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Assessment of Hepatoprotective Potential of *Sapindus emarginatus* Vahl. — A Pharmacognostic and Phytochemical Study

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Abstract

This study aimed to evaluate the hepatoprotective potential of drugs from herbal origins. Using a stepwise pharmacognostic and phytochemical approach, plant material was standardized to improve the purity, safety, and efficacy of herbal medicine for treating Drug-Induced Hepatotoxicity (DIHT). The literature review identified plants from folklore medicine that have hepatoprotective and nephroprotective effects but are not documented. So, the present research focused on the hepatoprotective activity of the drug by evaluating the alanine aminotransferase, alanine phosphatase, total protein, cholesterols and bilirubin levels. First the bark of the plant *S. emarginatus* Vahl., was studied for pharmacognostic evaluation and phytochemical evaluation by using various tests. Animals were divided into 5 groups with six animals in each group namely, control, standard control, toxicity control, ethanolic extract and water extract for pharmacological evaluation. Treatment with hepatotoxic (paracetamol) led to the depletion of glutathione and reduction in non-protein sulfhydryl moiety, which is further increased, were observed by treating with ethanolic and water extracts compared to the positive standard group. The key findings of this study highlight the potential of traditional folk medicine, in treating drug-induced hepatotoxicity. The findings of this study highlight the need for further research in identifying natural alternative medicines to treat drug-induced hepatotoxicity.

Major Findings: The novelty of this research lies in its comprehensive exploration of the hepatoprotective potential of *Sapindus emarginatus* Vahl. bark, which has not been documented despite its traditional use. The study employs a systematic standardization approach to ensure the quality and efficacy of the plant material, bridging traditional knowledge with modern scientific validation. Additionally, the in vivo evaluation against paracetamol-induced liver toxicity provides novel experimental evidence, offering insights into the plant's mechanisms of action and supporting its potential as a source of hepatoprotective phytochemicals.

Keywords: Alanine Aminotransferase, Alanine Phosphatase, Bilirubin, Drug-Induced Hepatotoxicity, Hepatoprotective Activity

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1. Introduction

The liver plays a crucial role in maintaining homeostasis and detoxification within the body. However, it is susceptible to damage from various factors such as toxins, infections, and metabolic disorders. Hepatoprotective drugs are essential in preventing and treating liver damage, and the search for effective hepatoprotective agents has led to the exploration of herbal sources[1,2]. Herbal drugs have been used for centuries in traditional medicine systems and are increasingly recognized for their potential in modern pharmacology. This study aims to evaluate the hepatoprotective potential of herbal drugs through a comprehensive pharmacognostic and phytochemical study[3]. Traditional medicine systems, including Ayurveda, Traditional Chinese Medicine, and Indigenous systems, have long relied on herbal remedies for the treatment of various ailments, including liver disorders. The rich biodiversity of plant species has provided a vast source of potential therapeutic agents, many of which have been used empirically for hepatoprotective purposes. However, the acceptance of herbal medicines in developed countries has been hindered by the lack of documentation and stringent quality control[4]. This highlights the need for research and documentation of the therapeutic potential of herbal drugs, particularly in the context of hepatoprotective agents. Standardization and documentation of traditional medicines are essential to ensure their safety, efficacy, and reproducibility[5]. The process of standardization involves comprehensive pharmacognostic and phytochemical studies to identify and characterize the active constituents of herbal drugs. These studies provide valuable information about the macroscopic and microscopic characteristics of plant parts, as well as their chemical composition and pharmacological properties. By establishing standardized methods for the identification and quality assurance of herbal medicines, their purity, safety, and efficacy can be ensured[6]. This study aims to contribute to the growing body of scientific knowledge on the hepatoprotective potential of herbal drugs. By conducting a rigorous pharmacognostic and phytochemical evaluation of the bark, *S. emarginatus* Vahl. The study seeks to identify the active constituents responsible for its hepatoprotective activity. The findings of this research will not only provide valuable insights into the potential of the herbal drug *S. emarginatus* Vahl. as a hepatoprotective agents but also contribute to the standardization and documentation of traditional medicines. In summary, the importance of hepatoprotective drugs from herbal sources cannot be overstated, especially in the context of the global burden of liver diseases[7]. The need for documentation and standardization of traditional medicines is critical to ensure their safety, efficacy, and acceptance in modern pharmacology. This study aims to address these needs by evaluating the hepatoprotective potential of herbal drugs through a scientifically and statistically sound approach, contributing to the advancement of herbal medicine research and practice. Based on the literature review, it is evident that *S. emarginatus* Vahl. has been extensively studied for a wide range of pharmacological activities[8]. The studies have explored various aspects of its therapeutic potential, including hepatoprotective, antidiabetic, antihyperlipidemic, CNS activity, antibacterial, antimosquito, antioxidant, anti-obesity, and more. These investigations have provided valuable insights into the plant's bioactive compounds and their potential applications in treating various health conditions. The justification for selecting these plants from folklore medicine and conducting a significant literature review aligns with the need to understand their ethnopharmacological therapeutic activity and chemical constituents for assessing their hepatoprotective and nephroprotective effects. The focus on hepatoprotective drugs is crucial, as they play a vital role in providing defense against oxidative stress and injury in liver cell membranes[9]. The evaluation of oxidative stress markers such as glutathione, glutathione peroxidase, superoxide dismutase, lipid peroxidation, and γ-glutamyl transpeptidase provides a comprehensive understanding of the mechanisms involved in hepatoprotection. Furthermore, the assessment of serum markers such as SGPT, SGOT, bilirubin, alkaline phosphatase, and cholesterol in response to hepatotoxic agents like paracetamol offers valuable insights into the potential of *S. emarginatus* Vahl. as a hepatoprotective agent. Overall, the extensive literature review and the diverse range of studies conducted on *S. emarginatus* Vahl. underscore its significance as a potential source of bioactive compounds with therapeutic implications for various health conditions, particularly in the context of hepatoprotection[10]. This study aimed to evaluate the hepatoprotective potential of drugs from herbal origins through stepwise pharmacognostic and phytochemical studies. The study aimed to standardize the plant or its part of plant material to be used as medicine, which would contribute to the purity, safety, and efficacy of herbal medicine[11]. The focus was primarily on the hepatoprotective effects of the drugs and the identification of the parameters of DIHT. Therefore, the

resulting knowledge and findings of the research will aid in the development of safe and effective hepatoprotective medication.

2. Materials and Methods

The materials and methods employed in the study are outlined below[12,13]. The materials and methods of the study included the selection and preparation of plant materials, pharmacognostic and phytochemical studies, extraction techniques, and experimental procedures.

2.1 Selection and Preparation of Plant Materials

The bark of the plant of *S. emarginatus* Vahl. (Figure 1) was collected in the autumn, spring or early summer seasons. The plant was collected from the medicinal garden of QIS College of Pharmacy, Ongole. Macroscopical evaluation of different features, such as colour, odour, taste, size, shape, and texture, was performed botanically. Morphological and organoleptic features were measured with appropriate tools and compared with the reference test.

2.2 Pharmacognostic Study

A proper pharmacognostic screening of the plant material was carried[14]. Standard methods were utilized to estimate the phytochemicals that are present in the plant which include tannins, flavonoids, saponins, steroids, alkaloids, carbohydrates, and fats and oils.



Figure 1. Bark of *S. emarginatus* Vahl.

2.3 Extraction Techniques

An initial pulverization of the plant powder was carried out. The phytochemicals were extracted using both polar and non-polar solvents to isolate different plant secondary metabolites, resulting in a crude extract. Sequential partitioning was carried out until no more phytochemicals were observed. The concentration of the crude extract resulted in the obtaining of semi-solid drugs.

2.4 Experimental Procedure

Paracetamol was used to induce liver toxicity in experimental rats. The animals were divided into five groups of six rats each, namely; control-2ml/kg, toxicity control (Paracetamol-50mg/kg), standard control (Sylimarin-200mg/kg, sample tested group (Ethanolic extract-200mg/kg and sample tested group (water extract 200mg/kg) drugs were administered through oral route, and the route of administration for both paracetamol and the *Sapindus emarginatus* extracts in rats was oral. The control group received saline, the toxicity control received paracetamol, and standard control received silymarin. The sample tested groups were given the semi-solid drugs from *S. emarginatus* Vahl. The research work has been approved by the IAEC committee QISCP/IAEC/02/2024.

Forty-eight-hours after the induction of liver toxicity, the animals' blood samples were collected and analyzed for different liver function parameters such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and bilirubin[15,16]. Phytochemical screening of the extracted crude material was used to identify the protective and therapeutic potential of the semi-solid drugs against liver injury. These semi-solid drugs were tested for hepatoprotective effects against liver toxicity induced by paracetamol in experimental rats[17,18].

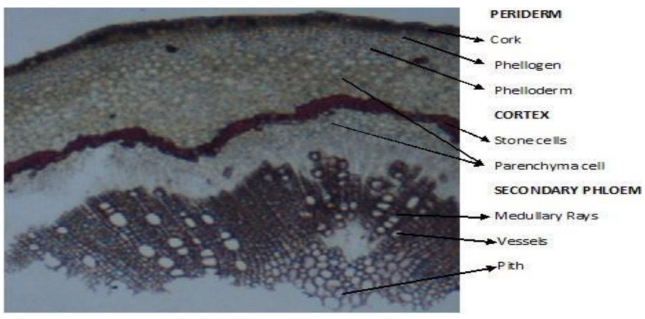


Figure 2. The transfer section bark of *S. emarginatus* Vahl.

3. Results

3.1 Pharmacognostic Evaluation

3.1.1 Macroscopic and Microscopic Evaluation

The macroscopic examination of the plant material (Figure 2) revealed the presence of periderm, cortex, and secondary phloem contents[19-21]. The microscopic evaluation revealed the presence of parenchyma, fibres and vessels (Table 1).

3.1.2 Proximate Analysis

The proximate composition of *S. emarginatus* Vahl. bark includes extractive values, moisture content, and ash values. Among the extractives, the bark demonstrates 5.12% solubility in petroleum ether, 14.72% in alcohol, and 17.6% in water, reflecting its varying solvent affinities. The total moisture content is 5.8%, suggesting moderate water retention. Ash analysis reveals a total ash content of 9.5%, with specific values for water-soluble ash at 4.05%, acid-insoluble ash at 1.45%, and sulfated ash at 8%. Together, these measurements provide a comprehensive understanding of the bark's chemical properties (Table 2).

3.2 Phytochemical Evaluation

The phytochemical analysis of the extracts[22-24] revealed the presence of various bioactive compounds, including flavonoids, tannins, steroids, and phenolic compounds shown to ethanolic and water extract thus both are administered to animals (Table 3).

Table 1. Macroscopic characters of the barks of *Sapindus emarginatus* Vahl.

S. No.	Features	Observation
1.	Color	Outer bark -grey color
		Inner bark - yellow to brown color
2.	Odor	Odorless
3.	Taste	Bitters, Slightly saccharine.
4.	Size	Length - 2 cm. to 15 cm.
		Width - 1.5 cm. to 7 cm.
		Thickness - Up to 1 cm.
5.	Texture	Hard
6.	Shape	Curved – channeled
7.	Special	Short fracture

3.3 Pharmacological Evaluation

3.3.1 Animal Studies

The rats were divided into five groups, with six rats in each group. Groups I-V received various treatments, including silymarin as a standard drug and doses of the ethanol and water extract of the bark.

The results of the study showed that the ethanol and water extract of the bark exhibited significant hepatoprotective potential against paracetamol-induced hepatotoxicity in rats indicated by an increase in the levels of liver function parameters (Figure 3) such as ALT, AST, and bilirubin[25]. Also showed an increase in triglycerides, cholesterol, albumin and total protein levels when compared with the toxicity control. The hepatoprotective action may be attributed to the free radical scavenging potential of the bioactive compounds present in the extract[26,27] (Table 4). In the treatment groups receiving *S. emarginatus* extracts, there was a significant decrease in liver function parameters (*e.g.*, SGPT, SGOT, bilirubin, and alkaline phosphate) compared to the paracetamol toxicity control group. This decrease indicates a protective effect of the extracts, as the elevated liver markers induced by paracetamol were reduced closer to normal levels following treatment with the extracts. The standard control group treated with silymarin also demonstrated a similar decrease in these parameters, supporting the hepatoprotective potential of the extracts.

Table 2. Proximate values of barks of *Sapindus emarginatus* Vahl.

S. No.	Parameter	Determined Value (% w/w)
A. Extractive values		
1.	Petroleum ether soluble extractive value	5.12
2.	Alcohol soluble extractive value	14.72
3.	Water soluble extractive value	17.6
B. Moisture contents		
4.	Total Moisture content	5.8
C. Ash values		
5.	Total ash	9.5
6.	Water soluble ash	4.05
7.	Acid insoluble ash	1.45
8.	Sulfated ash	8

Table 3. Qualitative phytochemical tests of *Sapindus emarginatus* Vahl.

Method		Hot				Cold
Phyto constituents	**Chemical tests**	**PE Extract**	**CHLF Extract**	**MTOH Extract**	**ETOH Extract**	**H_2O Extract**
Carbohydrate	Molisch's test	- ve	- ve	+ ve	+ ve	+ ve
	Fehling test	- ve	- ve	+ ve	+ ve	+ ve
Protein and Amino acids	Millon's tests	- ve	- ve	+ ve	+ ve	+ ve
	Ninhydrin tests	- ve	- ve	- ve	- ve	- ve
Sterol and triterpenoids	Salkowski test	+ ve	+ve	+ ve	- ve	- ve
	Liebermann-Burchardt tests	+ ve	+ve	- ve	- ve	- ve
Glycoside	Water solution	- ve	- ve	+ ve	+ ve	+ ve
	Sodium hydroxide Solution	- ve	- ve	+ ve	+ ve	+ ve
Saponin	Haemolysis test	- ve	- ve	+ ve	+ ve	+ ve
	Foam tests	- ve	- ve	+ ve	+ ve	+ ve
Alkaloids	Dragendroff's test	- ve	+ ve	+ ve	- ve	- ve
	Wagner's test	- ve	+ ve	+ ve	- ve	- ve
Flavonoids	Shinoda test	- ve	- ve	+ ve	+ ve	+ ve
	Ferric chloride test	- ve	- ve	+ ve	+ ve	+ ve
Tannins	Phenazone test	- ve	- ve	+ ve	+ ve	+ ve
	Gelatin test	- ve	- ve	+ ve	+ ve	+ ve
Phenols	Zinc-Hydrochloride reduction test	- ve	- ve	+ ve	+ ve	+ ve
Fixed oil and Fat	Spot test	+ ve	- ve	+ ve	+ ve	- ve

Where, (+ ve) = Present and (- ve) = Absent

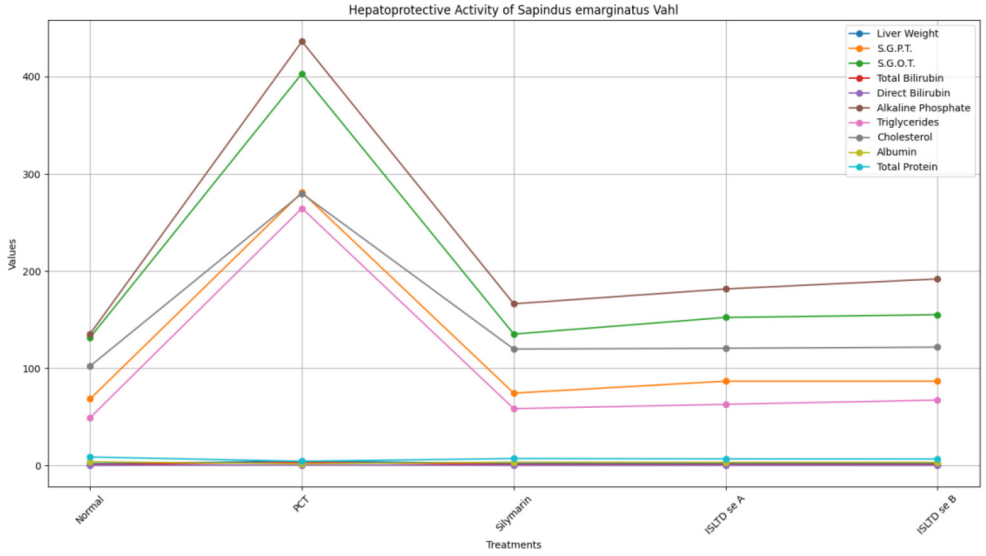


Figure 3. Hepatoprotective activity of extracts of *S. emarginatus* Vahl.

Table 4. Hepatoprotective activity of extracts of *Sapindus emarginatus* Vahl

Treatment	Group 1 Normal	Group 2 PCT	Group 3 Silymarin	Group 4 (ISTD se A)	Group 5 (ISLTD se B)
Liver weight	1.988 ± 0.023	4.505 ± 0.022###	2.273 ± 0.006***	2.440 ± 0.011***	2.443 ± 0.008***
S.G.P.T.	68.59 ± 0.016	281.2 ± 0.015###	74.57 ± 0.007***	86.87 ± 0.008***	86.88 ± 0.014***
S.G.O.T.	131.8 ± 0.009	403.2 ± 0.040###	135.3 ± 0.043***	152.4 ± 0.013***	155.2 ± 0.012***
Total Bilirubin	0.931 ± 0.007	3.423 ± 0.013###	1.065 ± 0.007***	1.053 ± 0.010***	1.055 ± 0.010***
Direct Bilirubin	0.255 ± 0.012	0.680 ± 0.005###	0.280 ± 0.015***	0.321 ± 0.006***	0.306 ± 0.008***
Alkaline Phosphate	135.6 ± 0.021	436.3 ± 0.017###	166.4 ± 0.011***	181.7 ± 0.013***	192.0 ± 0.007***
Triglycerides	49.32 ± 0.008	264.7 ± 0.009###	58.64 ± 0.012***	63.05 ± 0.011***	67.43 ± 0.012***
Cholesterol	102.4 ± 0.008	279.9 ± 0.012###	119.9 ± 4.223***	120.7 ± 0.013***	121.9 ± 0.012***
Albumin	3.963 ± 0.007	1.953 ± 0.010###	3.555 ± 0.010***	3.385 ± 0.010***	3.412 ± 0.007***
Total protein	8.840 ± 0.011	4.502 ± 0.023###	7.302 ± 0.006***	6.943 ± 0.003***	6.865 ± 0.005***

PCT-Paracetamol Toxicity; (ISLTD se A) - isolated ethyl acetate fraction compound ethanol extract; (ISLTD se B) -isolated ethyl acetate fraction compound water extract

In this study, statistical analysis was applied to compare the effects of treatments on various biochemical parameters indicative of liver function across the treatment groups. Specifically, data are presented as mean±standard deviation (SD), and significance levels are indicated with symbols (### for paracetamol-treated groups compared to normal control, and *** for treatment groups compared to the toxicity control). Typically, such studies employ statistical tests like one-way ANOVA followed by post hoc tests Dunnett's to evaluate the significance of differences between groups. This ensures that comparisons between multiple treatment groups (*e.g.*, normal control, paracetamol-induced toxicity control, silymarin-treated, and extract-treated groups) are statistically validated.

4. Discussion

The present investigation aimed to research and evaluate the hepatoprotective potential of drugs from herbal origins. The pharmacognostic screening protocols employed in this study were used to identify active constituents in the plant material to be used as hepatoprotective drugs[28-34]. The results demonstrated the presence of flavonoids, tannins, steroids, and phenolic compounds which are bioactive components and have therapeutic value. These findings support the efficacy of alternative medicine in treating drug-induced hepatotoxicity.

The hepatoprotective activity of the semi-solid drugs prepared from the plant material was studied in laboratory animals. *In-vivo* studies showed that the semi-solid drugs were effective hepatoprotective agents. The rats administered paracetamol exhibited a significant increase in ALT, AST, and bilirubin levels, which indicated liver stress. The key findings of this study identified several parameters that indicate DIHT; increased levels of alanine aminotransferase, alanine phosphatase, and bilirubin were common indicators. Intended for the therapeutic regimen of liver diseases, we explicitly investigated the phytochemical characteristics of the semi-solid drugs. Saponins have been linked with many pharmacological effects, including hepatoprotective effects against liver damage, injury, and oxidative stress. Almost 60% of quinols isolated from the plant material display hepatoprotective activity. Flavonoids, including catechin, and naringenin, among others, hold essential chemo-preventive activity against hepatocellular injury. Flavonoids demonstrate significant activity against free radicals, including reactive oxygen species, which are hectic players of oxidative stress in hepatocytes.

The study highlighted the identification of potentially valuable sources of hepatoprotective drugs and provided valuable insight into how plant-derived remedies are so beneficial in treating liver diseases. The results obtained from animal studies demonstrated that the semi-solid drugs mitigate liver injury induced by paracetamol. Flavonoid content present corroborates with their hepatoprotective activity. Hence, these findings portrayed promising therapeutic potential in the fight against drug-induced hepatotoxicity. The results of this study signify the importance of alternative medicine systems, such as traditional herbal medicines in treating drug-

induced hepatotoxicity. The findings of this study will aid the scientific community in bridging the gap between modern and traditional medicine[35-37].

5. Conclusion

In conclusion, the study highlights the potential of *S. emarginatus* Vahl. as a source of bioactive compounds with therapeutic implications for various health conditions, particularly in the context of hepatoprotection. The results of the study provide a scientific basis for the traditional use of *S. emarginatus* Vahl. bark as a medicinal plant. Further studies are warranted to explore the full therapeutic potential of the plant extract and its active constituents.

6. Acknowledgement

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