RESEARCH PAPER

In-vivo Investigation of the Hepatoprotective Effects of *Jatropha tanjorensis* Root Extract: A Pharmacognostical and Phytochemical Study

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ABSTRACT

Introduction:
In many developing countries, herbal medicine continues to play a significant role in primary healthcare, serving approximately 75–80% of the population. In addition to their nutritional importance, many plant species have been reported to possess valuable medicinal properties and are widely utilized in traditional medicine for the prevention and treatment of various diseases. *Jatropha tanjorensis*, a medicinal plant belonging to the family Euphorbiaceae, is one such species recognized for its therapeutic potential. In Nigeria, it is commonly referred to by several local names, including "Hospital Too Far," "Catholic Vegetable," "Iyana-Ipaja," and "Lapalapa," reflecting its widespread acceptance and use in indigenous healthcare practices.

Methods:
The phytochemical composition, pharmacognostic characteristics, and acute toxicity of *Jatropha tanjorensis* were evaluated using established standard procedures. Furthermore, the hepatoprotective potential of the plant's hydroalcoholic root extract was assessed in vivo using a rat model of hepatotoxicity induced by rifampicin and isoniazid.

Results:
The hydroalcoholic root extract of *Jatropha tanjorensis* produced a percentage yield of 3.883%. Preliminary phytochemical analysis demonstrated the presence of several bioactive constituents, including carbohydrates, glycosides, phenolic compounds, tannins, alkaloids, terpenoids, sterols, saponins, flavonoids, and proteins. Physicochemical evaluation of the root revealed a total ash value of 5.78% w/w, acid-insoluble ash value of 1.68% w/w, water-soluble ash value of 3.83% w/w, water-soluble extractive value of 6.81% w/w, and alcohol-soluble extractive value of 5.90% w/w. The levels of foreign matter and loss on drying were determined to be 1.03% and 6.5% w/w, respectively. In the in vivo hepatoprotective study, rats administered rifampicin and isoniazid (Group B) exhibited a significant reduction ($P < 0.001$) in body weight, decreasing from 217.72 ± 7.42 g in the normal control group (Group A) to 164.38 ± 5.11 g. Treatment with the hydroalcoholic root extract of *J. tanjorensis* at doses of 100 mg/kg and 200 mg/kg body weight (Groups C and D) significantly improved body weight to 198.70 ± 6.44 g and 208.39 ± 8.28 g, respectively, compared with the hepatotoxic control group ($P < 0.01$–$P < 0.001$). Similarly, administration of the standard hepatoprotective drug silymarin at a dose of 100 mg/kg significantly ($P < 0.001$) increased body weight to 212.80 ± 4.56 g relative to the hepatotoxic group.

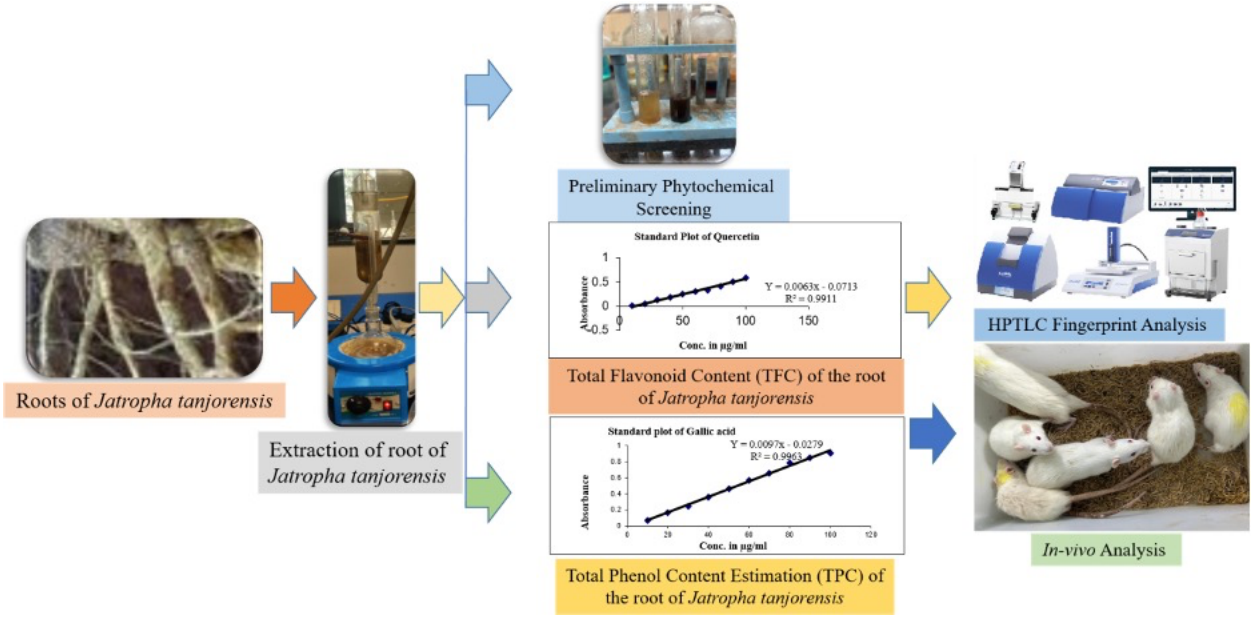
Keywords: *J. tanjorensis*, Phytochemical screening, Pharmacognostic profile, Hepatoprotective, Rifampicin (R) and Isoniazid (I).

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Graphical representation of Abstract

Introduction

Medicinal plants have been utilized since ancient times to promote human health, extend longevity, and manage a wide range of diseases. Over the years, scientific investigation of plant-derived materials has remained an important approach in the search for novel therapeutic agents with improved efficacy (1). The use of plants as remedies for the prevention and treatment of illnesses predates recorded history and has been an integral part of healthcare across different civilizations. Traditional knowledge, accumulated and transmitted through generations, has played a vital role in preserving and disseminating information on the medicinal properties of plants. Consequently, this indigenous knowledge continues to serve as a valuable source of healthcare for numerous communities and ethnic populations worldwide (2).

Green plants are essential sources of proteins, carbohydrates, fats, vitamins, and minerals required for the growth and survival of humans and animals (3). In addition to their nutritional importance, numerous plant species possess pharmacological properties that make them valuable in the prevention and treatment of diseases. Medicinal plants contain a diverse range of bioactive compounds capable of managing both chronic and infectious health conditions. The therapeutic effectiveness of these plants is largely attributed to their naturally occurring phytochemicals, which exert specific physiological effects on the human body. In many developing countries, herbal medicine continues to serve as the primary form of healthcare for approximately 75–80% of the population (4). Its widespread use is often associated with its cultural acceptance, accessibility, perceived compatibility with the human body, and relatively low incidence of adverse effects compared with many conventional medicines (5).

The family Euphorbiaceae is one of the largest families of flowering plants, comprising approximately 300 genera and about 7,500 species. Although the majority of its members are herbaceous plants, many species found in tropical regions occur as shrubs or trees. Plants belonging to this family are widely utilized for nutritional, medicinal, and ornamental purposes.

The genus name Jatropha is derived from the Greek words jatrós, meaning "doctor," and trophé, meaning "food," reflecting the long-recognized medicinal importance of plants within this genus (6). *Jatropha tanjorensis* belongs to the family Euphorbiaceae and is widely valued for its medicinal and nutritional properties. In Nigeria, it is commonly known by several indigenous names, including "Hospital Too Far," "Catholic Vegetable," "Iyana-Ipaja," and "Lapalapa," depending on the region (3). Among the Igbo people of southeastern Nigeria, the

plant is popularly referred to as "Ugu-Oyibo." The genus Jatropha comprises several other species, such as *Jatropha curcas*, *Jatropha gossypiifolia*, *Jatropha podagrica*, *Jatropha glandulifera*, *Jatropha multifida*, and *Jatropha integerrima* (6).

In recent decades, research into medicinal plants as sources of bioactive compounds has expanded considerably due to the increasing interest in natural therapeutic agents and the rising challenge of resistance to synthetic drugs (7). Plant-derived compounds continue to play a significant role in pharmaceutical research, with nearly 40% of currently available medicines originating from or being developed based on natural plant products, highlighting their enduring importance in drug discovery and development (8). *Jatropha tanjorensis* is a perennial shrub that has attracted considerable interest in traditional medicine because of its reported therapeutic potential, including anti-inflammatory, antimicrobial, and antioxidant activities (9). Different parts of the plant, particularly the leaves and roots, have been widely used by traditional practitioners in the management of various ailments, such as malaria, diabetes mellitus, hypertension, and wounds, reflecting its important role in indigenous healthcare systems (9). Although *Jatropha tanjorensis* has been extensively used in traditional medicine, there is still limited scientific evidence supporting many of its reported pharmacological properties. Existing studies have largely concentrated on the phytochemical composition and biological activities of the leaves, while comparatively little research has been conducted on the roots, despite their frequent use in traditional herbal preparations (10). The roots of medicinal plants often possess phytochemical constituents that differ from those found in the aerial parts, suggesting that they may contain unique bioactive compounds with significant therapeutic potential (11).

Oxidative stress arises when the generation of reactive oxygen species (ROS) exceeds the capacity of the body's antioxidant defense system, resulting in cellular damage that contributes to the development of several diseases, including cardiovascular disorders, neurodegenerative diseases, cancer, and age-related conditions (12). Plant-derived natural antioxidants have attracted considerable research interest because of their ability to scavenge free radicals, reduce oxidative damage, and regulate oxidative stress-related pathways, making them promising candidates for therapeutic applications (13). The efficiency of extracting bioactive constituents from medicinal plants is largely determined by the extraction method employed and the type of solvent used. Because solvents differ in their polarity, they vary in their ability to dissolve and recover specific classes of phytochemicals, which in turn influences the chemical composition and biological activities of the resulting extracts (14). Consequently, a systematic evaluation of different extraction solvents is essential for maximizing the yield of bioactive compounds and gaining a better understanding of their distribution within plant materials.

Materials and Methods

1.1 Collection, identification and preparation of plant material

Fresh roots of Jatropha tanjorensis were collected from the Meerut district of Uttar Pradesh, India, during March 2023. The plant material was taxonomically authenticated by Prof. (Dr.) Mohammed Arif, Plant Pharmacognosist, Faculty of Pharmacy, Integral University, Lucknow, India. Voucher specimens were deposited in the Herbarium Unit of the Faculty of Pharmacy, Integral University, Lucknow, under accession numbers IU/PHAR/HRB/24-13 and IU/PHAR/HRB/24-14 for future reference. The collected root samples were thoroughly cleaned to remove adhering foreign matter, air-dried at room temperature for 5–6 days, and subsequently pulverized into a coarse powder. The powdered material was then stored in clean polyethylene bags under appropriate conditions until further analysis.

1.2 Extraction of root of *Jatropha tanjorensis*

The hydroalcoholic extract of Jatropha tanjorensis roots was prepared using the Soxhlet extraction technique (9). Briefly, 500 g of the powdered root material was packed into a cellulose extraction thimble and placed in the Soxhlet apparatus. A hydroalcoholic solvent consisting of ethanol and water in a ratio of 75:25 (v/v) was used as the extraction medium. The extraction process was continued for approximately 72 hours, allowing repeated cycles of solvent evaporation, condensation, and percolation through the plant material to ensure efficient extraction of bioactive constituents. Upon completion of the extraction, the extract was filtered through Whatman No. 42 filter paper to remove insoluble residues. The filtrate was then concentrated under reduced pressure using a rotary evaporator maintained at 40°C to remove the solvent. The resulting dried extract was stored at room temperature in an airtight container until further phytochemical and biological analyses were performed (9).

1.3 Preliminary Phytochemical Screening

Preliminary qualitative phytochemical analysis of the extract was performed using standard procedures described by Harborne (15) and Trease and Evans (16) with slight modifications, to detect the presence of the major classes of secondary metabolites.

1.3.1 Alkaloids - Wagner's test: Approximately 2 ml of the plant extract was mixed with a few drops of Wagner's reagent (iodine–potassium iodide solution). The appearance of a brown or reddish-brown precipitate was considered a positive indication of the presence of alkaloids.

1.3.2 Saponins - Foam test: One ml of the extract was diluted with 2 ml of distilled water in a test tube and shaken vigorously. The formation of a stable, persistent

froth that remained for several minutes was taken as evidence of the presence of saponins.

1.3.3 Steroids - Liebermann–Burchard test: Two ml of the extract were mixed with 2 ml of acetic anhydride, followed by the careful addition of a few drops of concentrated sulfuric acid. The development of a violet color that gradually changed to blue was considered indicative of the presence of steroids.

1.3.4 Phenols – Ferric chloride test: One ml of the plant extract was treated with a 5% ferric chloride solution. The appearance of a deep blue coloration was interpreted as a positive result for the presence of phenolic compounds.

1.3.5 Quinones - Test for quinones: A few ml of the plant extract were treated with 5 ml of hydrochloric acid (HCl). The formation of a yellow precipitate was considered indicative of the presence of quinones.

1.3.6 Flavonoids - Test for flavonoids: A few ml of the plant extract were mixed with an appropriate volume of diluted sulfuric acid. The development of an orange coloration was taken as a positive indication of the presence of flavonoids.

1.3.7 Proteins: Biuret and ninhydrin tests

1.3.8 Glycosides - Keller–Kiliani test: One ml of the plant extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. Subsequently, 1 ml of concentrated sulfuric acid was carefully added along the side of the test tube. The formation of a distinct brown ring at the junction of the two layers was considered a positive indication of the presence of cardiac glycosides.

1.3.9 Terpenoids (Noller's test) - A few mg of the dried extract were placed in a dry test tube containing a small piece of tin foil. Approximately 0.5 ml of thionyl chloride was added, and the mixture was gently warmed when necessary. The appearance of a pink coloration was considered indicative of the presence of terpenoids.

1.3.10 Test for Resins - The extract was diluted with 5 ml of distilled water and observed for the development of turbidity, which was considered indicative of the presence of resins. In a confirmatory test, 3 ml of the extract was mixed with 3 ml of acetone and hydrochloric acid (HCl), followed by heating in a water bath for 30 minutes. The appearance of a pink coloration after heating was recorded as a positive result.

1.4 Pharmacognostical Evaluation

1.4.1 Fluorescence analysis - Fluorescence analysis of the dried root powder was performed according to the standard procedure described by Kokate. The powdered drug was treated with different reagents and subsequently examined under ultraviolet light at wavelengths of 254 nm and 366 nm to observe its fluorescence characteristics.

1.4.2 Physico-chemical evaluation of the root of *Jatropha tanjorensis* - The physicochemical properties of the powdered root, including moisture content, ash values (total ash, acid-insoluble ash, and water-soluble ash), extractive values, loss on drying, and the determination of foreign organic matter, were evaluated using the standard procedures outlined in the World Health Organization (WHO) guidelines for quality control of medicinal plant materials (17), along with the methods described by Kokate (18).

1.5 Total Flavonoid Content (TFC) of the root of *Jatropha tanjorensis* - A stock solution of the extract was prepared by dissolving the sample in methanol to obtain a final concentration of 10 mg/ml. For the assay, 0.5 ml of the stock solution was transferred into a test tube and combined with 1.5 ml of methanol. Thereafter, 0.1 ml each of aluminum chloride ($AlCl_3$) and sodium acetate (CH_3COONa) were added, followed by the addition of 2.8 ml of distilled water. The reaction mixture was thoroughly mixed and allowed to stand at room temperature for 30 minutes before its absorbance was measured at 415 nm using a UV–Visible spectrophotometer.

Quercetin was used as the reference standard for calibration. Aliquots (0.5 ml) of the prepared quercetin standard solutions were treated using the same procedure, with the addition of 1.5 ml of methanol, 0.1 mL of aluminum chloride, 0.1 mL of sodium acetate, and 2.8 mL of distilled water.

Following incubation for 30 minutes at room temperature, the absorbance of each standard solution was recorded at 415 nm. The blank solution contained 2 ml of methanol, 0.1 ml of aluminum chloride, 0.1 ml of sodium acetate, and 2.8 ml of distilled water. A standard calibration curve was generated from the absorbance values of the quercetin standards (Fig. 2), and the total flavonoid content of the sample was calculated by reference to the corresponding calibration curve (19).

1.6 Total Phenolic Content (TPC) of the root of *Jatropha tanjorensis*

A 10% Folin–Ciocalteu reagent was prepared using distilled water, and a 1 M sodium carbonate (Na_2CO_3) solution was similarly prepared. A stock solution of gallic acid (1 mg/ml) was prepared in methanol and subsequently diluted with ethanol to obtain working standard solutions within the concentration range of 25–300 µg/ml. The extract was dissolved in ethanol to obtain a final concentration of 10 mg/ml. An appropriate aliquot of the sample solution was mixed with 5 ml of Folin–Ciocalteu reagent, followed by the addition of 4 ml of 1 M sodium carbonate solution. The reaction mixture was allowed to incubate at room temperature for 15 minutes before its absorbance was measured at 765 nm using a UV–Visible spectrophotometer. For preparation of the calibration curve, 0.5 ml aliquots of each gallic acid standard solution were treated with 5 ml of Folin–Ciocalteu reagent and 4 ml of sodium carbonate solution under identical experimental conditions. After 15 minutes of incubation, the absorbance of each standard solution was determined at 765 nm. The blank solution consisted of 0.5 ml of methanol, 5 mL of Folin–Ciocalteu reagent, and 4 ml of

sodium carbonate solution. A standard calibration curve was constructed from the absorbance values of the gallic acid standards (Fig. 1), and the total phenolic content of the sample was calculated from the calibration plot. The results were expressed as gallic acid equivalents (GAE), where applicable (20).

1.7 Total Saponins Content of the root of *Jatropha tanjorensis* - The total saponin content was estimated according to the method of Obadoni and Ochuko (21), with minor modifications where necessary. Briefly, 20 g of finely powdered plant material was placed in a conical flask containing 100 ml of 20% aqueous ethanol. The mixture was heated in a water bath at approximately 55°C for 4 hours with continuous stirring to facilitate extraction. After filtration, the residual plant material was re-extracted with 200 ml of 20% aqueous ethanol under the same conditions. The combined filtrates were concentrated to approximately 40 ml by evaporation in a water bath maintained at about 90°C.

The concentrated extract was transferred into a 250 mL separating funnel, and 20 ml of diethyl ether was added. The mixture was shaken thoroughly to separate the phases, after which the ether layer was discarded while the aqueous layer was retained. This purification step was repeated to ensure the removal of non-polar impurities. Subsequently, 60 ml of *n*-butanol was added to the aqueous phase to extract the saponins. The combined *n*-butanol extracts were washed twice with 10 mL portions of 5% aqueous sodium chloride solution to remove residual impurities. The purified extract was evaporated to dryness in a water bath and further dried in an oven until a constant weight was achieved. The total saponin content was then calculated and expressed as a percentage of the original sample weight (22).

1.8 Total Alkaloidal Content of the root of *Jatropha tanjorensis* - The total alkaloid content was determined using the method approximately 5 g of the powdered plant sample was placed in a 250 mL beaker, and 200 ml of 10% acetic acid prepared in ethanol was added. The mixture was covered and left to stand for 4 hours to ensure adequate extraction of alkaloids. Following extraction, the solution was filtered, and the filtrate was concentrated on a water bath until its volume was reduced to about one-quarter of the original volume. Concentrated ammonium hydroxide was then added gradually, with continuous stirring, until complete precipitation of the alkaloids was achieved. The mixture was allowed to stand to facilitate complete settling of the precipitate. The precipitated alkaloids were separated by filtration, washed with dilute ammonium hydroxide to remove residual impurities, and filtered again. The recovered residue was dried to a constant weight, and the total alkaloid content was calculated based on the weight of the dried precipitate (23).

1.9 High-Performance Thin Layer Chromatography (HPTLC) Activity of the root of *Jatropha tanjorensis*

High-performance thin-layer chromatography (HPTLC) analysis was carried out using silica gel 60 F254 100 × 100 mm plates (Merck). A mobile phase consisting of toluene, ethyl acetate, and formic acid in the ratio of 5:4.5:0.5 (v/v/v) was employed for the chromatographic separation of both the reference standards (gallic acid and quercetin; 0.5–5 µl) and the *Jatropha tanjorensis* extract (10 µl). Sample application was performed using a CAMAG Linomat V sample applicator (Muttenz, Switzerland) fitted with a 100 µl Hamilton syringe. The samples were applied as 8 mm bands with an application volume of 10 µl at a rate of 160 nl/s. The spacing between adjacent tracks was maintained at 10 mm, with a distance of 15 mm from the edge of the plate, while the solvent migration distance was set at 70 mm. Following chromatographic development, the plates were scanned densitometrically using a CAMAG TLC Scanner III controlled by WinCATS software (version 1.2.0). Detection was performed in absorption/reflection mode at wavelengths of 254 nm, 366 nm, and within the visible region. Peak integration was carried out using the Gaussian (legacy) algorithm with the sensitivity, separation, and threshold parameters set at 0.1, 1, and 0.1, respectively (24).

1.10 Animals - Adult Wistar rats weighing 140–180 g and Swiss albino mice weighing 25–40 g were obtained from the Laboratory Animal Centre (LAC), Lloyd Institute of Management and Technology, Greater Noida, Uttar Pradesh, India. The animals were housed individually in polypropylene cages under standard laboratory conditions, with a controlled temperature of 25 ± 2°C, relative humidity of 50–60%, and a 12-hour light/12-hour dark cycle. Before the commencement of the study, the animals were acclimatized to the laboratory environment for one week. Throughout the experimental period, they were provided with standard laboratory rodent pellet feed (Dayal Industries Limited, Barabanki, Uttar Pradesh, India) and clean drinking water *ad libitum*. All experimental procedures involving animals were conducted in accordance with the established guidelines for the care and use of laboratory animals.

1.10.1 Acute toxicity test - Twenty-four healthy Swiss albino mice of both sexes were randomly assigned to four experimental groups, with six animals in each group (n = 6). Animals in Group A served as the control and received distilled water orally at a dose of 10 ml/kg body weight. Groups B, C, and D were administered the hydroalcoholic root extract of *Jatropha tanjorensis* (HREJT) orally at doses of 50, 300, and 2000 mg/kg body weight, respectively. Following treatment, the animals were closely monitored for a period of 48 hours to detect any signs of acute toxicity, behavioral abnormalities, or mortality.

1.10.2 Effect of hydroalcoholic root extract of *J. tanjorensis* on rifampicin (R) and isoniazid (I) Induced Hepatotoxicity

The animals were randomly divided into 5 groups of

male wistar rats 6 animals per group for 28 days and treated as follows.

Group A: Animals received no treatment and served as normal control for 28 days

Group B: Animals received selected dose of anti-tubercular drugs R+I (50 mg/kg body wt. each, p.o) for 28 Days.

Group C: Animals were treated orally with 100 mg/kg of silymarin (a standard hepatoprotective drug) and R+I (50 mg/kg body wt. each, p.o) for 28 days.

Group D: Animals were orally treated with (R+I) + HREJT (100 mg/kg for 28 days separately). **Group E:** Animals were orally treated with (R+I) + HREJT (200 mg/kg for 28 days separately). Male Wistar rats were randomly allocated into five experimental groups, with six animals in each group (n = 6), and the study was conducted over a period of 28 days. Group A served as the normal control and received only purified water containing 1% carboxymethyl cellulose (CMC). Hepatotoxicity was induced in Groups B, C, D, and E by oral administration of isoniazid and rifampicin, each at a dose of 50 mg/kg body weight. Following induction, Groups D and E were treated orally with the hydroalcoholic root extract of *Jatropha tanjorensis* (HREJT) at doses of 100 mg/kg and 200 mg/kg body weight, respectively, prepared as an aqueous suspension. Group C received silymarin (100 mg/kg body weight) as the standard hepatoprotective treatment.

Forty-eight hours after the induction of hepatotoxicity, blood samples were collected from each rat via the retro-orbital venous plexus at the medial canthus of the eye using sterile capillary tubes. The collected blood samples were centrifuged at 200 rpm for 10 minutes to separate the serum, which was carefully transferred into sterile plain sample tubes and stored for subsequent biochemical analysis.

The separated serum was analyzed to determine various biochemical markers of liver function, including serum bilirubin (SBL) (24), cholesterol (CHL) (25), albumin (ALB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT) (26), alkaline phosphatase (ALP) (27), and total protein (TP) (28). In addition, the supernatant obtained from the liver homogenate was subjected to colorimetric assays to evaluate antioxidant status by measuring the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) (29), as well as the level of thiobarbituric acid reactive substances (TBARS), an indicator of lipid peroxidation (30).

Statistical analysis - All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test for multiple comparisons between groups. Differences were considered statistically significant when the *p*-value was less than 0.05.

Result

To determine the actual extraction yield, the crude extract obtained from the powdered roots of *Jatropha tanjorensis* by Soxhlet extraction was concentrated by completely removing the hydroalcoholic solvent composed of 75% ethanol and 25% water. Following solvent evaporation, the dried extract was weighed, and the percentage yield of the hydroalcoholic root extract was calculated. The extraction process produced a percentage yield of 3.883% for the root of *Jatropha tanjorensis.*

Preliminary phytochemical Screening

Preliminary phytochemical screening of the hydroalcoholic root extract of *Jatropha tanjorensis* revealed the presence of several important phytoconstituents, including carbohydrates, glycosides, phenolic compounds, tannins, alkaloids, terpenoids, sterols, saponins, flavonoids, and proteins. The results of the qualitative phytochemical analysis are presented in Table 1.

S. No.	Phytoconstituents	*J. tanjorensis*
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	+
4	Phenolic compounds and tannins	+
5	Flavonoids	+
6	Terpenoids	-
7	Saponins	+
8	Sterols	+
9	Proteins	+
10	Resins	-
11	Mucilage	-
12	Lipids/Fats	-

Positive = Present, **Negative** = Absent

Pharmacognostical Evaluation

Fluorescence analysis: The result of fluorescence analysis for roots of *J. tanjorensis* are tabulated in tables 2.

Table 2: Fluorescence analysis of roots of *J. tanjorensis*

Chemical used	Daylight hours	Ultra violet light	
		254nm	366nm
Crude herbal drugs	Whitish	Colorless	Greenish
1N Hydrochloric acid	Light yellow	Colorless	Light green
50% Hydrochloric acid	Light yellow	Colorless	Light green
HNO_3 (50%)	Light yellow	Colorless	Greenish
H_2SO_4 (50%)	Faintly white	Colorless	Greenish
1N Sodium hydroxide	Yellowish	Yellowish	Light green
Alum. Sodium hydroxide	Light yellow	Yellowish	Olive green
CH_3OH	Light yellow	Colorless	Purple
C_6H_6	Yellowish	Colorless	Light green
$CH_3COOC_2H_5$	Light yellow	Colorless	Yellowish
$FeCl_3$	Light yellow	Colorless	Light green
1% Potassium hydroxide	Light yellow	Colorless	Olive green
$(Pb(CHCOO)_2)$	Colorless	Colorless	Colorless
Distilled H_2O	Whitish	Colorless	Greenish

Physico-chemical evaluation of the root of *Jatropha*

tanjorensis: The physicochemical characteristics of the root of *Jatropha tanjorensis* were evaluated by determining parameters such as total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive value, and alcohol-soluble extractive value. The values obtained were 5.78%, 1.68%, 3.83%, 6.81%, and 5.90% w/w, respectively. In addition, the contents of foreign matter and loss on drying were found to be 1.03% and 6.5% w/w, respectively, indicating the quality and purity of the plant material.

Phytochemical Analysis: Quantitative phytochemical analysis of the hydroalcoholic root extract of *Jatropha tanjorensis* (HREJT) confirmed the presence of several bioactive secondary metabolites, including flavonoids, saponins, alkaloids, and phenolic compounds, as presented in Table 3. The extract exhibited appreciable levels of flavonoids, saponins, and alkaloids, while the total phenolic content was determined to be 80.72 ± 0.62 µg GAE/ml, expressed as gallic acid equivalents (GAE).

Table 3 Phytochemical constituent of *Jatropha tanjorensis* hydro alcoholic roots extract

S. No.	Phytochemicals	Amount (µg/ml)
1	Flavonoid	58.60 ± 1.202
2	Saponin	0.79 ± 0.057
3	Alkaloid	1.94 ± 0.026

Total Phenol Content Estimation (TPC): The total phenolic content (TPC) of the hydroalcoholic root extract of *Jatropha tanjorensis* (HREJT) was expressed as micrograms of gallic acid equivalents (GAE) per milliliter of dry extract and calculated using the calibration equation Y = 0.0097X + 0.0279 (R^2 = 0.9963), where Y represents the absorbance and X corresponds to the gallic acid equivalent concentration. Based on the calibration curve, the TPC of the extract was determined to be 80.72 ± 0.62 µg GAE/mL, as illustrated in Figure

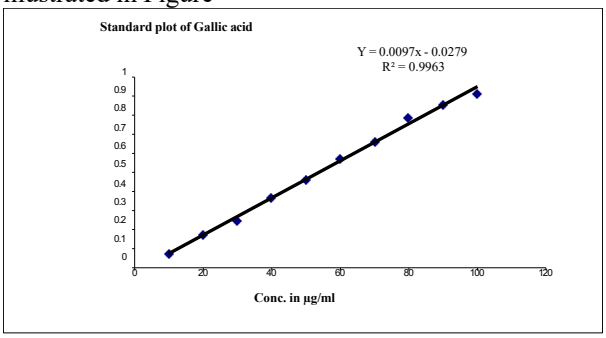


Figure 1: Calibration curve for total phenolic content in reference to Gallic acid

Total Flavonoids Content Estimation (TFC): The total flavonoid content (TFC) of the hydroalcoholic root extract of *Jatropha tanjorensis* (HREJT) was determined and expressed as micrograms of quercetin equivalents (QE) per milliliter of extract. Quantification was performed using the calibration equation Y = 0.0063X + 0.0713 (R^2 = 0.9911), where Y denotes the absorbance and X represents the quercetin equivalent concentration. Based on the standard calibration curve, the TFC of the extract was calculated to be 58.60 ± 1.202 µg QE/ml. The quantitative results are presented in Table 3 and Figure 2.

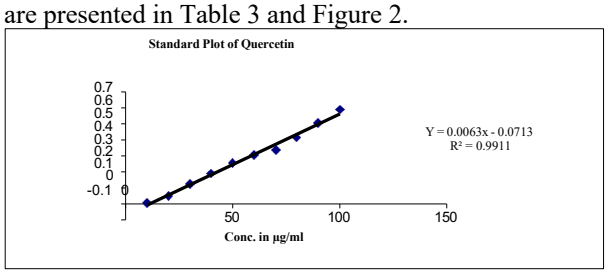


Figure 2: Calibration curve of total flavonoidal contents in reference to quercetin.

HPTLC Fingerprint Analysis: HPTLC fingerprint analysis of the hydroalcoholic root extract of Jatropha tanjorensis (HREJT) revealed a chromatographic profile consisting of fourteen distinct peaks, indicating the presence of multiple phytochemical constituents. The reference standard, quercetin, produced a well-defined and compact peak with an Rf value of 0.51, detected at 290 nm, as illustrated in Figures 3 and 4. Analysis of the HREJT chromatogram at the same wavelength confirmed the occurrence of several phytoconstituents with different retention factors. A total of fourteen spots were identified with Rf values of 0.15, 0.19, 0.25, 0.32, 0.36, 0.42, 0.49, 0.51, 0.57, 0.64, 0.68, 0.73, 0.79, and 0.85. Notably, one of the peaks in the extract exhibited an Rf value of 0.51, corresponding to that of the quercetin standard, suggesting the presence of quercetin or a closely related compound in the extract. The HPTLC chromatographic profile of HREJT and the corresponding quercetin standard are presented in Figures 3 and 4, respectively.

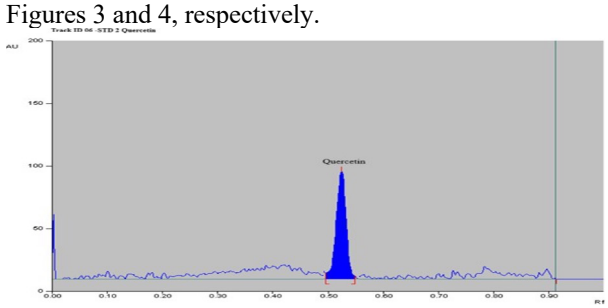


Figure 3. Standard Quercetin HPTLC Chromatogram at Rf value 0.51, Scanned at 290 nm.

Figure 4. HPTLC Chromatogram of a Hydro-Alcoholic Root Extract of *Jatropha tanjorensis* (HREJT) (Peak 1-14) Scanned at 290 nm.

Acute toxicity study - The acute oral toxicity study demonstrated that administration of the hydroalcoholic root extract of *Jatropha tanjorensis* (HREJT) did not produce any observable behavioral abnormalities or mortality in mice during the first 24 hours or throughout the subsequent 14-day observation period. No treatment-related deaths were recorded during the study, indicating that the median lethal dose (LD_{50}) of the extract was greater than 2000 mg/kg when administered orally. Based on these findings, oral doses of 100 mg/kg

and 200 mg/kg body weight were selected for subsequent pharmacological investigations.

Impact of HREJT on rats subjected to rifampicin (R) and isoniazid (I)-induced toxicity: body weight, liver weight, and relative liver weight.

At the commencement of the experiment, no significant differences were observed in the mean body weights among the five experimental groups, indicating a uniform baseline. Rats in the normal control group (Group A) maintained a steady increase in body weight throughout the four-week study period and exhibited no signs of abnormality.

In contrast, rats administered rifampicin and isoniazid (Group B) showed a significant reduction ($P < 0.001$) in body weight, decreasing from 217.72 ± 7.42 g in the normal control group to 164.38 ± 5.11 g, indicating the development of hepatotoxicity. Oral administration of the hydroalcoholic root extract of *Jatropha tanjorensis* (HREJT) at doses of 100 mg/kg and 200 mg/kg body weight significantly improved body weight to 198.70 ± 6.44 g ($P < 0.01$) and 208.39 ± 8.28 g ($P < 0.001$), respectively, compared with the hepatotoxic control group. Similarly, treatment with the standard hepatoprotective agent, silymarin (100 mg/kg), produced a significant ($P < 0.001$) increase in body weight to 212.80 ± 4.56 g relative to Group B. These findings are presented in Figure 5.

Administration of rifampicin and isoniazid resulted in a significant ($P < 0.001$) increase in liver weight in the hepatotoxic control group (Group B), with the mean liver weight rising from 5.76 ± 0.58 g in the normal control group (Group A) to 8.55 ± 0.72 g. Treatment with the hydroalcoholic root extract of Jatropha tanjorensis (HREJT) at doses of 100 mg/kg (Group C) and 200 mg/kg (Group D), as well as the standard drug silymarin (Group E), significantly ($P < 0.05$–$P < 0.001$) reduced liver weight to 6.98 ± 0.39 g, 5.70 ± 0.33 g, and 5.12 ± 0.28 g, respectively, when compared with the hepatotoxic control group. These results are presented in Figure 6.

Similarly, the relative liver weight of rats in Group B increased significantly ($P < 0.001$) from 3.20 ± 0.08 g in the normal control group to 5.12 ± 0.22 g, indicating marked hepatic enlargement following rifampicin and isoniazid administration. Oral treatment with HREJT at 100 mg/kg and 200 mg/kg, as well as silymarin, significantly ($P < 0.05$–$P < 0.001$) decreased the relative liver weight to 4.69 ± 0.27 g, 3.41 ± 0.18 g, and 3.34 ± 0.10 g, respectively, compared with Group B. The findings are illustrated in Figures 6 and 7.

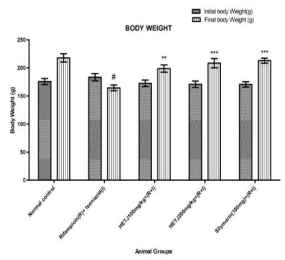


Figure 5: Effect hydro-alcoholic root extract of *J. tanjorensis* (HREJT) on body weight of R+I induced hepatotoxicity in rats. Values are expressed as mean ± SEM of 6 rats in each group. P values: #<0.001, Compared with respective normal control group. P values: *<0.05, **<0.01, ***<0.001 Compared with group II (R+I).

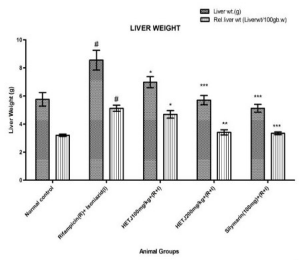


Figure 6: Effect of hydro-alcoholic root extract of *J. tanjorensis* (HREJT) on liver weight and relative weight liver of control and R+I induced hepatotoxicity in rats.

Effect of HREJT on Serum bilirubin (SBL), Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT)), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), Total protein (TP), Albumin (ALB), Serum bilirubin (SBL) Cholesterol (CHL) and Triglyceride (TG) levels in Rifampicin (R) + Isoniazid (I) Induced-Hepatotoxic rats.

Figure 8 (A–I) illustrates the effects of the hydroalcoholic root extract of *Jatropha tanjorensis* (HREJT) on biochemical parameters in rats with rifampicin- and isoniazid-induced hepatotoxicity. Compared with the normal control group (Group A), oral administration of rifampicin and isoniazid at 50 mg/kg each produced a significant ($P < 0.001$) elevation in serum bilirubin (SBL), serum glutamate pyruvate transaminase (SGPT/ALT), serum glutamate oxaloacetate transaminase (SGOT/AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and cholesterol levels, indicating marked hepatic injury.

Pretreatment with HREJT at doses of 100 mg/kg and 200 mg/kg body weight, administered one hour before rifampicin and isoniazid, significantly reduced the elevated levels of SBL ($P < 0.01$–$P < 0.001$), SGPT ($P < 0.001$), SGOT ($P < 0.01$–$P < 0.001$), ALP ($P < 0.001$), LDH ($P < 0.01$–$P < 0.001$), and cholesterol ($P < 0.05$–$P < 0.001$) when compared with the hepatotoxic control group (Group B). These findings suggest that HREJT effectively restored the altered hepatic biochemical markers toward normal values.

In contrast to the normal control group, rats treated with rifampicin and isoniazid exhibited a significant ($P < 0.001$) reduction in serum total protein (TP) and albumin (ALB) concentrations. However, oral administration of HREJT at both 100 mg/kg and 200 mg/kg significantly ($P < 0.001$) increased the levels of total protein and albumin relative to the hepatotoxic control group, indicating an improvement in liver synthetic function.

Similarly, treatment with the standard hepatoprotective drug silymarin (100 mg/kg) significantly ($P < 0.001$) decreased serum bilirubin and the activities of liver marker enzymes, including SGPT, SGOT, ALP, LDH, and cholesterol, compared with the rifampicin- and isoniazid-treated group. Silymarin also produced a significant ($P < 0.001$) increase in serum total protein

and albumin levels, demonstrating its protective effect against drug-induced hepatotoxicity.

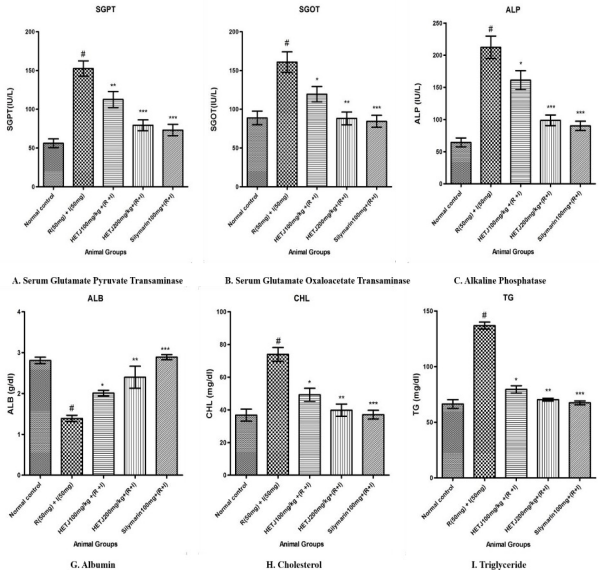


Figure 8. Show different biochemical estimation (A-I).

Conclusion

The findings of the present study demonstrate that the hydroalcoholic root extract of *Jatropha tanjorensis* (HREJT) exerts a beneficial effect on hepatic function by improving key biochemical markers, including serum glutamate pyruvate transaminase (SGPT/ALT), serum glutamate oxaloacetate transaminase (SGOT/AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein (TP), albumin (ALB), serum bilirubin (SBL), cholesterol (CHL), and triglycerides (TG) in experimental rats. These improvements indicate the hepatoprotective potential of the extract against liver injury. Nevertheless, prolonged administration of high doses may raise concerns regarding possible hepatotoxic effects, suggesting that its use should be approached with caution and appropriate dosage considerations. Overall, the results support the potential application of *J. tanjorensis* root extract in the prevention and management of liver disorders. Furthermore, the phytochemical constituents present in the extract may serve as valuable lead compounds for drug discovery, providing molecular templates for the development of novel therapeutic agents and pharmacological research.

Abbreviations

HREJT: Hydroalcoholic root extract of *Jatropha tanjorensis,* LSD: Least significance difference, TFC: Total Flavonoids Content, TPC: Total Phenolic Content, SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, SBL: Serum bilirubin, ALP:

Alkaline phosphatase, LDH: Lactate dehydrogenase, TP: Total protein, ALB: Albumin, SBL: Serum bilirubin, CHL: Cholesterol and TG: Triglyceride

Authors' contributions

AA, GK and AAS designed the protocol. NEA and AAS collected the seeds for preparation of extracts. AAS carried out the laboratory work. HMF and AAS wrote the manuscript. All authors read and revised the manuscript and confirmed its publication.

Conflict of interests

None

Ethical considerations

All animal procedures included in this protocol were approved by Institutional Animal Ethics Committee (IAEC) of Lloyd Institute of Institute of management & Technology, Greater Noida
U.P. India. (IAEC Approval certificate no-1206//PO/Re/S/08/CPCSEA/09/2024/10/21).

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Plant authentication

The root part was identified by Prof. (Dr.) Mohammed Arif, Plant Pharmacognosist at the Faculty of Pharmacy, Integral University, Lucknow, India. A voucher specimen number (IU/PHAR/HRB/24-13 and IU/PHAR/HRB/24-14) was deposited at the Herbarium Unit, Faculty of Pharmacy Integral University, Lucknow, India

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