

Comparative Evaluation of Hepatoprotective Activity of *Syzygium cumini* Leaf and *Moringa oleifera* Flower Extracts Against Hepatotoxicity Using HepG2 Cell Line

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Abstract - Hepatotoxicity, or drug/chemical-induced liver injury, remains a major cause of liver dysfunction and drug withdrawal worldwide. Medicinal plants with antioxidant potential offer a promising alternative for hepatoprotection. This study evaluated and compared the hepatoprotective activity of ethanolic extracts of *Syzygium cumini* (Jamun) leaf and *Moringa oleifera* (drumstick) flower using the HepG2 human hepatocellular cell line. Extracts were prepared by Soxhlet extraction (1:10 ratio) and subjected to preliminary phytochemical screening, which revealed the presence of carbohydrates, alkaloids, glycosides, phytosterols, phenolic compounds, and gums/mucilages in both extracts. Cell viability and cytotoxicity were assessed by the MTT colorimetric assay at concentrations of 6.25-100 µg/mL after 24 h of treatment. Both extracts showed no significant cytotoxicity toward HepG2 cells at the tested concentrations, indicating a favourable safety profile. These findings support the traditional use of *S. cumini* and *M. oleifera* as natural hepatoprotective agents and warrant further in-vivo and mechanistic investigation.

Key Words: Hepatotoxicity; *Syzygium cumini*; *Moringa oleifera*; HepG2 cell line; MTT assay; Hepatoprotective activity; Phytochemical screening

1. INTRODUCTION

Hepatotoxicity refers to liver damage caused by pharmaceutical drugs, environmental toxins, or herbal supplements, manifesting as enzyme elevation, hepatocellular injury, cholestasis, fibrosis, or liver failure. Drug-induced liver injury (DILI) is a leading cause of drug withdrawals and regulatory warnings worldwide. The liver plays a central role in drug metabolism via Phase I (oxidation, reduction, hydrolysis) and Phase II (conjugation) reactions mediated largely by the cytochrome P450 system; reactive metabolites generated during this process can themselves cause hepatocyte injury.

Hepatotoxicity is broadly classified as intrinsic (dose-dependent, e.g. acetaminophen-induced injury via the toxic metabolite NAPQI), idiosyncratic (unpredictable, immune- or genetically-mediated, e.g. isoniazid hepatotoxicity), cholestatic (impaired bile flow, e.g. chlorpromazine), hepatocellular (direct hepatocyte damage, e.g. methotrexate), or mixed. Common mechanisms converge on oxidative stress, glutathione depletion, mitochondrial dysfunction, and release of inflammatory cytokines, culminating in elevated ALT, AST and ALP, hepatitis, fibrosis, and potentially acute or chronic liver failure. Clinical presentation ranges from fatigue, anorexia and nausea to jaundice, ascites and hepatic encephalopathy, and diagnosis relies on history, liver function tests, imaging, and, where needed, biopsy.

Given the limitations and adverse effects of synthetic hepatoprotective drugs, plant-derived antioxidants have gained attention. *Moringa oleifera* (drumstick tree) and *Syzygium cumini* (Jamun) are rich in flavonoids, phenolic acids and other antioxidant phytochemicals reported to reduce oxidative stress, restore antioxidant enzyme levels

(SOD, CAT, GPx), and protect hepatocytes from toxin-induced injury. The present work uses the HepG2 human hepatocellular carcinoma-derived cell line - a well-established in-vitro model for studying liver metabolism, toxicity and cytoprotection - to evaluate and compare the hepatoprotective potential of these two plants.

2. LITERATURE REVIEW

Several reviews and experimental studies support the hepatoprotective potential of both plants. Ayyanar and Subash-Babu (2012) reported that *S. cumini* leaves are rich in flavonoids, phenolic acids, gallic acid and quercetin, which reduce lipid peroxidation and strengthen antioxidant defence. Baliga et al. (2011) and Sharma et al. (2013) showed that *S. cumini* leaf extracts scavenge free radicals, enhance SOD, CAT and GPx activity, and restore normal liver architecture in CCl₄-induced injury models. Kumar et al. (2014) additionally found reduced TNF- α and IL-6 levels following treatment, indicating anti-inflammatory action, and a 2025 systematic review confirmed significant reductions in serum ALT, AST and ALP across multiple animal studies.

For *M. oleifera*, Stohs and Hartman (2015) and Bhattacharya et al. (2018) reported that its flavonoids (quercetin, kaempferol), chlorogenic acid and vitamin C confer strong antioxidant and anti-inflammatory activity with a favourable safety profile. Hassan et al. (2021) showed increased SOD, CAT and GPx with reduced malondialdehyde, while Saki et al. (2023), using HepG2 cells specifically, found improved cell viability, reduced intracellular ROS, and preserved mitochondrial function under oxidative stress. Rani et al. (2023) summarised that multiple plant parts of *M. oleifera* exhibit hepatoprotective, antioxidant and anti-inflammatory activity attributable to its flavonoid and phenolic content.

Collectively, this literature supports the rationale for evaluating both plants together against a common HepG2 model.

3. PLANT PROFILE

3.1 *Moringa oleifera*

Moringa oleifera, the "miracle tree," is believed native to the Indian subcontinent and now thrives across tropical and subtropical regions worldwide. It belongs to the family Moringaceae and is valued for its nutrient-rich leaves, flowers, pods and bark, which are traditionally used to treat ulcers, toothache, hypertension and malnutrition. Its flowers are creamy-white, fragrant and borne in axillary panicles, and phytochemically the plant is a rich source of flavonoids (quercetin, kaempferol, myricetin), phenolic acids (chlorogenic, gallic, caffeic acid), glucosinolates/isothiocyanates, phytosterols, carotenoids and vitamins C and E - constituents widely linked to its antioxidant, anti-inflammatory and hepatoprotective properties.

3.2 *Syzygium cumini*

Syzygium cumini (Jamun, Java plum), family Myrtaceae, is an evergreen tree indigenous to the Indian subcontinent and Southeast Asia, valued in Ayurveda for its astringent, digestive and wound-healing properties. Its leaves are traditionally used for stomach ailments, dermatopathy, fever and constipation, while the fruit and seeds are used for pharyngitis and ringworm. Phytochemically, *S. cumini* leaves contain alkaloids (jambosine, jamboline), flavonoids (quercetin, myricetin, rutin), anthocyanins, phenolic acids (gallic, ellagic, ferulic acid), tannins and triterpenoids (oleanolic and ursolic acid), which together confer strong free-radical scavenging and antioxidant activity.

3.3 HepG2 Cell Line

HepG2 is a well-differentiated human hepatocellular-carcinoma-derived cell line, isolated in 1975 and maintained by the ATCC. It retains many functional characteristics of normal hepatocytes, including Phase I/II drug-metabolising activity and polarized bile-canalicular/sinusoidal membrane domains, making it a widely used and reproducible in-vitro model for studying hepatotoxicity, drug metabolism, and the cytoprotective effects of natural compounds without the ethical and logistical constraints of animal or primary human hepatocyte models.

4. AIM AND OBJECTIVES

Aim: To evaluate and compare the hepatoprotective activity of *Syzygium cumini* leaf extract and *Moringa oleifera* flower extract against chemically induced hepatotoxicity using the HepG2 cell line.

Objectives:

- Collect, authenticate and prepare *S. cumini* leaves and *M. oleifera* flowers for extraction.
- Prepare ethanolic extracts using the Soxhlet method (1:10 ratio).

- Perform preliminary phytochemical screening of the extracts.
- Culture and maintain HepG2 cells under standard conditions.
- Determine non-toxic concentrations using the MTT viability assay.
- Evaluate and compare the hepatoprotective/cytotoxicity profile of both extracts on HepG2 cells.

5. MATERIALS AND METHODS

4.1 Plant Material and Extraction

Fresh *S. cumini* leaves and *M. oleifera* flowers were collected, shade-dried, and pulverized. Extraction was carried out by continuous hot Soxhlet extraction using ethanol (solvent-to-material ratio 1:10). The solvent was cycled through repeated siphoning until exhaustive extraction was achieved, after which the extract was concentrated by evaporation and stored in labelled containers for further use.

4.2 Phytochemical Screening

Standard qualitative tests (Molisch, Fehling's, Benedict's, Mayer's, Dragendorff's, Hager's, Wagner's, Liebermann-Burchard, Salkowski, saponification, 90% alcohol, foam, Ninhydrin, ferric chloride and aqueous NaOH tests) were performed to screen for carbohydrates, glycosides, alkaloids, phytosterols, gums/mucilages, saponins, proteins/amino acids, phenolics and flavonoids.

4.3 Cell Culture

HepG2 cells (NCCS, Pune) were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution, maintained at 37 °C in a humidified incubator with 5% CO₂, and sub-cultured every three days.

4.4 Materials and Equipment

Key materials used included HepG2 cell lines (NCCS, Pune), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, MTT reagent (5 mg/mL), D-PBS, 96-well and T25 culture flasks, centrifuge tubes and micropipette tips. Equipment comprised a centrifuge, calibrated micropipettes (2-1000 µL range), an inverted binocular biological microscope, a 37 °C CO₂ incubator with humidified atmosphere (5% CO₂), and a 96-well microplate reader for absorbance measurement.

4.5 MTT Assay Protocol

Cells were seeded in 96-well plates (~20,000 cells/well) and allowed to adhere for 24 h. Extracts were added at 6.25, 12.5, 25, 50 and 100 µg/mL and incubated for a further 24 h. MTT reagent (0.5 mg/mL final concentration) was added, plates were protected from light and incubated for 3 h, and the resulting formazan crystals were solubilised in DMSO. Absorbance was measured at 570 nm, and cell viability (%) was calculated as [mean absorbance of treated cells / mean absorbance of untreated control] × 100.

6. RESULTS

5.1 Phytochemical Screening - *Moringa oleifera*

S.No	Phytochemical	Test	Result
1	Carbohydrates	Molisch Test	+
2	Glycosides	Fehling's Test	+
3	Glycosides	Benedict's Test	+
4	Alkaloids	Mayer's Reagent	+
5	Alkaloids	Dragendorff's Reagent	–
6	Alkaloids	Hager's Reagent	+
7	Alkaloids	Wagner's Reagent	+
8	Phytosterols	Liebermann-Burchard	+
9	Phytosterols	Salkowski Test	+
10	Phytosterols	Saponification Test	–
11	Gums & Mucilages	90% Alcohol Test	+
12	Saponins	Foam Test	–
13	Proteins/Amino acids	Ninhydrin Test	+
14	Phenolic compounds	Ferric Chloride Test	+
15	Flavonoids	Aqueous NaOH Test	–

5.2 Phytochemical Screening - *Syzygium cumini*

S.No	Phytochemical	Test	Result
1	Carbohydrates	Molisch Test	+
2	Glycosides	Fehling's Test	–
3	Glycosides	Benedict's Test	+
4	Alkaloids	Mayer's Reagent	+
5	Alkaloids	Dragendorff's Reagent	–
6	Alkaloids	Hager's Reagent	–
7	Alkaloids	Wagner's Reagent	+
8	Phytosterols	Liebermann-Burchard	+
9	Phytosterols	Salkowski Test	–
10	Phytosterols	Saponification Test	–
11	Gums & Mucilages	90% Alcohol Test	+
12	Saponins	Foam Test	–
13	Proteins/Amino acids	Ninhydrin Test	–
14	Phenolic compounds	Ferric Chloride Test	+
15	Flavonoids	Aqueous NaOH Test	–

Both extracts were positive for carbohydrates, alkaloids (Mayer's/Wagner's), phytosterols, phenolic compounds and gums/mucilages, while saponins and flavonoids (by the aqueous NaOH test) were not detected in either extract.

5.3 Concentration-wise Cell Viability Observation

S.No	Conc. (µg/mL)	<i>M. oleifera</i>	<i>S. cumini</i>
1	6.25	Non-toxic	Non-toxic
2	12.5	Non-toxic	Non-toxic
3	25	Non-toxic	Non-toxic
4	50	Non-toxic	Non-toxic
5	100	Non-toxic	Non-toxic

Microscopic examination at each concentration showed intact cell morphology comparable to the untreated control, with no visible signs of rounding, detachment or reduced confluency, further supporting the absence of cytotoxicity across the tested dose range.

5.4 HepG2 Cytotoxicity / Hepatoprotective Screening

Microscopic evaluation of treated HepG2 cells after 24 h showed no significant reduction in viable, morphologically normal cells at any of the five tested concentrations (6.25-100 µg/mL) for either extract. *M. oleifera* flower extract showed no significant cytotoxicity against HepG2 cells at all tested concentrations. Similarly, *S. cumini* leaf extract was found to be non-cytotoxic to HepG2 cells under identical experimental conditions, supporting the safety of both extracts within the tested dose range.

7. DISCUSSION

Phytochemical screening confirmed that both *S. cumini* leaf and *M. oleifera* flower extracts contain alkaloids, glycosides, phytosterols and, notably, phenolic compounds - constituents widely reported to underlie antioxidant and hepatoprotective activity through free-radical scavenging, restoration of endogenous antioxidant enzymes (SOD, CAT, GPx) and suppression of pro-inflammatory cytokines. The absence of cytotoxicity in the MTT assay across the tested concentration range (6.25-100 µg/mL) indicates that both extracts are well tolerated by HepG2 cells and do not themselves induce hepatocellular damage, a prerequisite for further evaluation of protective efficacy against a chemical insult.

Comparing the two extracts, both showed a similar phytochemical profile - positive for carbohydrates, alkaloids, phytosterols, phenolic compounds and gums/mucilages - while differing slightly in glycoside and protein/amino-acid content, which may account for subtle differences in their reported antioxidant potency in the literature. Since flavonoids and phenolics are the principal drivers of hepatoprotection through radical scavenging and membrane stabilisation, the confirmed presence of phenolic compounds in both extracts (by the ferric chloride test) is particularly significant, even though the aqueous NaOH test for flavonoids was negative, possibly reflecting the specific flavonoid sub-types present or their concentration falling below the detection threshold of this qualitative method.

These findings are consistent with prior literature attributing the hepatoprotective potential of both plants primarily to their phenolic and flavonoid content, and support their continued investigation as natural hepatoprotective agents. Future work should extend this screening-level safety assessment to a full hepatoprotective evaluation, in which HepG2 cells are first challenged with a hepatotoxic agent (e.g., paracetamol or a CCl₄ metabolite model) and then co-treated with each extract, so that biochemical markers of protection (cell viability recovery, ALT/AST/ALP normalisation, ROS reduction) can be directly compared between the two plants and correlated with their respective phytoconstituent profiles.

8. CONCLUSION

The present study demonstrated that *Syzygium cumini* leaf and *Moringa oleifera* flower extracts possess a favourable safety profile in the HepG2 cell line, showing no significant cytotoxicity at concentrations of 6.25-100 µg/mL over 24 h. Preliminary phytochemical screening revealed the presence of phenolic compounds, alkaloids, glycosides and phytosterols in both extracts, constituents associated with antioxidant and hepatoprotective activity. These results

support both plants as promising natural sources of hepatoprotective agents, warranting further in-vivo and mechanistic studies to establish their protective efficacy against chemically induced liver injury.

Overall, the study strengthens the scientific basis for the traditional use of *Moringa oleifera* and *Syzygium cumini* in liver-related ailments and provides a validated baseline safety concentration range for future hepatoprotective and mechanistic investigations, including toxin-challenge models, dose-response biochemical assays and comparative antioxidant enzyme profiling, which together could support the eventual development of a standardized polyherbal hepatoprotective formulation from these two locally available plants.

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