



Evaluation of Antioxidant and Hepatoprotective Activity of *Abutilon indicum* (L.) Sweet

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ABSTRACT

Medicinal plants play a significant role in traditional medicine and continue to be a valuable source of therapeutic agents. *Abutilon indicum* (L.) Sweet, commonly known as Indian mallow, belongs to the family Malvaceae and has been widely used in traditional systems of medicine for the treatment of various diseases including inflammation, ulcers, fever, and liver disorders. The present study aimed to evaluate the antioxidant and hepatoprotective potential of the ethanolic extract of *Abutilon indicum* seeds. The seeds were collected, dried, powdered, and extracted using ethanol by cold maceration technique. Preliminary phytochemical screening was conducted to identify the presence of biologically active constituents. Antioxidant activity was determined using the DPPH radical scavenging assay, while hepatoprotective activity was evaluated using the MTT assay on HepG2 liver cell lines. The phytochemical analysis revealed the presence of flavonoids, phenols, saponins, steroids, proteins, amino acids, and cardiac glycosides. The extract demonstrated concentration-dependent antioxidant activity with a maximum inhibition of 50.33% at 500 µg/ml. The IC₅₀ value of the extract was determined to be 114.9 µg/ml. The MTT assay showed dose-dependent effects on HepG2 cell viability. The results suggest that *Abutilon indicum* possesses considerable antioxidant and hepatoprotective potential. These activities may be attributed to the presence of phenolic compounds and flavonoids in the extract. Further studies are necessary to isolate the active constituents and evaluate their therapeutic efficacy.

Keywords: *Abutilon indicum*, Antioxidant activity, Hepatoprotective activity, DPPH assay, MTT assay

INTRODUCTION

Traditional medicine remains one of the most affordable and accessible sources of healthcare, especially in resource-poor communities. The use of plants for medicinal purposes dates back thousands of years, with evidence suggesting therapeutic applications as early as 4000–5000 BC. In India, references to medicinal plants appear in ancient texts such as the Rigveda. Over time, traditional systems like Ayurveda, Unani, and Siddha developed structured knowledge on plant-based treatments. According to the World Health Organization (WHO), traditional medicine includes knowledge, skills, and practices based on cultural beliefs and experiences used for maintaining health and treating illnesses. WHO classifies herbal medicines into four categories: indigenous herbal medicines, herbal medicines in systems, modified herbal medicines, and imported herbal products. Indigenous herbal medicines are locally used and well-known within communities, while system-based medicines like Ayurveda are documented and widely practiced. Modified herbal medicines involve changes in formulation or dosage and must meet regulatory standards. Imported herbal products require safety and efficacy validation before use.

Historical Perspective

The use of plants as medicine predates recorded history. Evidence from ancient civilizations such as Babylon, China, India, and Egypt shows extensive use of herbal remedies. Ayurveda, dating back over 5000 years, emphasizes health promotion and disease prevention. Classical texts like Charaka Samhita and Sushruta Samhita describe thousands of herbs and formulations. Globally, traditional medicine systems such as Traditional Chinese Medicine, Kampo (Japan), and Unani continue to play a vital role in healthcare. Even modern medicine has its roots in plant-based compounds; for example, aspirin (willow bark), quinine (cinchona), and morphine (opium poppy). Growing Popularity of Herbal Medicine, Herbal medicine is used by nearly 75–80% of the global population, especially in developing countries, due to its affordability and accessibility. In recent years, there has been a global shift toward natural therapies, often referred to as a “return to nature.” Increasing concerns about side effects of synthetic drugs and the belief that natural products are safer have contributed to this trend. In many rural areas, traditional medicine remains the primary healthcare system due to limited access to modern medical facilities. Additionally, herbal medicine is widely used for chronic



conditions such as diabetes, arthritis, and cancer, where patients seek alternative or complementary therapies.

Global Trends and Market Growth, The global herbal medicine market is rapidly expanding, with increasing demand for plant-based pharmaceuticals, cosmetics, and health supplements. Herbal products are widely used in regions such as Asia, Africa, Europe, and North America. Countries like India and China are major contributors to the global herbal market due to their rich biodiversity and traditional knowledge. The market growth is driven by factors such as increased awareness of natural therapies, aging populations, and rising healthcare costs. Herbal medicines are also gaining popularity in developed countries as complementary and alternative medicine (CAM).

Antioxidants and Their Importance, Antioxidants are substances that prevent or delay cellular damage caused by free radicals. Free radicals are generated through environmental exposure, radiation, and metabolic processes, leading to oxidative stress and various diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions.

Antioxidants are classified into: **Enzymatic antioxidants**: Superoxide dismutase, catalase, glutathione peroxidase **Non-enzymatic antioxidants**: Vitamins C and E, carotenoids, selenium These antioxidants work by neutralizing free radicals and protecting cellular components. Nutrient antioxidants such as vitamin C, vitamin E, and beta-carotene play a crucial role in maintaining health and preventing disease.

Liver and Hepatoprotective Agents, The liver is a vital organ responsible for metabolism, detoxification, and storage of nutrients. It processes both endogenous substances (like hormones) and exogenous substances (like drugs). Due to its central role in detoxification, the liver is highly susceptible to damage from toxins, drugs, and infections. Hepatitis, an inflammation of the liver, can be caused by viruses (A, B, C, D, E), alcohol, toxins, or autoimmune conditions. It may lead to severe complications such as cirrhosis and liver cancer. Herbal hepatoprotective agents play an important role in protecting the liver and promoting its recovery. These agents are categorized as:

Antihepatotoxic agents – counteract liver toxins

Hepatoprotective agents – prevent liver damage

Hepatotropic agents – promote liver healing

Numerous medicinal plants have shown hepatoprotective activity, and over 700 herbal formulations are used globally for liver disorders. Commonly studied hepatotoxins in research include carbon tetrachloride, paracetamol, and galactosamine, which help evaluate the protective effects of herbal compounds.

Future Prospects, Herbal medicine continues to gain global importance due to its therapeutic potential and economic value. Approximately 25% of modern drugs are derived from plant sources, and many more are based on traditional knowledge. Advances in scientific techniques such as chromatography and molecular analysis have accelerated the discovery of bioactive compounds from plants.

PLANT PROFILE



TOXONOMY

Family : Malvaceae

Botanical name : *Abutilon indicum* (L.)



Sweet Kingdom	: Plantae – Plants
Subkingdom	: Tracheobionta – Vascular plants Super
division	: Spermatophyta – Seed plants
Division	: Magnoliophyta – Flowering plants
Class	: Magnoliopsida – Dicotyledons
Subclass	: Dilleniidae –
Order	: Malvales
Genus	: <i>Abutilon</i> Mill. – Indian mallow
Species	: <i>Abutilon indicum</i> (L.) Sweet – Monkeybush

VERNACULAR NAME

Tamil	: Perum Tutti, Paniyara Hutti, thuthi
Hindi	: Kanghi, Kakahi
English	: Country mallow
Kannada	: Tutti
Malayalam	: Vellula
Telugu	: Tutturu Benda, Duvvenakaya, Duvvena Kayalu

Traditional Uses, Pharmacological Activity and Phytoconstituents of *Abutilon indicum*

Abutilon indicum (L.) Sweet is widely used in traditional systems of medicine, especially in Siddha and Ayurveda, for treating various ailments such as piles, jaundice, leprosy, ulcers, and fever. Historically, plants of the Bala group—including *A. indicum*—were used to treat vata–pitta disorders, heart diseases, poisoning, and uterine problems. The plant contains important phytochemicals like flavonoids, alkaloids, saponins, amino acids, and hydrocarbons, contributing to its therapeutic potential.

Traditional Uses of Plant Parts

Leaves: Demulcent and used for bronchitis, diarrhea, fever, bladder inflammation, and ulcers. Leaf juice is used for jaundice, wound healing, and as an antidote for snakebite.

Flowers: Used to improve male fertility and applied externally for boils and ulcers.

Seeds: Possess laxative, expectorant, and aphrodisiac properties; used in cough, diabetes, urinary disorders, and skin diseases.

Roots: Act as cooling agents, diuretics, and sedatives; useful in fever, hematuria, and leprosy.

Stem bark: Used for urinary disorders, fever, and as an anthelmintic. **Fruits:** Treat piles, gonorrhoea, cough, and hemorrhagic conditions.

Whole plant: Exhibits anti-inflammatory, immunostimulatory, and aphrodisiac properties; widely used for fever, dysentery, and allergies.



Pharmacological Activities

Anti-inflammatory & Anti-proliferative: Ethanolic leaf extract shows significant inhibition of inflammation and cancer cell growth.

Antidiabetic: Enhances insulin sensitivity and glucose uptake via GLUT1 and PPAR γ pathways.

Anticancer & Antioxidant: Exhibits free radical scavenging activity and cytotoxic effects on cancer cells.

Antidiarrhoeal: Reduces intestinal motility and fluid secretion. Anticonvulsant: Delays onset of seizures due to flavonoids and fatty acids.

Larvicidal: Effective against mosquito larvae, especially petroleum ether extract.

Wound Healing: Accelerates wound closure and tissue regeneration. Antiasthmatic: Improves respiratory parameters like FVC and FEV1. Diuretic: Increases urinary output and sodium excretion.

Immunomodulatory: Enhances immune response and hypersensitivity reactions.

Anti-estrogenic & Anti-arthritic: Reduces inflammation and hormone-induced effects. Analgesic & Sedative: Shows central and peripheral pain relief activity.

Antimicrobial: Effective against bacteria like *Staphylococcus aureus* and *E. coli*. Hepatoprotective: Protects liver against toxicity and improves biochemical markers.

Phytoconstituents

Leaves: Contain tannins, flavonoids (quercetin, luteolin), alkaloids, sterols, and amino acids.

Aerial parts: Rich in phenolic acids, β -sitosterol, flavonoids, and organic acids. Roots: Contain asparagin, gallic acid, sterols, and terpenoids.

Flowers: Include flavonoids (luteolin, quercetin), sesquiterpene lactones, and essential oils. Seeds: Rich in fatty acids (oleic, linoleic, palmitic), proteins, mucilage, and essential oils. Fruits: Contain flavonoids and alkaloids.

Whole plant: Contains compounds like farnesol, geraniol, β -pinene, and lupeol.

MATERIALS AND METHODS

The seeds of *Abutilon Indicum* (L.) Sweet were collected from the local area of Komarapalayam in the month of May 2025, Namakkal District, Tamilnadu, India. The plant material was authenticated by Dr. P. RADHA; Research officer (Botany) Sci II, I/C; Siddha Medicinal Plants Garden / Mettur Dam, Tamilnadu - 636401 .and a voucher specimen{A1511250171I} was submitted at the SSM College of Pharmacy, Erode (638312).

EXTRACTION OF PLANTS: 10gm of sample (SS) was added in 100 ml of ethanol and incubated at 40C in the Refrigerator. The material immersed in the ethanol was separated and filtered by Muslin cloth, Filter paper, and Whatman No.1 paper and dried by evaporator.



SS Sample



Heated at 70°C



DPPH Radical scavenging activity

Principle

The DPPH assay is a simple and sensitive method used to evaluate antioxidant activity based on the ability of compounds to donate hydrogen and scavenge free radicals. DPPH, a stable purple radical, turns yellow when reduced by an antioxidant, showing decreased absorbance at 517 nm. The reduction in absorbance is proportional to the antioxidant capacity of the sample.

Procedure:

Briefly, prepare 0.1 mM of DPPH solution in methanol and add 100 μ l of this solution to 300 μ l of the solution of Sample (SS) at different concentration (500, 250, 100, 50, and 10 μ g/ml). The mixtures have to be shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical can be calculated by using the following formula.

$$\text{DPPH scavenging effect (\% inhibition)} = \left[\frac{(\text{absorbance of control} - \text{absorbance of reaction mixture})}{\text{absorbance of control}} \right] \times 100$$

MTT ASSAY

PRINCIPLE

The MTT assay measures cell viability based on the ability of mitochondrial enzymes in living cells to convert yellow MTT into dark blue formazan crystals. The amount of formazan formed is directly proportional to the number of viable cells. This color change is quantified using a spectrophotometer or plate reader.

PROCEDURE

The Test sample (SS) was tested for *in vitro* cytotoxicity, using Hep G2 by MTT assay. Briefly, the cultured Hep G2 were harvested by trypsinization and pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 μ L) into the 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the Test sample in a



serum-free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO₂ incubator for 24 h. After incubation, MTT (10 µL of 5 mg/ml) was added to each well and the cells were incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) was aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC₅₀ value were calculated using Graph Pad Prism 6.0 software (USA).

$$\text{Formula Cell viability \%} = \text{Test OD/Control OD} \times 100$$

RESULT AND DISCUSSION PRELIMINARY PHYTO CHEMICAL ANALYSIS TEST:

The Seed of *Abutilon Indicum* (L.) Sweet was subjected to systematic Phytochemical screening by successively extracting with ethanol and the extracts were subjected for Phytochemical investigation by qualitative chemical identification tests.

Qualitative analysis of phytochemicals:

Alkaloids (Mayer's Test): Add Mayer's reagent to the extract. Formation of cream or whitish-yellow precipitate indicates alkaloids.

Steroids (Liebermann–Burchard Test): Mix extract with acetic anhydride and concentrated sulphuric acid. Violet, blue, or green color confirms steroids.

Terpenoids (Salkowski Test): Add chloroform and sulphuric acid to the extract. Formation of a reddish-brown ring indicates terpenoids.

Flavonoids (Alkaline Reagent Test): Add ammonium solution followed by hydrochloric acid. Yellow coloration indicates flavonoids.

Saponins (Froth Test): Shake extract with distilled water. Persistent froth formation confirms saponins.

Phenols (Lead Acetate Test): Add lead acetate solution to extract. Formation of precipitate indicates phenols.

Tannins: Lead Acetate Test: White precipitate indicates tannins. **Ferric Chloride Test:** Blue-black or green color confirms tannins.

Cardiac Glycosides (Keller–Killiani Test): Treat extract with glacial acetic acid, ferric chloride, and sulphuric acid. Formation of brown ring with blue layer indicates glycosides.

Amino Acids (Ninhydrin Test): Heat extract with ninhydrin solution. Purple or blue color indicates amino acids.

Proteins (Biuret Test): Add sodium hydroxide and copper sulphate. Violet color confirms proteins.

Carbohydrates (Barfoed's Test): Heat extract with Barfoed's reagent. Reddish-brown precipitate indicates carbohydrates.

Reducing Sugars (Fehling's Test): Heat extract with Fehling's solutions A and B. Brick-red precipitate confirms reducing sugars.

Test	Observation
Alkaloids	-
Steroids	+
Flavanoids	+
Terpenoids	-
Saponins	+
Phenols	+
Tannis	-
Cardiac Glycosides	+
Amino Acids	+
Proteins	+
Carbohydrates	-



Phytochemical Compounds Identified

➤ Steroids, flavonoids, Saponin, Phenols, Cardiac glycosides, Amino Acids, Proteins are present in the ethanolic extract of *Abutilon indicum*.

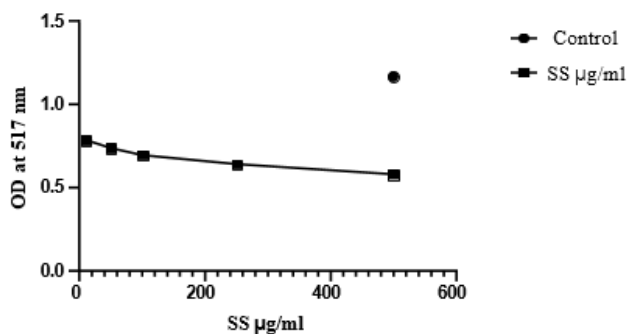
➤ Alkaloids, Terpenoids, Tannis, Carbohydrates are absent.



DPPH Radical scavenging activity:

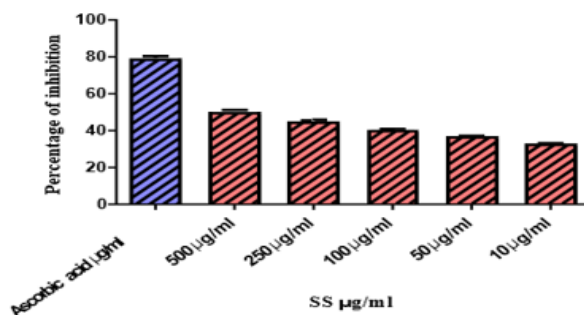
A. OD Value at 517 nm B. Control Mean OD value: 1.169

Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
	Control	1.189	1.147
500 µg/ml	0.582	0.574	0.587
250 µg/ml	0.637	0.644	0.646
100 µg/ml	0.695	0.692	0.698
50 µg/ml	0.737	0.741	0.735
10 µg/ml	0.782	0.792	0.788
Ascorbic acid	0.253	0.23	0.247



B. Percentage of inhibition

Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
	Ascorbic acid	78.370	80.336	
500 µg/ml	50.242	50.926	49.815	50.328
250 µg/ml	45.540	44.942	44.771	45.084
100 µg/ml	40.580	40.838	40.325	40.581
50 µg/ml	36.991	36.649	37.162	36.934
10 µg/ml	33.143	32.288	32.630	32.687



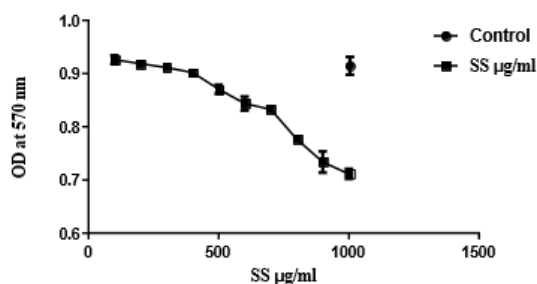
log(inhibitor) vs. normalized response --Variable slope	
Best-fit values	
LogIC50	2.060
HillSlope	-1.549
IC50	114.9
Std. Error	
LogIC50	0.02730
HillSlope	0.1448
95% CI (profile likelihood)	
LogIC50	2.000 to 2.120
HillSlope	-1.869 to -1.287
IC50	100.1 to 131.8
Goodness of Fit	
Degrees of Freedom	13
R squared	0.9740
Sum of Squares	476.4
Sy.x	6.054
Number of points	
# of X values	15
# Y values analyzed	15



MTT ASSAY

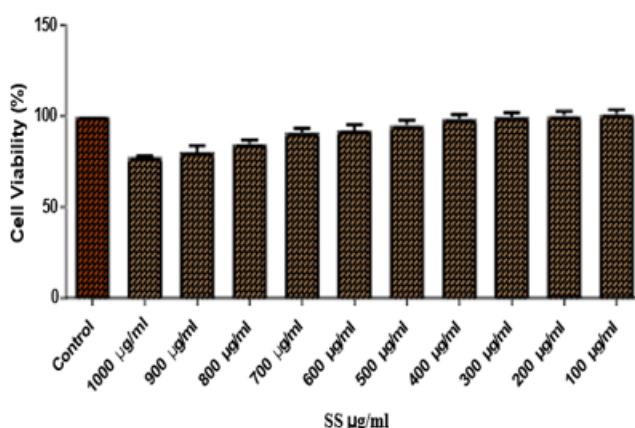
A. OD Value at 570 nm

Tested sample concentration (µg/ml)	OD value at 570 nm (in triplicates)		
	Control	0.898	0.932
1000 µg/ml	0.702	0.721	0.712
900 µg/ml	0.754	0.714	0.734
800 µg/ml	0.781	0.772	0.777
700 µg/ml	0.839	0.827	0.833
600 µg/ml	0.857	0.831	0.844
500 µg/ml	0.879	0.862	0.871
400 µg/ml	0.907	0.897	0.903
300 µg/ml	0.916	0.907	0.912
200 µg/ml	0.921	0.912	0.923
100 µg/ml	0.925	0.918	0.935



B. Cell Viability (%)

Tested sample concentration (µg/ml)	Cell viability (%) (in triplicates)			Mean Value (%)
	Control	100	100	
1000 µg/ml	78.1737	77.3605	77.8142	77.782814
900 µg/ml	83.9644	76.6094	80.2186	80.264129
800 µg/ml	86.971	82.8326	84.918	84.907233
700 µg/ml	93.4298	88.7339	91.0383	91.067334
600 µg/ml	95.4343	89.1631	92.2404	92.279275
500 µg/ml	97.8842	92.4893	95.1913	95.188238
400 µg/ml	101.002	96.2446	98.6885	98.645129
300 µg/ml	102.004	97.3176	99.6721	99.664727
200 µg/ml	102.561	97.8541	100.874	100.42988
100 µg/ml	103.007	98.4979	102.186	101.23011

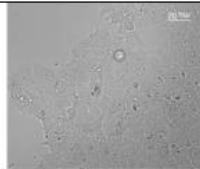
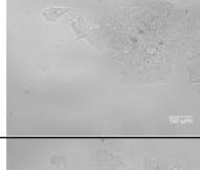
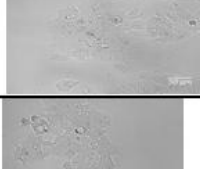
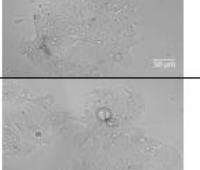
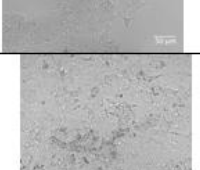
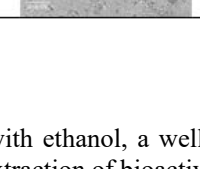


C. IC50 Value of tested sample: 706.9 µg/ml

log(inhibitor) vs. normalized response	
Best-fit values	
LogIC50	2.849
IC50	706.9
95% CI (profile likelihood)	
LogIC50	2.697 to 3.008
IC50	498.0 to 1018
Goodness of Fit	
Degrees of Freedom	29
R squared	0.5674
Sum of Squares	15783
Sy.x	23.33
Number of points	
# of X values	30
# Y values analyzed	30



D. Images of control cells and treated cells.

CONC.	IMAGE
CONTROL	
1000 µg/ml	
700 µg/ml	
500 µg/ml	
300 µg/ml	
100 µg/ml	

SUMMARY AND CONCLUSION

Abutilon indicum seed parts were extracted using cold extraction with ethanol, a well-established technique for obtaining plant extracts. The process was performed for 3- 4 days ensuring efficient extraction of bioactive compounds from the plant material. The yield of the ethanolic extract was calculated to be 68.09%, which indicates a high concentration of phytochemicals in the Abutilon indicum seeds. Preliminary phytochemical screening revealed the presence of compounds such as steroids, flavonoids, saponin, phenols, cardiac glycoside amino acids, protein, reducing sugar are present in the ethanolic extract of Abutilon indicum. These compounds are known for their antioxidant and hepatoprotective effects. The results of the **DPPH radical scavenging assay** clearly demonstrate that the tested sample possesses significant antioxidant activity, as evidenced by changes in optical density (OD), percentage inhibition, and IC_{50} value. A marked decrease in optical density at 517 nm was observed with increasing concentrations of the sample, indicating effective scavenging of DPPH free radicals. This reduction in absorbance confirms the ability of the sample to donate hydrogen atoms or electrons to neutralize DPPH radicals. The percentage inhibition values increased in a concentration-dependent manner, with the highest inhibition observed at 500 µg/ml ($\approx 50.33\%$) and the lowest at 10 µg/ml ($\approx 32.69\%$). Although the scavenging activity of the sample was lower than that of the standard antioxidant ascorbic acid ($\approx 79.19\%$), the results indicate appreciable free radical scavenging potential. The IC_{50} value of the tested sample was found to be 114.9 µg/ml, suggesting a moderate antioxidant potency. A lower IC_{50} value reflects higher antioxidant activity, and the obtained value supports the effectiveness of the sample in scavenging free radicals. The high R^2 value (0.9740) further indicates a good fit of the dose-response curve and reliability of the results. In conclusion, the tested sample exhibits moderate but significant DPPH radical scavenging activity, which may be attributed to the presence of antioxidant phytoconstituents such as phenolic compounds and flavonoids. These findings support the potential of the sample as a natural source of antioxidants. The **MTT assay** results indicate that the tested sample exhibited concentration-dependent effects on cell viability in Hep G2 cells. A gradual decrease in optical density (OD) values at 570 nm was observed with increasing concentrations of the test sample, reflecting reduced mitochondrial activity and hence decreased cell viability at higher doses. The cell viability percentage decreased as the concentration increased, with the highest concentration (1000 µg/ml) showing approximately 77.78% viability, while lower concentrations (100–300 µg/ml) maintained viability close to



or above 100%. This suggests that the tested sample is less cytotoxic at lower concentrations and exhibits moderate cytotoxic effects only at higher concentrations. The IC_{50} value was found to be 706.9 $\mu\text{g/ml}$, indicating that a relatively high concentration of the sample is required to reduce cell viability by 50%. This high IC_{50} value reflects low cytotoxicity of the tested sample against Hep G2 cells under in-vitro conditions. The dose–response analysis supports the safety profile of the sample at lower concentrations. In conclusion, the tested sample demonstrated good cell compatibility and minimal cytotoxicity at lower and moderate concentrations, with observable cytotoxic effects only at higher doses.

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