHV-1033). The plant roots were washed in tap water and dried under shade for 6 hrs. Thereafter, the plant root barks were chopped into pieces with a machete and air-dried under shade for 8 days. Chunks of the dried root barks were pulverized with a grinding machine. Then, 400 g of the pulverized plant materials were macerated in 5 L of boiled water for 72 hrs with occasional stirring, and the mixture was filtered with filter paper (Whatman No. 1). The filtrate was concentrated with rotary evaporator and then dried with an oven regulated at 55 ^{o}C. The dried aqueous extract of root bark of *S. nitida* (ARBS) was then kept in a refrigerator at 4 ^{o}C until required for use. The yield of the extract was calculated with the formula:

Yield (%) = weight of extract/weight of powder plant material x 100.

Phytochemical analysis

The analysis of phytochemical compositions of the extract was done using gas chromatography-flame ionization detector (GC-FID). In this case, 1 g of ARBS was dispensed into a test tube, and 15 ml of ethanol and 10 ml of potassium hydroxide (KOH) were added. The mixture was left to stay for 1 hr at 60 ^{o}C in a water bath and then rinsed in a separatory funnel with ethanol (20 ml), cold water (10 ml), hot water (10 ml), and hexane (3 ml). The resultant mixtures were combined and re-washed three times with 10 ml of ethanol solution. Thereafter, the mixture was dried using anhydrous sodium sulfate. The sample was dissolved in pyridine (1000 µl), and 200 µl was dispensed into a vial for GC-FID analysis. Determination of phytochemical composition of the extract was performed suing a Gas chromatography (BUCK M910) that is coupled to flame ionization detector. The study deployed a RESTEK 15-meter MXT-1 (15 m x 250 µm x 0.15 µm) column. The system functioned on a 280 ^{o}C injector temperature, a 2 µl splitless sample injection, and a linear velocity of 30 cm/s. The carrier gas was helium (5.0 pa.s) with a flow rate of 40 ml/min. The initial temperature of the oven was kept at 200 ^{o}C, and further heated to 330 ^{o}C at a rate of 3 ^{o}C/min and maintained at this temperature for 5 min. The detector functioned at 320 ^{o}C. Phytochemical analysis of the extract was evaluated by calculating the ratio between the area and mass of the internal standard and the area of the identified phytocompounds. The concentrations of the various identified phytocompounds were presented in µg/g.

Oral toxicity test

The acute oral toxicity study (LD_{50}) of ARBS was performed according to the method of Lorke (1983).

Experimental design

Thirty-six (36) Wistar rats weighing between 165.00±8.90 g and 230.45±10.30 g of both sexes, obtained from the animal house of the Department of Biochemistry, University of Port Harcourt, were used for the study. Animals were kept in plastic cages under standard housing conditions of a 12/12 hr light/dark cycle, 30 ± 3 ^{o}C temperature, and 55–73% humidity for 10 days and were allowed free access to feed (grower's marsh) and clean water *ad libitum*. The study protocol adhered strictly to the guidelines for the care and use of laboratory animals (ILAR, 2011), as approved by the University of Port Harcourt Ethics Committee (Approval Number: UPH/BCH/AEC/2017/023D).

The 36 rats were randomly grouped into 6, with 6 rats per group and treatment were done according to the protocol below: Normal Control (NC) group, which did not receive any treatment or ethanol; Induced Control (IC) group that was exposed to 20 mg/kg body weight (BW) of ethanol without treatment; Induced groups treated with either 700 mg/kg (IT700ARBS), 900 mg/kg (IT900ARBS) and 1100 mg/kg (IT1100ARBS) BW of ARBS, respectively. Lastly, an induced group was administered 25 mg/kg BW of SILY (IT25SM).

All the animals were weighed at the end of the experiment, anesthetized with formalin, and sacrificed by cervical dislocation. Blood samples were taken by venipuncture into lithium-heparinized specimen bottles for the evaluation of biochemical parameters. The sacrificed animals were then dissected, and their livers were collected for histopathological study.

Biochemical assays

Blood samples were centrifuged at 4000 rpm for 15 minutes, and the supernatants were used for assessment of the levels of total proteins (TP), albumin (A), total bilirubin (TB), total cholesterol (TC), triacylglycerol (TAG) using standard experimental techniques and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and gamma glutamyltransferase (GGT) using standard experimental techniques. The liver tissue homogenates were also centrifuged at 4000 rpm for 15 minutes, and the supernatants were used to assess the level of malondialdehyde (MDA), and activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) using standard experimental protocols. The level of globulin (G), A/G ratio, AST/ALT, and GGT/ALP ratios were determined mathematically (Vasudevan and Sreekumari, 2007).

Histopathological studies

Portions of the liver collected from the experimental rats were fixed in a 10% formal-saline solution for 48 hrs and then cut into 3 mm x 3 mm slices and dehydrated in different concentrations of ethanol for about 14 hrs and xylene for 4 hrs and thereafter embedded in paraffin wax. The embedded tissues were then sectioned into 5 μm slices with a microtome. The sections were stained with haematoxylin solution for about 10 min, followed by eosin solution for 30 seconds (H & E). They were mounted in dibutylphthalate polystyrene xylene (DPX) and viewed with a microscope using 400x magnification.

Data analysis

The statistical software SPSS version 22 was used for one-way analysis of variance (ANOVA) of the data. The Scheffe's post hoc test was used for multiple comparisons, and differences were deemed significant at the 95% confidence level ($p < 0.05$). Mean values ± standard deviation (SD) was used to express results.

RESULTS

The yield of aqueous extract of root bark of *S. nitida* (ARBS) was 18.33%. The LD_{50} shows that ARBS was very safe even at 5000 mg/kg BW. The GC-FID results (Figure 1) show that ARBS is composed of 4.12% alkaloids (sparteine and lunamarin), tannin (7.72%), sapogenin (7.90%), 52.49% flavonoids (naringenin, flavan-3-ol, rutin, anthocyanin, naringin, flavanones, kaempferol, epicatechin, flavone, and proanthocyanin), 10.18% glycosides (cyanogenic glycoside and cardiac glycoside), steroid (9.02%); phytate and oxalate (8.47%); and resveratrol (0.10%) (Table 1).

Table 1: GC-FID phytochemical Compositions of Aqueous Extract of Root Bark of *Salacia nitida*

Phytochemical constituents	Retention	Area	Height	Concentration (ppm)
Resveratrol	0.080	232.8066	139.940	0.1743
Lunamarin	0.280	3400.3980	117.455	4.3563
Naringenin	2.390	12252.8106	301.042	10.6788
Flavan-3-ol	4.120	6344.5478	157.568	4.4226
Rutin	6.016	18154.0688	442.689	11.2653
Anthocyanin	7.470	8442.9838	206.428	11.6218
Tannins	10.366	19598.0668	476.646	13.0881
Sparteine	12.970	6238.3258	152.341	2.6332
Sapogernin	15.460	4967.5639	121.273	6.2414
Naringin	17.966	11339.2568	276.424	10.5424
Flavanones	20.313	12756.4840	307.630	7.5489
Steroids	22.730	9573.1408	233.186	15.3121
Kaempferol	25.650	10008.8176	245.115	9.0101
Cyanogenic glycoside	27.536	11458.0104	280.295	15.9139
Phytate	29.860	5478.4406	133.723	7.1794
Flavone	32.996	14337.0482	348.877	8.8967
Epicatechin	34.600	6059.7940	147.836	7.8364
Oxalate	36.876	6988.5601	170.310	7.1845
Sapogenin	39.200	10234.6024	249.263	7.1529
Cardiac glycoside	42.276	3473.1416	85.310	1.3621
Proanthocyanin	44.170	10509.6768	256.782	7.1937
Total		191848.5454		169.6154

The levels of total protein, albumin and globulin of IT700ARBS, IT900ARBS, IT1100ARBS and IT25SM groups were significantly ($p < 0.05$) increased compared to the IC group (Figure 2). In contrast, the total bilirubin, total cholesterol and triacylglycerol concentrations of IT700ARBS, IT900ARBS, IT1100ARBS and IT25SM groups were significantly ($p < 0.05$) reduced compared to the IC group (Figure 3).

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and gamma glutamyltransferase (GGT) of IT700ARBS, IT900ARBS, IT1100ARBS and IT25SM groups were significantly ($p < 0.05$) decreased compared to the IC group (Figure 4).

Similarly, A/G ratio of IT700ARBS, IT900ARBS, IT1100ARBS and IT25SM groups significantly ($p < 0.05$) increased compared to the IC group, while the AST/ALT, and GGT/ALP ratios of IT700ARBS, IT900ARBS, IT1100ARBS and IT25SM groups were significantly ($p < 0.05$) reduced compared to the IC group (Figure 5).

The level of malondialdehyde (MDA) of IT700ARBS, IT900ARBS, IT1100ARBS and IT25SM groups were significantly ($p < 0.05$) decreased compared to the IC group (Figure 6). In contrast, the activities of superoxide desmutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) of IT700ARBS, IT900ARBS, IT1100ARBS and IT25SM groups were significantly ($p < 0.05$) increased compared to the IC group (Figure 7). The body weights of IT700ARBS, IT900ARBS, IT1100ARBS and IT25SM groups were significantly ($p < 0.05$) increased compared to the IC group (Figure 8).

The photomicrographs showing the effects of the extract from ARBS on the liver architecture of experimental animals are shown in figures 9.1 to 9.6. Figure 9.1 showed the normal histologic features of the liver of rats in NC, showing normal hepatocyte cords (H) radiating from the central vein (CV), and the sinusoids (S). Photomicrograph of hepatocytes in the IC rats showed liver tissues with hepatocytic distortions, characterized by intracytoplasmic vacuolation and microvesicular steatosis (arrow in Figure 9.2) compared to those of the NC (Figure 9.1) and the RC (Figure 9.6). Experimental rats in IT700ARBS, IT900ARBS, IT1100ARBS that were given different graded doses of the extract showed liver tissues with normal histological architectures when compared to those of the IC (Figures 9.3-9.5).

Figure 1: Chromatogram Showing the Phytochemical Composition of Aqueous Extract of Root Bark of *Salacia nitida*

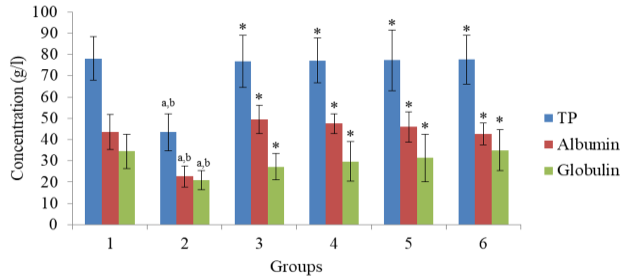

Figure 2: Effects of Aqueous Extract of Root Bark of *Salacia nitida* on Total Protein, Albumin, and Globulin Concentrations in Rats with Alcohol-Induced Hepatotoxicity
a,b,* = values are significant ($p < 0.05$); n = 6.

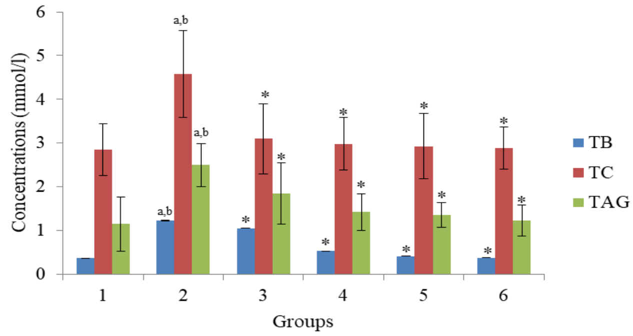

Figure 3: Effects of Aqueous Extract of Root Bark of *Salacia nitida* on Total Bilirubin (TB), Total Cholesterol (TC), and Triacylglycerol (TAG) Concentrations in Rats with Alcohol-Induced Hepatotoxicity.
a,b,* = values are significant ($p < 0.05$); n = 6.

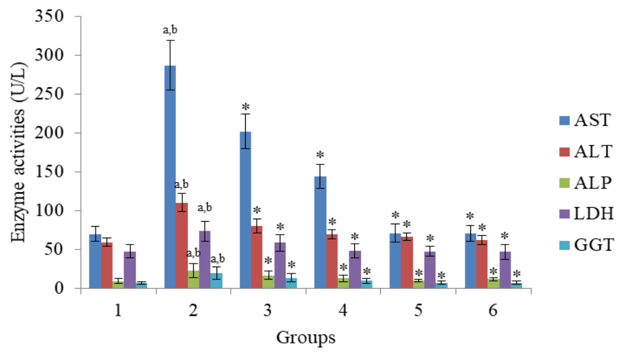

Figure 4: Effects of Aqueous Extract of Root Bark of *Salacia nitida* on Activities of Aspartate Aminotransferase (AST),

Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), and Gamma Glutamyltransferase (GGT) in Rats with Alcohol-Induced Hepatotoxicity
a,b,* = values are significant ($p < 0.05$); n = 6.

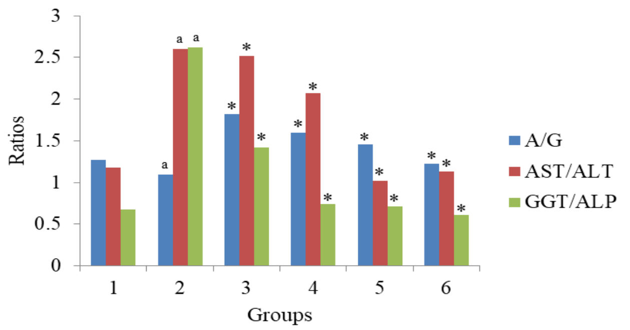

Figure 5: Effects of Aqueous Extract of Root Bark of *Salacia nitida* on A/G, AST/ALT, GGT/ALP Ratios in Rats with Alcohol-Induced Hepatotoxicity
a,b,* = values are significant ($p < 0.05$); n = 6.

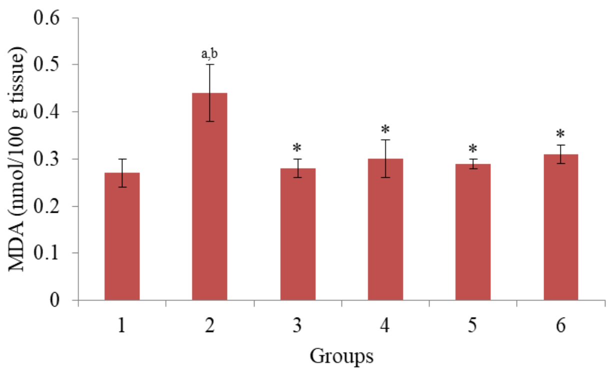

Figure 6: Effects of Aqueous Extract of Root Bark of *Salacia nitida* on Malondialdehyde (MDA) in Rats with Alcohol-Induced Hepatotoxicity
a,b,* = values are significant ($p < 0.05$); n = 6.

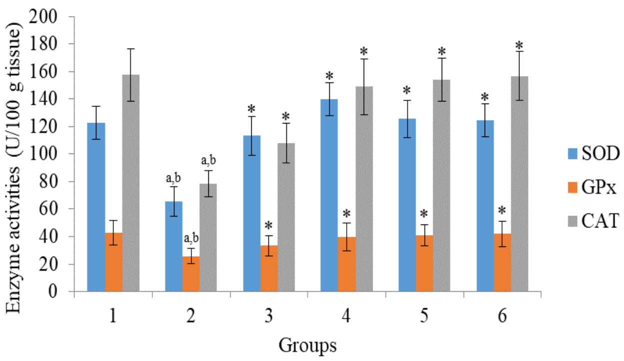

Figure 7: Effects of Aqueous Extract of Root Bark of *Salacia nitida* on the Activities of Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Catalase (CAT) in Rats with Alcohol-Induced Hepatotoxicity
a,b,* = values are significant ($p < 0.05$); n = 6.

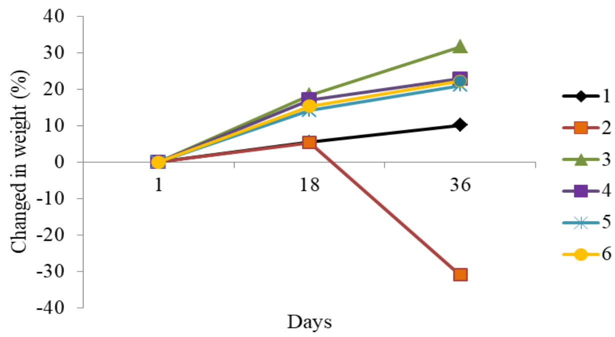

Figure 8: Effects of Aqueous Extract of Root Bark of *Salacia nitida* on Body Weights of Rats with Alcohol-Induced Hepatotoxicity

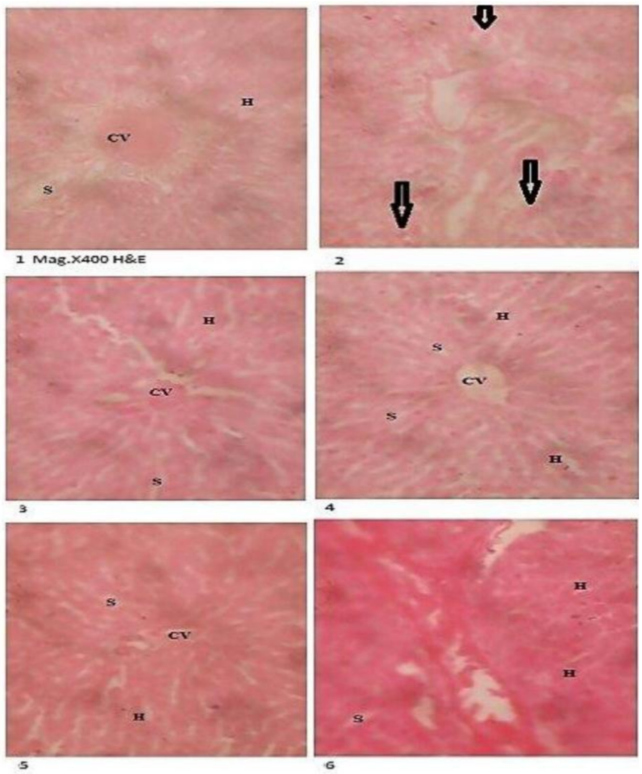

Figure 9: Photomicrograph of Liver Sections Showing Histopathological Changes in Liver Cells of Experimental Rats Showing Hepatocyte Cords (H), Central Vein (CV), and Hepatic Sinusoid (S).

DISCUSSION

In most parts of the world, medicinal plants contribute greatly to the healthcare of the people, since many rural populations with low socioeconomic status depend on plant materials for their well-being. The yield of the plant material was good, though factors like temperature, polarity, type of solvent used, rate of evaporation might affect the yield.

Medicinal plants are very rich in a variety of bioactive compounds. The relationship between the phytochemical compounds in plant materials and their pharmacological activities has been proven (Farooq *et al.*, 2022). Our GC-FID results (Figure 1) showed that ARBS is rich in phytochemicals with hepatoprotective, anti-oxidative, anti-inflammatory, and lipid-lowering effects (Kim *et al.*, 2024). The hepatoprotective potential of secondary metabolites are due to their antioxidant and anti-inflammatory activities. Polyphenols like tannins and flvonoids have been reported to exert hepatoprotective effect by scavenging free radicals and suppressing the generation of reactive oxygen species (ROS) (Koka *et al*., 2024). It has been shown that polyphenolic compounds can lower the risk of onset and progression of serious disease conditions like liver diseases, as a result of their inherent antioxidant potentials (Yousef *et al*., 2016). Tannins, a polyphenol present in the extract (Figure 1) have been proven to exhibit a protective effect on the liver by significantly decreasing the levels of hepatic enzymes and inhibiting oxidative stress (Sobeh *et al*., 2017; Abu *et al*., 2022). Also, the antioxidant and anti-inflammatory activities of tannins have been unraveled (Da Silva *et al*., 2022). Flavonoids have been reported to show hepatoprotective, enzyme modulating, lipid lowering, anti-inflammatory, antioxidative, and free radical scavenging functions (Kim *et al*., 2024; Wan *et al*., 2024; Jomova et al., 2025). The anti-inflammatory effect of flavonoids is due their abilities to reduce pro-inflammatory compounds by decreasing cytokines such as TNF-α, IL-1β, IL-6, NF-kB, prostaglandins, reducing the activities of lipoxygenases (LOX), cyclooxygenases (COX), and inducible nitric oxide synthases (iNOS), and modulating the production of ROS (Muhammad *et al*., 2024). Rutin and kaempferol are reportedly used for treating liver problems (Pan *et al*., 2014; Zang *et al*., 2017), while naringenin, naringin, flavan-3-ol, flavones, epicatechin, flavanones, and anthocyanins are said to exhibit hepatoprotective, antioxidant, anti-inflammatory, and hypocholesterolemic properties (Frattaruolo *et al*., 2019; Banach *et al*., 2020; De Araujo *et al*., 2020; Chagas *et al*., 2022; Mohammed & Khan, 2022; Yang *et al*., 2022; Mansour *et al*., 2023; Muhammad *et al*., 2024; Fayaz *et al*., 2025; Wang *et al*., 2025) and to attenuate lipid peroxidation (Al-amer *et al.*, 2023; Peng *et al.*, 2024). Steroid has been shown to exhibit hypocholesterolemic properties (Tenorio *et al*., 2021). Phytate and oxalate are phytoconstituents with antioxidative properties (Liguori *et al*., 2018; Da Silva *et al.*, 2019). It was generally observed that the protective effects of ARBS on the biochemical indices studied were dose-dependent and that the rich phytochemicals in ARBS are responsible for its protective actions against ethanol-induced hepatic injury. Also, that the synergistic and polyvalent actions of these secondary metabolites make it suitable for the pretreatment of hepatic damages.

Perturbations in biochemical indices was observed in this study. Raised levels of TB, TC, and TAG, with low levels of TP, albumin, globulin, and A/G ratio, are indications of hepatic injuries (Yue *et al*., 2006; Vasudevan & Sreekumari, 2007; Deshpande *et al*., 2014; Thakur *et al*., 2024). Raised levels of AST, ALT, GGT, ALP, LDH, AST/ALT, and GGT/ALP ratios recorded in this work are indications of hepatic injuries (Yue *et al*., 2006). AST level higher than ALT is an indication of alcoholic liver disease (ALD) (Vasudevan & Sreekumari, 2007). Our result for A/G ratio is in tandem with the report of Das and Vasudevan (2005), which states that a low A/G ratio is also caused by alcoholic liver disease (ALD). Pre-treatments of hepatic injury with different doses of ARBS attenuated hepatic damages in the animals. Our findings are in line with that of Padmanabhan and Jangle (2014). AST, ALT, ALP, GGT, and LDH are cytosolic enzymes of the liver that can leak into circulation when hepatic cell membranes are damaged. AST/ALT ratio greater than 2 and GGT/ALP ratio greater than 2.5 are quite suggestive of ALD (Mendenhall, 1981; Goldberg *et al*., 1986; Vasudevan & Sreekumari, 2007).

Chronic alcohol consumption activates the formation of free radicals, including lipid peroxidation (Cederbaum, 2001). Das and Vasudevan (2005), reported that too much consumption of alcohol disrupts the antioxidant system, and our results show that SOD, GPx, and CAT were downregulated. Elevation of GGT activity in our work is an indication of increased oxidative stress related to excess alcohol consumption (Bai *et al*., 2022). Also, Nwiloh *et al*. (2021) have reported that *S. nitida* root bark ameliorates lipid peroxidation and hepatomegaly in *Plasmodium berghe*i malaria infected rats. The significant decrease in body weight observed in our study might be due to excessive consumption of ethanol which affects digestion and nutrients intake (Thomson and Pratt, 1992; Chao *et al*., 2019). It was seen that pre-treatments of animals with the ARBS improved the body weights of the animals, an indication that the extract is protective and did not cause any biochemical abnormality.

Alcohol consumption causes distortion in liver architecture. The distortion of hepatic cells is associated with inflammation, fatty infiltration, and necrosis of hepatocytes (Osna *et al*., 2017; Subramaniyan *et al*., 2021). This feature was observed in animals induced with ethanol. However, the extract was able to reversed the ethanol-induced deformation of the hepatic tissues, suggesting that ARBS has antioxidant potential.

CONCLUSION

This study confirmed that ARBS exhibits hepatoprotective properties, as the perturbations in biochemical indices, body weights, and distortions in liver architecture were attenuated by the extract, which lends credence to the folkloric claims regarding the use of the plant's root bark in treating liver diseases. Also, ARBS was shown to contain bioactive phytochemicals that could be explored in the pharmaceutical industry for the production of novel plant-derived drug formulations and supplements for the treatment and management of liver diseases.

AUTHORS' CONTRIBUTIONS

BIN - conceptualisation, methodology, original draft preparation of manuscript, formal data analysis, project administration, project supervision, and funding; POO - formal data analysis, writing-review and editing of manuscript, supervision; UOU - data curation, supervision, writing - review and editing of manuscript. All authors have read and agreed to the published version of the manuscript.

FUNDING STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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