IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
onal Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 6, Issue 1, July 2026 Impact Factor: 8.2

In Vitro Assessment of Hepatoprotective Activity of Hibiscus arnottianus Leaf Extract

Avantika Kamble, Asif Mulani, Harshada Kadam, Neha Sakale, Kajal Padlkar
Nootan College of Pharmacy, Kavathemahankal, Sangli, Maharashtra, India
avantikakamble2405@gmail.com

Abstract: *The present study aimed to evaluate the in vitro hepatoprotective activity of Hibiscus arnottianus leaf extract against CCl□-induced toxicity in HepG2 cells. Fresh leaves were collected, shade-dried, powdered, and extracted using ethanol by the maceration method. Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, steroids, and alkaloids. TLC analysis confirmed the presence of phytochemical constituents with Rf values of 0.56 and 0.25. Hepatoprotective activity was assessed using the MTT assay. The extract showed dose-dependent protection against CCl□-induced liver cell damage, with cell viability increasing from 36.78% at 50 µg/ml to 61.26% at 200 µg/ml. Although the activity was lower than the standard drug Silymarin, the results demonstrated significant hepatoprotective potential. The study suggests that Hibiscus arnottianus leaves may serve as a promising natural source of hepatoprotective compounds.*

Keywords: Hibiscus arnottianus, Hepatoprotective Activity, HepG2 Cells, Phytochemical Screening, MTT Assay.

I. INTRODUCTION

The liver is one of the most important organs in the human body and performs a wide range of physiological functions, including metabolism, detoxification, protein synthesis, and regulation of biochemical processes essential for maintaining health. It plays a vital role in eliminating toxins, drugs, and harmful metabolites from the body. However, continuous exposure to environmental pollutants, alcohol, drugs, viral infections, and toxic chemicals can lead to liver damage and various hepatic disorders.[1] Liver diseases such as hepatitis, cirrhosis, fibrosis, and hepatocellular carcinoma are major causes of morbidity and mortality worldwide. Although several synthetic drugs are available for the treatment of liver disorders, their long-term use is often associated with adverse effects and limited therapeutic efficacy. Therefore, there is a growing interest in the development of safer and more effective hepatoprotective agents from natural sources. Medicinal plants have been used for centuries in traditional systems of medicine for the prevention and treatment of various diseases. They contain numerous bioactive compounds such as flavonoids, phenolic compounds, alkaloids, tannins, glycosides, saponins, and terpenoids, which exhibit a wide range of pharmacological activities. Among these activities, antioxidant properties are particularly important because oxidative stress plays a significant role in the initiation and progression of liver injury. Plant-derived antioxidants can neutralize free radicals, reduce oxidative damage, and protect liver cells from toxic insults.[2-3]

Hibiscus arnottianus, a member of the family Malvaceae, is a medicinal plant native to Hawaii and known for its traditional therapeutic applications. Various species of the genus *Hibiscus* have been reported to possess antioxidant, antimicrobial, anti-inflammatory, anticancer, and hepatoprotective activities.[4] Previous phytochemical investigations have demonstrated that *Hibiscus arnottianus* contains several bioactive constituents, including flavonoids, phenolic compounds, tannins, anthocyanins, and other antioxidant molecules. These phytochemicals are believed to contribute to the medicinal value of the plant and may provide protection against cellular damage induced by oxidative stress. Carbon tetrachloride (CCl□) is a well-known hepatotoxic agent commonly used in experimental models to induce liver injury.[5] It generates reactive oxygen species and free radicals that cause lipid peroxidation, membrane damage, and cell

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death. HepG2 cells, a human liver-derived cell line, are widely employed as an in vitro model for evaluating hepatoprotective activity because they closely resemble normal hepatocytes in their metabolic functions. The MTT assay is a reliable and commonly used method for determining cell viability and assessing the protective effects of test compounds against toxic agents.[5-7]

The present study was designed to investigate the in vitro hepatoprotective activity of ethanolic leaf extract of *Hibiscus arnottianus* against CCl□-induced toxicity in HepG2 cells. In addition, preliminary phytochemical screening and thin-layer chromatographic analysis were performed to identify the presence of bioactive constituents. The findings of this study may provide scientific evidence supporting the traditional use of *Hibiscus arnottianus* and contribute to the development of novel plant-based hepatoprotective agents.

II. MATERIAL AND METHOD

Collection of Material

Fresh leaves of Hibiscus arnottianus were collected from Bhose village, Sangli district, Maharashtra. The leaves were washed thoroughly with water, shade-dried for about one and a half weeks, and then powdered using a mechanical grinder. The coarse powder obtained was stored in an airtight container and used for extraction and further studies. The materials used in this study included powdered Hibiscus arnottianus leaves, ethanol (95%), distilled water, conical flasks, beakers, glass rods, a magnetic stirrer/shaker, Whatman No. 1 filter paper, a rotary evaporator or water bath, and a hot air oven/desiccator for extraction and processing of the plant material.

Extraction (Maceration)[8-11]

Procedure

1. Fresh leaves of *Hibiscus arnottianus* were collected, washed, shade-dried, and powdered.
2. Twenty grams of the powdered sample was accurately weighed and transferred into a clean conical flask.
3. Two hundred milliliters of 95% ethanol (1:10 w/v) was added to the flask.
4. The flask was sealed with aluminum foil or a cotton plug to prevent solvent evaporation.
5. The mixture was kept on a magnetic stirrer/shaker at room temperature for 24–48 hours.
6. After maceration, the extract was filtered through Whatman No. 1 filter paper.
7. The plant residue was re-extracted once or twice with fresh solvent to obtain maximum extraction yield.
8. All filtrates were combined and collected in a clean container.
9. The solvent was removed using a rotary evaporator under reduced pressure at 40–45°C.
10. The concentrated extract was evaporated until a thick semisolid mass was obtained.
11. The dried extract was stored in an airtight container and used for further phytochemical screening and hepatoprotective activity studies.

In Vitro Hepatoprotective Assay

The screening of hepatoprotective activity was based on the protection of human liver-derived HepG2 cells againstCCl4-induced damage determined by estimating mitochondrial synthesis using the tetrazolium assay. The HepG2 cells were routinely grown and sub cultured as monolayers in DMEM supplemented with 10% fb the experiments in this investigation were conducted with cells. At this stage, the cells were harvested and plated at approximately 30,000 cells/well in 96well-microtitre plates (Nunclon) and left to rest for 24 h at 37°C in a humidified atmosphere of 5% CO2. The cells were then exposed to toxicant (medium containing 1% CCl4) medium alone (as normal) standard Silymarin. At the end of the period, cytotoxicity was assessed by estimating the viability of the HepG2 cells by the MTT reduction After 1 h incubation, the test solution from each well was removed by aspiration and replaced with50 µl of MTT prepared in MEM without phenol red (MEM-PR) The plates were gently shaken and incubated for 3 h at 37°C in a humidified 5% CO2 atmosphere. The supernatant was removed and 50 µl of propanol

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was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at 540 nm.[12-15]

III. RESULT

Authentication of Plant Material

The leaves of *Hibiscus arnottianus* used in this study were collected from Bhose village, Sangli district, Maharashtra, India, and authenticated by a qualified botanist. The authenticated plant specimen was identified as *Hibiscus arnottianus* belonging to the family Malvaceae. A voucher specimen was preserved for future reference.

Phytochemical analysis

Hibiscus arnottianus leaf extract has successfully had its starch extracted using a straight forward extraction method. The Phytochemical analysis of extracted starch carried out and result are as follow,

Table No. 1 Phytochemical analysis by extract

Sr. No.	Phytochemical Test	Result
1	Alkaloids (Dragendorff's Test)	Positive (+)
2	Flavonoids (Shinoda Test)	Positive (+)
3	Phenolic Compounds (Ferric Chloride Test)	Positive (+)
4	Tannins (Ferric Chloride Test)	Positive (+)
5	Saponins (Foam Test)	Positive (+)
6	Glycosides (Keller-Killiani Test)	Positive (+)
7	Terpenoids (Salkowski Test)	Positive (+)
8	Steroids (Liebermann-Burchard Test)	Positive (+)

Maceration Extraction Procedure

Maceration is a simple and widely used method for extracting active constituents from plant materials. In this method, the powdered plant material is soaked in a suitable solvent for a specific period to dissolve the bioactive compounds. It is commonly used in herbal research because it is easy, cost-effective, and does not require special equipment.

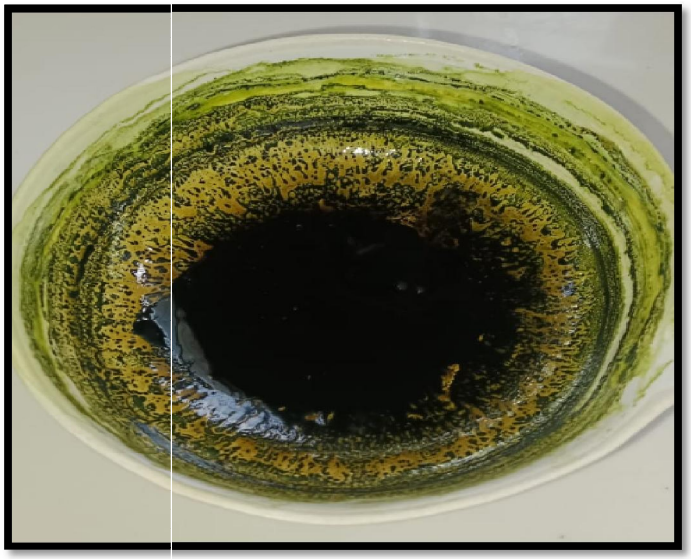

Fig. No. 1. Maceration Extraction

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TLC

The TLC analysis of *Hibiscus arnottianus* leaf extract was carried out using silica gel plates as the stationary phase and a suitable solvent system (e.g., Toluene:Ethyl acetate).

Fig. 2. TLC analysis of *Hibiscus arnottianus* leaf extract

Spot	Distance (cm)	Rf Value
Upper spot	4.5	0.56
Lower spot	2.0	0.25

Rf = Distance traveled by compound / Distance traveled by solvent front

In Vitro Hepatoprotective Assay

Table No 2. Effect of *Hibiscus arnottianus* leaf extract and standard Silymarin on CCl□-induced HepG2 cells determined by MTT assay.

Sr. No.	Concentration (µg/ml)	Absorbance				Cell Viability (%)
		1	2	3	Average Absorbance (OD)	
1	Control	2.326	2.325	2.328	2.326 ± 0.001	33.64
2	CCl□ Induced HepG2	0.788	0.812	0.748	0.782 ± 0.0263	53.16
3	Standard (Silymarin) 50	1.233	1.239	1.238	1.236 ± 0.0026	65.47
	100	1.521	1.522	1.526	1.523 ± 0.00216	82.70
	200	1.923	1.922	1.926	1.923 ± 0.0017	82.70
4	Sample Extract 50	0.852	0.856	0.859	0.855 ± 0.003512	36.78
	100	1.254	1.256	1.253	1.254 ± 0.001528	53.91
	200	1.425	1.425	1.426	1.425 ± 0.000577	61.26

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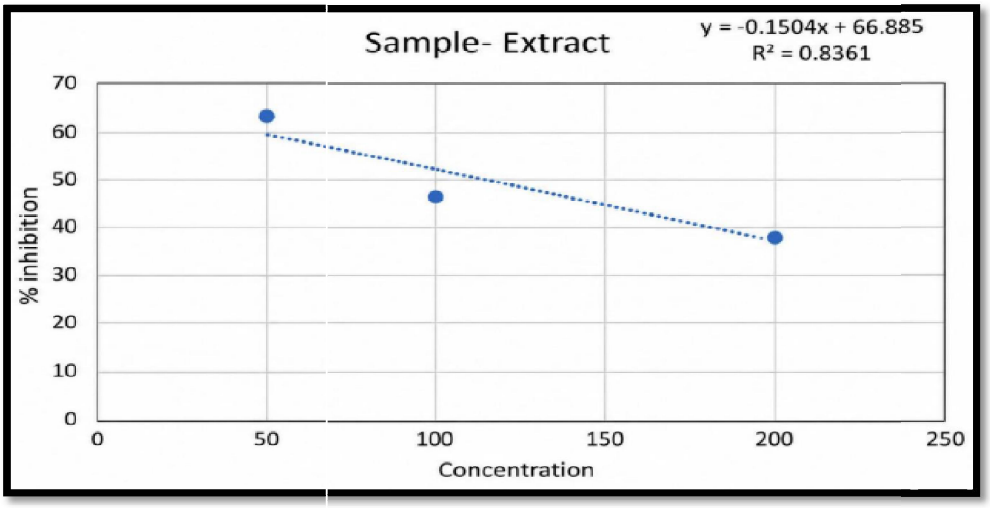

Fig. 3. Effect of **Hibiscus arnottianus** Leaf Extract on Cell Viability of CCl□-Induced HepG2 Cells.

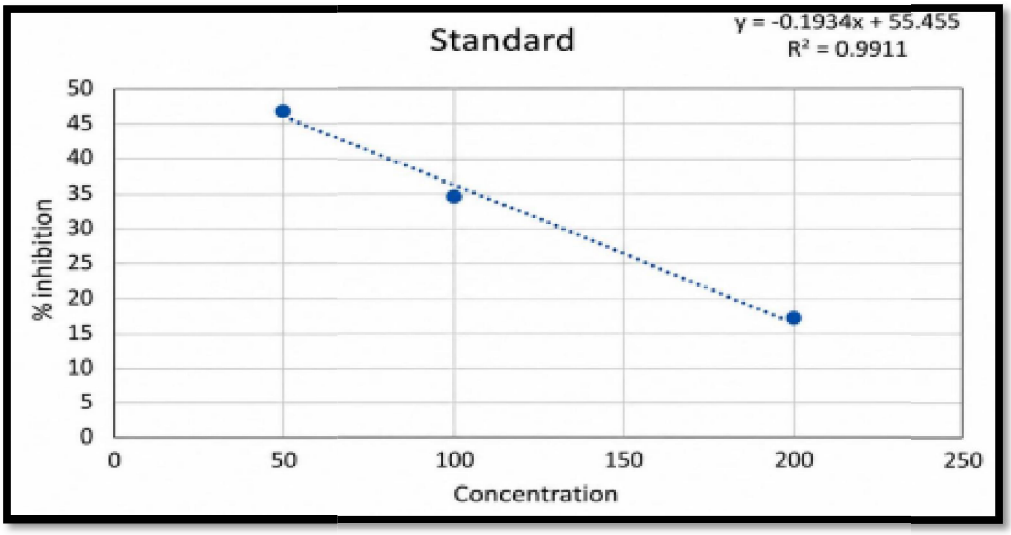

Fig. 4. Effect of Standard Silymarin on Cell Viability of CCl□-Induced HepG2 Cells.

The results of both graphs indicate that Hibiscus arnottianus leaf extract possesses significant hepatoprotective activity against CCl□-induced liver cell damage. The activity increased with increasing concentration of the extract, suggesting a dose-dependent response. Although the extract was less effective than the standard drug Silymarin, it demonstrated considerable protection of HepG2 cells, likely due to its antioxidant phytoconstituents. These findings support the traditional medicinal use of Hibiscus arnottianus and suggest its potential application in the development of herbal hepatoprotective formulations.

IV. CONCLUSION

The present study successfully evaluated the in vitro hepatoprotective activity of *Hibiscus arnottianus* leaf extract against CCl□-induced liver cell damage using the HepG2 cell line model. Phytochemical screening confirmed the

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presence of important bioactive constituents such as flavonoids, phenolic compounds, tannins, alkaloids, saponins, glycosides, steroids, and terpenoids, which are known for their antioxidant and protective properties. TLC analysis further indicated the presence of phytochemical components in the extract. The hepatoprotective activity was assessed using the MTT assay, and the results demonstrated that the ethanolic leaf extract provided significant protection against CCl□-induced cytotoxicity in a concentration-dependent manner. Cell viability increased progressively with increasing concentrations of the extract, indicating its ability to protect liver cells from toxic injury. Although the activity of the extract was lower than that of the standard drug Silymarin, it exhibited promising hepatoprotective effects. The observed activity may be attributed to the antioxidant properties of the phytoconstituents present in the extract, which help reduce oxidative stress and cellular damage. The final findings suggest that *Hibiscus arnottianus* leaves possess significant hepatoprotective potential and may serve as a valuable natural source for the development of herbal formulations for liver protection. Further in vivo studies and isolation of active compounds are recommended to confirm its therapeutic efficacy.

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