



HEPATO-PROTECTIVE ACTIVITY OF AN ETHANOLIC EXTRACT OF AN FRUIT OF *SOLANUM NIGRUM* FROM THEIR PHARMACOGNOSTICAL, PHARMACOLOGICAL AND PHYTOCHEMICAL SCREENING

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ABSTRACT

Solanum nigrum Linn commonly known as black nightshade, is a traditional medicinal plant with notable hepatoprotective potential. This review compiles and analyzes pharmacognostical, phytochemical, in vitro, and in vivo studies related to its liver-protective effects. Phytochemical screening reveals the presence of key bioactive constituents such as alkaloids, flavonoids, saponins, tannins, and phenolic compounds, which are associated with antioxidant and anti-inflammatory activity. In vivo experiments using D-galactosamine- induced hepatic fibrosis in rats demonstrated that *S. nigrum* extract significantly reduced liver enzyme levels (ALT, AST, ALP), lipid peroxidation, and histopathological damage, while enhancing antioxidant enzymes (SOD, CAT, GSH). In vitro assays confirmed strong free radical scavenging capacity, ferric reducing power, and total antioxidant potential. These findings support the traditional use of *S.nigrum* as a hepatoprotective agent and indicate its potential for

development into a natural therapeutic. Further clinical studies are warranted to establish its safety, efficacy, and standardized formulations for therapeutic use.

KEYWORDS: Hepatoprotective activity, Phytochemical studies, *S.nigrum*, Antioxidant potential, Pharmacognostical studies, Liver protective effects.

INTRODUCTION

One kind of flowering plant is the European black nightshade, *Solanum nigrum*, which is a member of the Solanaceae family. In Tamil, it's called Manathakkali. Because of its many pharmacological qualities, this medicinal plant is employed in many different medical systems, such as Ayurveda, Unani, and Chinese medicine. Different components of the plant, including as the leaves, fruits, and roots, are utilized as traditional medicines in some places, while ripe berries and cooked leaves of edible strains are consumed as food.

The herbaceous plant *Solanum nigrum* has a maximum height of one meter. Widely found in both tropical and temperate locations, the plant is well-known for its anti-inflammatory, antipyretic, hepatoprotective, antioxidant, and anti-cancer properties. The main purpose of *solanum nigrum*'s leaves and fruits is its hepatoprotective properties. Alkaloids, flavonoids, tannins, saponins, glycosides, proteins, carbohydrates, coumarins, and phytosterols are found in the entire plant.



Numerous chemicals that have demonstrated pharmacological relevance to the observed effects of *solanum nigrum* prepared from entire plants have been identified from various fractions of the plant. Particularly, the leaf extract shows strong liver-protective properties against chemically produced hepatoprotectives (such as those brought on by paracetamol or carbon tetrachloride).^[1,2]

COMPONENTS

STEMS

Stems can be erect, ascending, or prostrate, and vary from herbaceous in ephemeral plants to somewhat woody or even shrubby in those that make it to the biennial stage. Stems can be circular or angular, green to purplish, smooth or somewhat hairy.



LEAVES

The alternating, oval leaves, which range in texture from smooth-edged to shallowly lobed, are held on short stalks that are 2 to 8 cm long. They are either smooth or finely hairy, opaque, and dark green on the underside as well as the top.



FLOWERS

Straight from the stems between the leaves, the tiny, white, star-shaped blooms are carried in umbels on thin stalks. There are typically five to ten flowers in each cluster, which open one after the other over a few days. The golden centers of the 5-8 mm-diameter flowers are easily noticeable.



FRUITS

A large number of flattened, finely pitted, yellow to dark brown, woody seeds, about 1.5 mm long, are contained within the globular, dark green, matt berries, which are 5–13 mm wide and matt black when ripe.



SEEDS

The tiny, woody, flattened seeds range in color from yellow to dark brown. Their surface is finely pitted, and they are usually 1.5 mm long. The dark green, globular berries (fruits), which turn black when ripe, contain these seeds.^[3-13]



CHEMICAL CONSTITUENTS

➤ ALKALOIDS

Solanine Solamargine Solasonine

Solasodine -antimicrobial, anti-inflammatory, and anticancer properties.

➤ FLAVONOIDS

Quercetin Kaempferol Luteolin

Apigenin – antioxidant, anti-inflammatory, hepatoprotective effect.

➤ PHENOLIC COMPOUNDS

Chlorogenic acid Caffeic acid

Gallic acid – antioxidant potential.

➤ SAPONINS

These contribute to the plant's cytotoxic and antimicrobial properties.

➤ TANNINS

These compounds have astringent and antioxidant effects.

➤ STEROIDS

Beta-sitosterol

Stigmasterol- support anti-inflammatory and cholesterol lowering activities.

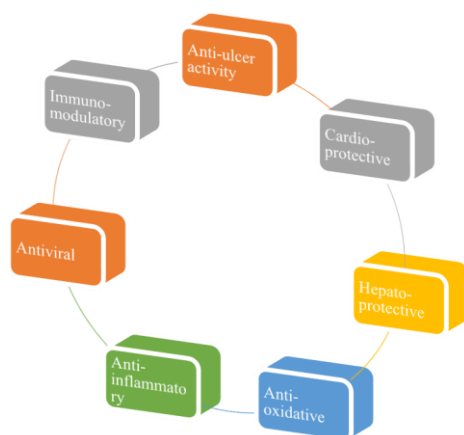
➤ VITAMINS AND MINERALS

Vitamin C, Vitamin A, Iron, Calcium and Phosphorus.

➤ CAROTENOIDS

Beta-carotene – These support antioxidant and vision-protective effects.

MEDICINAL EFFECT OF BLACK NIGHTSHADE



INTRODUCTION ABOUT LIVER DISEASES

Medical terminology pertaining to the liver frequently begin with hepato or hepatic because the Greek word for liver is hepar. On the right side of the abdomen, directly behind the rib cage, is an organ called the liver. The liver is responsible for hundreds of vital bodily processes, but one of its most significant roles is to remove poisons from your blood.

The liver plays a crucial role in metabolism, secretion, and storage. It is sometimes referred to as the body's "great chemical factory" because it is essential for the regulation, synthesis, storage, and secretion of numerous vital proteins, nutrients, and chemicals as well as for the removal of toxins and other unnecessary substances.^{[14][15]}



SOURCE OF THE PLANT MATERIAL

Synonym - Black nightshade, Makoi.

Biological source - It consist of the dried and full-grown berries of *solanum nigrum*.

Family - Solanaceae.

Geographical sources

- Medicinal Botany
- National Institute of Siddha
- Chennai
- Tami Nadu
- India^[20]

COLLECTION OF PLANT MATERIAL

Whole plants of the black- and orange-fruits varieties of *Solanum nigrum* L. were gathered from Perambalur.

EXTRACTION

Preparation Of Aqueous Extract

Fruits from *Solanum nigrum* were cleaned, shade-dried, and ground into a fine powder. After two hours of suspension in 250 ml of water, the 100 grams of powder was heated for 30 minutes at 60 to 65 °C The extract was collected independently, and the procedure was carried out three times using the leftover powder, collecting the extract each time. After being gathered, the extracts were combined and run through a fine cotton fabric. Under low pressure, the filtrates were evaporated in a rota vapour at 40–50 °C.^[24]

Preparation Of Ethanolic Extract

Fresh fruits were gathered and cleaned to get rid of any dirt. To prepare them for grinding, all sections were first sun-dried and then baked at a temperature below 50 °C. A second grinder was used to grind the dried portion to a powder. For seven days, while being shaken and stirred occasionally, the powdered ingredients were immersed in an adequate amount of ethanol in a sealed, flat-bottomed container. After filtering, the extracts were dried in an electrical water bath. Until they were needed again, the dried extracts were kept in the freezer in their corresponding airtight vials

PHARMACOGNOSTICAL STUDIES**1. MACROSCOPICAL STUDIES****• BLACK FRUITED**

HABIT: Erect and branched 1.5m tall

LEAVES: Broadly ovate – elliptic 6- 12 x 4 -7 cm Chartaceous

FLOWERS: Small,

Umbel Ca. 7 flowered, extra axillary

FRUIT: Berry

SIZE - 1-1.25 cm, Ripening

COLOUR - Black (or) purplish black, globose, smooth, shining

SEED: discoid, minutely pitted,

1.5mm diameter, Yellow

• ORANGE FRUITED

HABIT- Erect and sparsely branched 2-3m tall

LEAVES: Deltoid to elliptic ovate 5.5 – 12 x 3.5 – 6.5 cm chartaceous

FLOWERS: Racemes, axillary FRUIT: Berry

SIZE- 1.5cm across COLOUR – orange when ripe

SEED: Minutely scaly

2. MICROSCOPICAL STUDIES

Varieties of *Solanum nigrum* fruits were preserved in FAA (90 ml of 70% ethanol, 5 ml of formalin, and 5 ml of acetic acid). After a few days in FAA, the materials were dehydrated using the Tertiary Butyl Alcohol (TBA) series in accordance with Sass.^[27] The standard procedure was used for paraffin infiltration and embedding in wax blocks.^[28] Using a Spencer Rotary Microtome, serial paraffin slices with a thickness of 10–12 m were created. Toluidine blue was used to dye certain portions. Due to its metachromatic feature, toluidine blue stain was discovered to be highly suitable for microscopic observation of cells. Fast green was also used to stain certain sections.^[29]

Microscopic investigations were conducted in both polarized and non- polarized lighting conditions.

Seed of *Solanum nigrum* L. variant black fruited.



Fig. 1: T.S of Seeds possessing embryos.

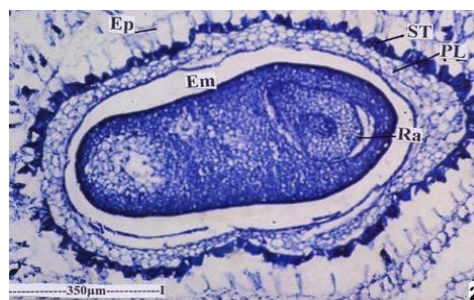


Fig. 2: A seed with an embryo.

Em – Embryo; Ep – Epidermis; SC – Seed Coat; Ra – Radicle; ST – Sarcotesta; PL – Parenchyma Layers.

Fruit of *Solanum nigrum* L. variant orange fruited.

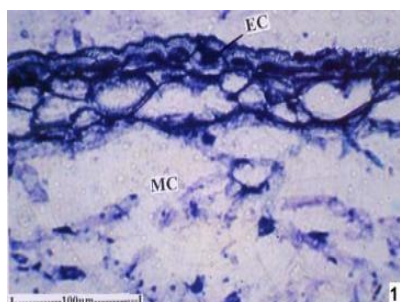


Fig. 1: T.S of fruit showing epicarp and mesocarp.

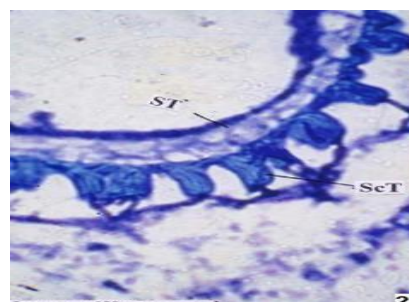


Fig. 2: Seed coat in sectional view.

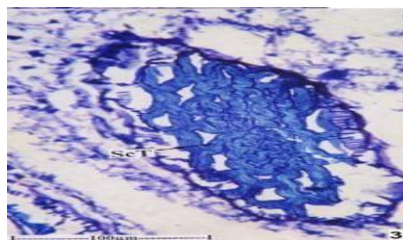


Fig. 3: Seed coat in surface view.

EC – Epicarp; MC – Mesocarp; ST – Sarcotesta; ScT – Sclerotesta

QUALITATIVE PHYTOCHEMICAL ANALYSIS

1. Test for Alkaloids

Hager's test: A saturated aqueous solution of picric acid was added to the extract and thoroughly shaken. There was no yellow precipitate.

2. Test of Saponins

Foam test: 3 mL of distilled water was added to the extract and violently shaken. There was foam discovered.

3. Test for Tannins

Lead acetate test: A few drops of a 5% Pb (CH₃COO)₂ solution were added to the extract and thoroughly shaken. A white color was discovered.

4. Test for Glycosides

Legal's test: Dissolving the extract solution in pyridine and then adding sodium nitroprusside solution to make it alkaline. Glycosides were indicated by their pink-red color. A blood-red color was discovered.

5. Test for terpenoids

Salkowski test: Chloroform was added to the extract and thoroughly stirred. A few drops of concentrated H₂SO₄ were then added to the test tube's sidewalls. A red ring was discovered.

6. Test for Steroids

Libermann-Burchard's test: 1 ml of acetic anhydride was added to the extract and thoroughly shaken. Conc. H₂SO₄ was applied in little droplets along the test tube's sidewalls. A dark violet hue was discovered.

7. Test for Flavonoids

Alkaline Reagent Test: A few drops of a 2% NaOH solution were added to the extract, and it was thoroughly shaken. A yellow hue was discovered.

8. Test for carbohydrate

Molisch test: Two drops of alcoholic α -naphthol solution were added to the extract and thoroughly shaken. A few drops of Conc.H₂SO₄ were added to this. A violet ring was discovered.^[30,31]

INVIVO STUDIES

10-week-old male Wistar rats weighing 200–250 g were used to test the n butanol fraction of *S. nigrum* berries' hepatoprotective ability against D GalN (D GalN)- induced hepatic fibrosis. In accordance with the rules of the Committee for the purpose of Control and Supervision of Experiments on Animals, Central Animal House Facility of Hamdard University, the experimental techniques and protocols were authorized by the Institutional Animal Ethical Committee (Approval No: 1017).

Standard housing with controlled settings (25°C $\pm$ 2°C, 60% $\pm$ 10% humidity, and a 12-hour light-dark cycle) was used to acclimate the rats for a week. Rats were randomly assigned to five groups, each consisting of six animals, after a week of acclimation. As a control, Group I was given a regular diet and unlimited water. D GalN (400 mg/kg, i.p.) was administered to the animals in Group II V.

In addition to receiving D GalN therapy, Group II animals were given normal saline as a medication for ten days, acting as a toxic group. As a positive control, Group III was given silymarin orally for 10 days at a dose of 25 mg/kg. For ten days, Group IV received oral treatment with a 250 mg/kg crude berry extract. For ten days, each of Groups V and VI received a dose of 25 and 16 mg/kg of a steroidally enhanced fraction of berries. Rats with hepatic fibrosis were chosen for additional research after a liver biopsy was performed to ascertain whether the hepatic fibrosis model had been successfully developed.

INVITRO STUDIES

Antioxidant activity

DPPH[•] radical scavenging activity

The ethanol fruit extract of *S.nigrum* was tested for antioxidant activity using the stable 1, 1-

diphenyl 2 picrylhydrazyl (DPPH) radical scavenging activity as a foundation. 1 ml of a 0.1 mM DPPH solution in methanol was combined with 1 ml of fruit extract at different doses (10–60 µg/mL). After that, the mixture was left to stand in the dark for 30 minutes. The reference standard was ascorbic acid. The control was a mixture of 1 mL methanol and 1 mL DPPH solution. At 517 nm, the absorbance started to drop. As the standard reference, ascorbic acid was utilized.^[34]

The percentage of inhibition was calculated as:

$$\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Superoxide radical scavenging assay

After illumination, the absorbance at 590 nm was measured. The ascorbic acid scavenging activity test was based on the fruit extract's ability to prevent the formation of blue formazan by scavenging the superoxide radicals produced in the riboflavin-light-NBT system. Fruit extract at varying doses (20–120 µg/mL), 1.5 mM riboflavin (200 µL), 12 mM EDTA (100 µL), and 50 mM NBT (50 µL) were added in that order to the reaction mixture. A solution of 50 mM phosphate buffer (pH 7.6) should be used to produce the reagents. As a standard reference, the reaction was initiated by shining light on the reaction mixture.^[35]

The percentage of inhibition was calculated as:

$$\% \text{ of superoxide radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Ferric (Fe 3+) reducing power assay

The ethanol fruit extract of *S. nigrum*'s reducing capacity was assessed using a slightly altered potassium ferricyanide technique. Potassium ferricyanide [K₃Fe(CN)₆] (1%, w/v) solution and 1 mL of phosphate buffer (0.2 M, pH 6.6) were combined with 1 mL of fruit extract at varying doses (20–120 µg/mL). After that, the mixture was incubated for 20 minutes at 50°C in a water bath. Each mixture received 500 µL of 10% w/v trichloroacetic acid. Following a thorough shake and the addition of 100 µL of newly made FeCl₃ (0.1%, w/v) solution, the absorbance at 700 nm was measured. The standard reference was ascorbic acid.^[36]

The percentage of reduction was calculated as:

$$\% \text{ of Fe}^{3+} \text{ reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

Phosphomolybdenum reduction assay

The Prieto et al. technique was used to evaluate the antioxidant potential of the ethanol fruit

extract of *S. nigrum*. 1 ml of a reagent solution including ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulfuric acid (600 mM) was mixed with fruit extract at several doses (20–120 µg/mL). For 90 minutes, the reaction mixture was incubated at 95 degrees Celsius in a water bath. At 695 nm, the colored complex's absorbance was measured. Standard reference was ascorbic acid.^[37]

The percentage of reduction was calculated as:

$$\% \text{ of Mo6+ reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

RESULTS

EXTRACTION YIELD

The aqueous extraction of the *Solanum nigrum* yielded 14%.

CHEMICAL TEST

The results showed that Alkaloids, Terpenoids, Steroids, Flavonoids, Tannins, Glycosides, Carbohydrate, Saponins were detected in extract of *Solanum nigrum*. The result of qualitative phytochemical analysis of *Solanum nigrum* extract is summarised in table;

S.NO	PHYTOCHEMICALS	INFERENCE	RESULTS
01	Alkaloids	no yellow precipitate	+
02	Terpenoids	red ring appears	+
03	Steroids	dark violet colour appears	+
04	Flavonoids	yellow colour appears	+
05	Tannins	white colour appears	+
06	Glycosides	blood red colour appears	+
07	Carbohydrate	violet ring appears	+
08	Saponins	foam appears	+

INVIVO STUDIES

The in vivo study demonstrated that the n-butanol fraction and crude extract of *Solanum nigrum* berries exert significant hepatoprotective activity in D-galactosamine (D- GalN)-induced hepatic fibrosis in rats.

Liver Enzymes (ALT, AST, ALP)

D-GalN significantly elevated serum levels of ALT (259 ± 22.7 IU/L), AST (289 ± 25.4 IU/L), and ALP (441 ± 25.4 IU/L) compared to the normal control.

Total Bilirubin (STB)

D-GalN increased STB to 3.11 ± 0.15 mg/Dl.

AntiOxidant Enzymes (GSH, SOD, CAT)

D-GalN caused significant depletion.

Lipid Peroxidation (LPO)

Elevated by D-GalN, LPO levels were significantly decreased in treated groups, indicating reduced oxidative stress.

Histopathology

D-GalN caused necrosis, inflammation, and fibrosis in liver tissue.^[32,33]

INVITRO STUDIES**DPPH[•] radical scavenging activity**

The purple-colored stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was reduced to the yellow-colored 1,1-diphenyl-2 picrylhydrazine in order to assess the ethanol extract of *S. nigrum*'s capacity to scavenge DPPH. (1,1-diphenyl-2-picrylhydrazyl) radical. The reducing capacity increases as the extract concentration rises. At a dose of 120 µg/mL, the maximal DPPH[•] radical scavenging activity was $73.16 \pm 5.12\%$. *S. nigrum* fruit extract in ethanol had a good ability to scavenge DPPH free radicals; the IC₅₀ was 81.02 µg/mL. The standard ascorbic acid (IC₅₀ = 11.98 µg/ml) was used for comparison.^[38]

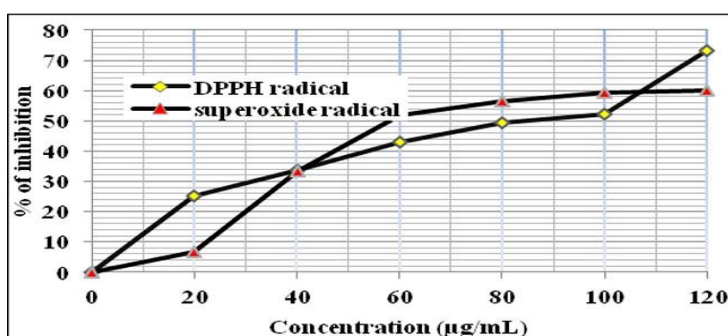
Superoxide radical scavenging activity

Superoxide anion is also extremely damaging to cells, and because it generates other types of free radicals and oxidizing agents, its effects can be amplified. Because they scavenge superoxide anions, flavonoids are powerful antioxidants. The riboflavin-light system's dissolved oxygen will produce superoxide anions, and these developing superoxide anions will lower NBT. This approach uses a UV-Vis spectrophotometer to measure the blue formazan at 590 nm, which is produced when the yellow dye (NBT²⁺) is reduced by the superoxide anion. The synthesis of blue NBT can be inhibited by antioxidants, and the reaction mixture's consumption of superoxide anion is shown by the antioxidants' decreased absorbance. At a dose of 120 µg/mL, *S. nigrum*'s maximal superoxide radical scavenging activity was $60.06 \pm 4.20\%$, and its IC₅₀ was 57.82 µg/mL. The ascorbic acid standard (IC₅₀ = 9.65 µg/mL concentration) was used for comparison.^[39]

DPPH[•] radical and superoxide radical scavenging activity of ethanol fruit extract of *S. Zigrum*

Concentration (µg/mL)	% of inhibition	
	DPPH radical	Superoxide radical
20	25.32±1.77	6.6±0.46
40	33.67±2.36	33.33±2.33
60	43.04±3.01	51.88±3.63
80	49.37±3.45	56.6±3.96
100	52.41±3.66	59.43±4.16
120	73.16±5.12	60.06±4.20

DPPH[•] radical and superoxide radical scavenging activity of ethanol fruit extract of *S. nigrum*.



Ferric (Fe³⁺) reducing power assay

The ability of the ethanol fruit extract of *S. nigrum* to reduce Fe³⁺ to Fe²⁺ was measured; the development of ferro-ferric complex causes the reduction ability to rise with extract concentration. An antioxidant's capacity to donate electrons is determined by its ferric ion reducing power activity. Strong antioxidant activity is demonstrated by the fruit extract's flavonoids and phenolic components' capacity to give electrons. At a concentration of 120 µg/mL, the highest Fe³⁺ reduction was 72.10±5.04%, and the RC₅₀ was 63.74 µg/mL. It was contrasted with the typical ascorbic acid concentration (RC₅₀ = 7.72 µg/mL).^[40]

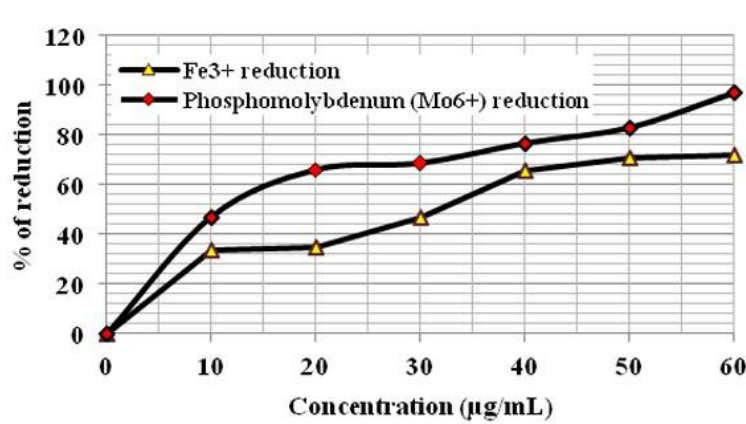
Phosphomolybdenum reduction activity

By using the phosphomolybdenum reduction test technique, which is based on the reduction of Mo (VI) to Mo(V) and the production of a green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm, the total antioxidant activity of the ethanol fruit extract of *S. nigrum* was determined.^[23] At a concentration of 120 µg/mL, the maximal phosphomolybdenum reduction was 96.77±6.77%, and the RC₅₀ was 21.25 µg/mL (Table 5; Figure 4). It was contrasted with the standard ascorbic acid concentration (RC₅₀ = 6.34 µg/mL).^[41]

Phosphomolybdenum reduction and Fe³⁺ reducing power activity of ethanol fruit extract of *S. nigrum*.

Concentration (µg/mL)	% of reduction	
	Phosphomolybdenum reduction	Fe ³⁺ reduction
20	47.05±3.29	33.72±2.36
40	65.9±4.613	34.73±2.43
60	68.53±4.79	47.06±3.29
80	76.56±5.35	65.87±4.61
100	82.82±5.79	70.77±4.95
120	96.77±6.77	72.10±5.04

Phosphomolybdenum reduction and Fe³⁺ reducing power activity of ethanol fruit extract of *S. nigrum*.



DISCUSSION

The review confirms that *Solanum nigrum* has strong hepatoprotective potential, supported by both in vivo and in vitro studies. Its phytochemicals—such as flavonoids, alkaloids, and phenolic compounds—exhibit antioxidant and liver-protective properties. In vivo tests on rats with liver damage showed reduced liver enzymes (ALT, AST, ALP) and improved antioxidant levels after treatment with *S. nigrum* extracts. Histopathology showed reduced liver tissue damage. In vitro antioxidant assays also demonstrated significant free radical scavenging and reducing power. These results support its traditional use in treating liver diseases. Overall, *Solanum nigrum* shows promise as a natural hepatoprotective agent.

CONCLUSION

Solanum nigrum (black nightshade) has shown remarkable hepatoprotective activity supported by phytochemical, in vitro, and in vivo studies. The plant contains bioactive compounds such as flavonoids, alkaloids, saponins, and phenolics that help reduce oxidative stress, protect liver cells, and enhance antioxidant defense systems. Experimental findings

reveal that extracts of *S. nigrum* significantly lower elevated liver enzymes, restore antioxidant levels, and minimize tissue damage in animal models of chemically induced liver injury. In vitro studies further confirm its strong radical scavenging and reducing power, highlighting its antioxidant capacity. These results provide scientific validation for its traditional use in treating liver-related ailments. While the evidence is promising, more clinical research is required to establish its therapeutic safety, effective dosage, and applicability in human patients. Therefore, *Solanum nigrum* holds great potential as a natural, safe, and affordable herbal alternative for managing liver disorders.

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