



PHYTOCHEMICAL INVESTIGATION AND IN VITRO ANTI-OXIDANT ACTIVITY OF DEFATTED HYDROALCOHOLIC EXTRACT OF ROOTS OF *JATROPHA GLANDULIFERA*

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ABSTRACT

Jatropha glandulifera Roxb., a member of the family Euphorbiaceae, is a medicinal shrub widely distributed in tropical and subtropical regions. The plant has been traditionally used in folk medicine for the treatment of inflammation, wounds, skin infections, and gastrointestinal disorders. The present study was undertaken to evaluate the pharmacognostic characteristics, phytochemical constituents, and antioxidant activity of *Jatropha glandulifera*. Macroscopic and microscopic examinations of the leaves were carried out to establish diagnostic features for identification and standardization. Preliminary phytochemical screening of different extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and phenolic compounds. The antioxidant activity of the plant extract was evaluated using standard in vitro methods such as DPPH radical scavenging assay, which demonstrated significant free

radical scavenging potential, indicating the presence of bioactive phenolic compounds. The plant also exhibits reported anti-inflammatory, antimicrobial, analgesic, and anthelmintic

activities. However, due to the presence of toxic constituents, especially in the seeds, proper toxicological evaluation is necessary before therapeutic use. The pharmacognostic standardization and antioxidant assessment of *Jatropha glandulifera* support its potential as a source of natural antioxidant agents and provide a basis for further pharmacological investigations.

1. INTRODUCTION

Jatropha glandulifera Roxb., a bushy shrub belonging to the Euphorbiaceae family and native to arid regions of India including Tamil Nadu, has been extensively utilized in traditional Ayurvedic and ethnomedicinal practices for managing inflammation, asthma, bronchitis, microbial infections, and pain.^[1-3] The roots of the plant have been particularly noted for their antimicrobial activity against pathogens such as *Salmonella typhi* and *Escherichia coli*.^[4,5] Phytochemical investigations of root extracts prepared using solvents such as petroleum ether, ethanol, chloroform, and ethyl acetate have revealed the presence of diverse bioactive secondary metabolites including alkaloids, flavonoids, tannins, saponins, phenolic compounds, triterpenoids, and glycosides.^[6,9] Unique constituents such as coumarino-lignans, jatrophenolone-A, and fraxetin have also been reported in *Jatropha* species, contributing to their broad pharmacological activities.^[10,11] Oxidative stress induced by reactive oxygen species (ROS) and free radicals plays a crucial role in the pathogenesis of chronic diseases, including cancer, by promoting cellular damage, lipid peroxidation, DNA mutations, and uncontrolled cell proliferation.^[12,14] Antioxidants counteract these deleterious effects by scavenging free radicals, chelating pro-oxidant metal ions, and modulating redox signaling pathways.^[15-17] Antioxidant and cytoprotective activities have been documented in related species such as *Jatropha gossypifolia*, supporting the therapeutic relevance of the genus.^[18,19] In view of these findings, the present study was undertaken to comprehensively evaluate the phytochemical composition and antioxidant activity of *J. glandulifera* roots, with particular emphasis on determining whether the observed antioxidant effect may contribute to inhibition of cell proliferation, thereby establishing a preliminary basis for its potential anticancer application.^[20-22]

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Fresh roots of *Jatropha glandulifera* Roxb. were collected from Palayamkottai, Tamil Nadu, India, during December 2025. The plant material was taxonomically authenticated by Dr.S.

Maheswaran, M.Sc., M.Phil., PhD Scientist and a voucher specimen (XCH.40796) was deposited in the departmental herbarium for future reference. Proper botanical identification and authentication procedures were carried out according to standard pharmacognostical methods. The collected roots were washed, shade-dried at room temperature (25–30°C) for 10–14 days, pulverized into coarse powder, passed through sieve No. 40, and stored in airtight containers until extraction. Drying and size reduction procedures were performed as per standard guidelines for crude drug preparation.

2.2 Preparation of Defatted Ethanolic Extract

2.2.1 Defatting by Maceration

Fifty grams (50 g) of powdered root material was defatted with petroleum ether (60–80°C) by maceration for 48 hours with intermittent shaking.^[23-24] Defatting removes non-polar constituents such as fats, waxes, fixed oils, and chlorophyll that may interfere with subsequent extraction and biological evaluation.^[25-26] The mixture was filtered, and the marc was air-dried to remove residual solvent.

2.2.2 Ethanolic Extraction

The defatted marc was extracted with 95% ethanol by cold maceration for 72 hours with occasional shaking to enhance solvent penetration and diffusion of phytoconstituents.^[25-27] The marc was re-macerated to ensure exhaustive extraction. Cold maceration and ethanolic extraction procedures were carried out according to standard pharmacognostic methods.^[24-28]

2.3 Concentration and Drying of Extract

Temperature not exceeding 40–45°C to prevent degradation of thermolabile constituents.^[29-30] The concentrated extract was further dried in a desiccator over anhydrous calcium chloride until constant weight was obtained and stored in amber-colored airtight containers at 4°C for further studies.^[23-29]

2.4 Determination of Percentage Yield

The percentage yield of the extract was calculated using the formula

$$\text{Yield (\%)} = (\text{Weight of dried extract} / \text{Weight of crude drug}) \times 100$$

Yield determination and extractive value calculations were performed according to standard WHO and pharmacognostic guidelines.^[31-32]

2.5 Expected Phytochemical Profile

Based on literature reports on the genus *Jatropha*, the 95% ethanolic extract is expected to contain flavonoids, phenolic compounds, tannins, alkaloids, glycosides, terpenoids, and saponins.^[6-9,18]



Fig.1: Roots of *Jatropha glandulifera*.

3. Qualitative Phytochemical Screening

Qualitative phytochemical screening of the extracts was carried out using standard phytochemical procedures to identify the presence of secondary metabolites such as steroids, triterpenoids, alkaloids, phenolics, tannins, saponins, flavonoids, and glycosides.^[33-35]

3.1 Test for Steroids and Triterpenoids

(a) Liebermann–Burchard Test

A small quantity of the extract was treated with a few drops of acetic anhydride, boiled gently, and allowed to cool. Concentrated sulphuric acid was then carefully added along the side of the test tube.^[33,34] Observation: Formation of a brown ring at the junction of the two layers with a green upper layer the presence of steroids. Development of a deep red coloration suggests the presence of triterpenoids.^[33]

(b) Salkowski Test

The extract was treated with concentrated sulphuric acid and allowed to stand undisturbed.^[34] Observation: A red coloration in the lower layer indicates the presence of steroids, while a yellow coloration indicates triterpenoids.^[34,36]

3.2 Test for Alkaloids

(a) Dragendorff's Test

To 2–3 mL of the filtrate, a few drops of Dragendorff's reagent (potassium bismuth iodide solution) were added.^[33-37]

Observation: Formation of an orange or orange-brown precipitate indicates the presence of alkaloids due to the formation of insoluble complex salts.^[33]

(b) Mayer's Test

To 2–3 mL of the filtrate, a few drops of Mayer's reagent (potassium mercuric iodide solution) were added.^[34]

Observation: Formation of a cream or white precipitate confirms the presence of alkaloids.^[34]

3.3 Test for Phenolic Compounds and Tannins

(a) Ferric Chloride Test

To 2–3 mL of the extract, a few drops of 5% ferric chloride solution were added.^[33-35]

Observation: A deep blue-black coloration indicates hydrolysable tannins, while a greenish-black.

(b) Dilute Iodine Test

The extract was treated with dilute iodine solution.^[35]

Observation: Appearance of transient red coloration supports the presence of tannins.^[35]

3.4 Test for Saponins

Foam Test

The extract was vigorously shaken with distilled water and allowed to stand for 10–15 minutes.^[33,38]

Observation: Formation of stable persistent froth indicates the presence of saponin glycosides.^[33]

3.5 Test for Flavonoids

(a) Shinoda Test

The extract was dissolved in ethanol, followed by addition of magnesium turnings and concentrated hydrochloric acid.^[33-39]

Observation: Development of pink, red, or magenta coloration indicates flavonoids.^[33]

(b) Lead Acetate Test

The extract was treated with lead acetate solution.^[34]

Observation: Formation of yellow precipitate confirms flavonoids.^[34]

3.6 Test for Glycosides**(a) Keller–Kiliani Test**

The extract was treated with glacial acetic acid containing ferric chloride, followed by careful addition of concentrated sulphuric acid.^[33-40]

Observation: Formation of reddish-brown ring at the junction indicates deoxy sugars characteristic of cardiac glycosides.^[33]

(b) Legal's Test

The extract was treated with sodium nitroprusside and pyridine.^[34]

Observation: Development of pink to red coloration indicates cardiac glycosides.^[34]

Table 1: PHYTOCHEMICAL SCREENING.

Sr. No.	TEST	DEFATTED HYDRO ALCOHOLIC EXTRACT OF <i>J. glandulifera</i>
1.	Alkaloids	+
2.	Flavonoids	+++
3.	Phenolic Compounds	++
4.	Tannins	++
5.	Saponins	+/-
6.	Glycosides	++/-
7.	Terpenoids (polar types)	Di/tri ++
8.	Carbohydrates	+
9.	Proteins/Aminoacids	-
10.	Fixed Oils	-
11.	Waxes	-
12.	Chlorophyll	-
13.	Non polar diterpenes	-

4. ANTI-OXIDANT ACTIVITY**4.1 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay**

DPPH radical scavenging activity -Antioxidant activity was determined based on the ability of the antioxidants to act as radical scavengers towards the stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). The ability of the various antioxidants to donate an electron or hydrogen radical to the stable DPPH free radical, prompting a colour change from purple colour to yellow colour solution. Decreased absorbance of DPPH indicates increased antioxidant effect.

Procedure

Sample with different concentrations, 2.9 mL of DPPH reagent (0.1mM in methanol) was added and mixed vigorously. The reaction mixture was stored in the dark for 30 minutes at room temperature and decolouration of DPPH was measured against a blank at 517 nm using an ultraviolet-visible (UV-Vis) spectrophotometer.^[43]

The inhibition % was calculated using the formula

$$\text{Inhibition\%} = \frac{A(\text{control}) - A(\text{test sample})}{A(\text{control})} \times 100$$

DPPH Solution and Test substances

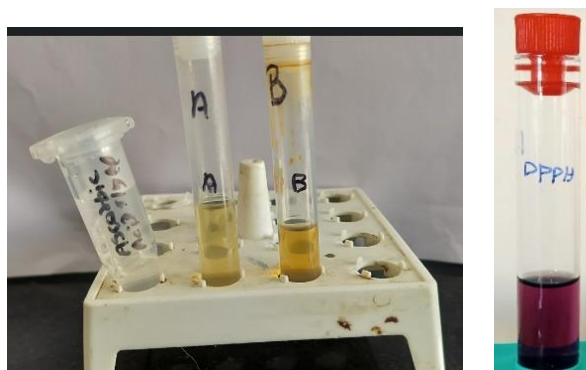


Fig.2: DPPH Solution and Test Substance.

INCUBATION OF COMPOUNDS WITH DPPH



Fig.3: TUBE METHOD.



Fig.4: MICROPLATE METHOD (to cross check we did)

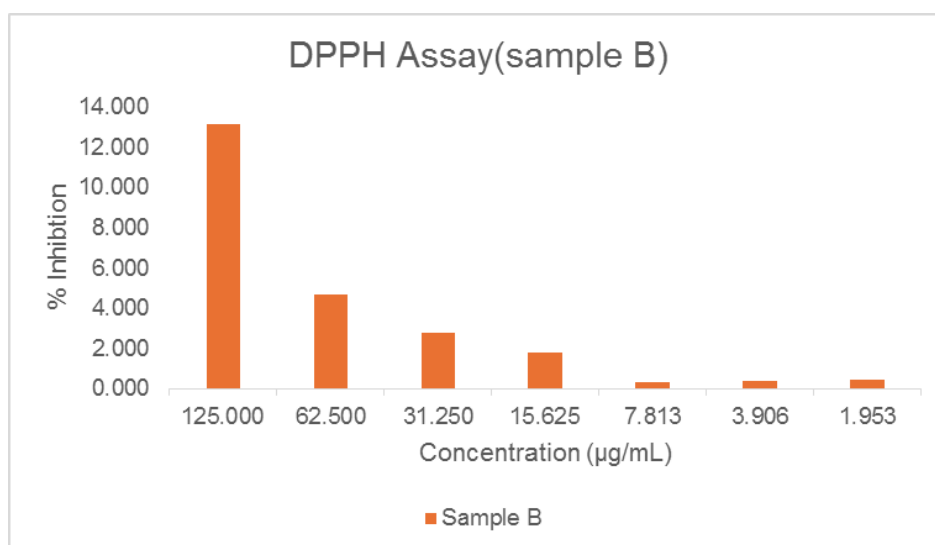
Table 2: Sample result for anti oxidant.

S.no	Samples (B)ug /ml	Inhibition (%) Well 1	Inhibition (%) Well 1	Mean	SD
1	125.00	13.202	13.103	13.153	0.070
2	62.50	4.729	4.631	4.680	0.070
3	31.25	2.857	2.660	2.759	0.139
4	15.63	1.872	1.773	1.823	0.070
5	7.81	0.099	0.493	0.296	0.279
6	3.91	0.394	0.394	0.394	0.000
7	1.95	0.394	0.493	0.443	0.070

Table 3: % inhibition mean of the sample.

S.no	Samples (B)ug/ml	% inhibition Mean Sample	SD
1	125.00	13.153	0.070
2	62.50	4.680	0.070
3	31.25	2.759	0.139
4	15.63	1.823	0.070
5	7.81	0.296	0.279
6	3.91	0.394	0.000
7	1.9	0.443	0.070

Sample showed higher inhibition at 125 ug/ml which is the highest tested concentration.



5. RESULT AND DISCUSSION

The antioxidant activity of the defatted hydroalcoholic root extract of *Jatropha glandulifera* was evaluated using the DPPH radical scavenging assay. The extract exhibited a concentration-dependent increase in percentage inhibition over the tested range of 1.95–125 µg/mL. The maximum radical scavenging activity observed was $13.153 \pm 0.070\%$ at 125 µg/mL, while lower concentrations demonstrated progressively reduced inhibition ($4.680 \pm 0.070\%$ at 62.50 µg/mL; $2.759 \pm 0.139\%$ at 31.25 µg/mL; $1.823 \pm 0.070\%$ at 15.63 µg/mL). Minimal inhibition was recorded at concentrations below 10 µg/mL. The IC_{50} value was extrapolated to be approximately 475 µg/mL, as 50% inhibition was not achieved within the tested concentration range, indicating comparatively low to moderate free radical scavenging potential under the experimental conditions.

6. CONCLUSION

The present study demonstrates that the defatted hydroalcoholic root extract of *Jatropha glandulifera* possesses concentration-dependent DPPH radical scavenging activity, with a

maximum inhibition of $13.153 \pm 0.070\%$ observed at $125 \mu\text{g/mL}$. Although the extract exhibited measurable antioxidant activity, the inhibition values remained relatively low across the tested concentration range, and 50% radical scavenging was not achieved, indicating modest antioxidant potency under the experimental conditions. The extrapolated IC_{50} value of approximately $475 \mu\text{g/mL}$ further supports the finding of mild free radical scavenging capacity. The observed activity may be attributed to the presence of phenolic compounds, flavonoids, tannins, and terpenoids identified during phytochemical screening. Overall, the results suggest that the root extract of *Jatropha glandulifera* exhibits limited but significant antioxidant potential, warranting further detailed pharmacological and mechanistic studies to better establish its therapeutic relevance.

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