International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 5, May 2026 ijppr.humanjournals.com ISSN: 2349-7203

Pharmacognostical Standardization and Comparative Antioxidant Investigation of *Raphanus sativus* and *Carica papaya* for Hepatic Disorders

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Received: 30 March 2026 Revised: 25 April 2026 Accepted: 30 April 2026

ABSTRACT

The aim of the present study was to evaluate therapeutic potential of methanolic extract of leaves of Raphanus sativus and *Carica papaya* in screening animals with hepatotoxicity. The plant extracts were evaluated for their antioxidant activity, qualitatively analysed for phytochemical compounds and molecularly characterized. Different solvents and extraction processes were used for the extraction of plant materials. This was followed by the physicochemical and qualitative phytochemical tests, which gave results in agreement with the pharmacopoeia. The HPTLC analysis of the methanolic extracts showed different chromatographic peaks for quercetin and rutin, creating the condition of presence of high level of important chemicals belonging to the flavonoids group. The quantitative analysis of total phenolic and flavonoid contents revealed that methanolic extract of Raphanus sativus leaves had significantly higher phenolic and flavonoids content than *Carica papaya* leaves. In vitro, the antioxidant activity of extracts was evaluated using various methods of free radical scavenging. The leaves methanolic extract of Raphanus sativus showed significant antioxidant capacity in DPPH radical scavenging assay, similar to the normal ascorbate. The methanolic extract of *Carica papaya* demonstrated considerable free radical scavenging activity, thus indicating good antioxidant activity. The results showed that both the extracts had appreciable antioxidant and hepatoprotective activities which could be beneficial in liver related ailments.

Keywords: *Raphanus sativus*, *Carica papaya*, Antioxidant, Hepatoprotective activity

1. INTRODUCTION

Herbal medicine refers to the use of plants and plant extracts to treat, treat and prevent diseases. It is one of the oldest healthcare systems which has been followed since ages in various traditional systems like Ayurveda, Traditional Chinese Medicine, and Unani medicine. Herbal medicines are made from different parts of plants, such as their leaves, roots, bark, stems, flowers, fruits and seeds, which contain physiologically active chemicals that have medicinal properties.[1,2] The medicinal plants have a rich source of phytochemicals such as alkaloids, flavonoids, tannins, glycosides, terpenoids, saponins, phenolic compounds and essential oils. The bioactive compounds exhibited a wide range of pharmacological activities such as antioxidant, anti-inflammatory, antibacterial, antidiabetic, hepatoprotective, cardioprotective, neuroprotective, and anticancer activities. Herbals may have synergistic therapeutic effects because of the multiple active ingredients, and they tend to have less side effects than much of synthetic medicines.[3,4]

Phytochemical screening, standardization, extraction and isolation of active substances, pharmacological and toxicological studies are the scientific steps involved in the assessment of herbal remedies. Bioactive chemical analysis is performed routinely using advanced analytical techniques for detection and characterization of bioactive chemicals from plant extracts. Experimental research using in vitro and in vivo models help to establish their therapeutic effectiveness and safety.[5,6]

Hepatoprotective activity

Hepatology places a focus on protecting the liver because the liver is essential to the body's metabolic system, detoxification, protein synthesis, storage of nutrients and biochemical regulation of the body. The liver is vital for carbohydrate, lipid, and protein metabolism, and for detoxification of toxic chemicals such as drugs, alcohol, pollutants in the environment, and metabolic products. The liver is especially susceptible to damage and loss of function from chronic exposure to toxic chemicals and xenobiotics.[7,8]

Hepatotoxicity is a chemical, drug, infectious, alcoholic, and oxidative liver damage. Acute liver toxicity is caused by a variety of hepatotoxic agents including carbon tetrachloride, paracetamol, alcohol and D-galactosamine, through mechanisms of oxidative stress, inflammation, lipid peroxidation and cell necrosis. Liver impairment may result in serious liver diseases such as hepatitis,

cirrhosis, fatty liver, fibrosis and liver failure. Rise in levels of hepatic enzymes is considered significant parameters of hepatic impairment.[9,10]

Raphanus sativus

Raphanus sativus, commonly known as radish, is a vegetable that is eaten for its root. It has a wide cultivation world-wide for its edible succulent roots and leaves. Annual or biennial herb with a short stem, lobed leaves, white to pinkish flowers and white, pink, red, purple or black roots, which are tuberous. R. sativus is a phytochemical plant containing different bioactive compounds such as glucosinolates, isothiocyanates, flavonoids, phenolic acids, anthocyanins, alkaloids, terpenoids and tannins. This is the reason why these compounds make it an effective powerful antioxidant, anti-inflammatory, anti-bacterial, hepatoprotective, and anti-cancer agent. The root contains high level of vitamin C, folate, potassium and dietary fibre, and is also a good source of proteins, minerals and secondary metabolites; the seeds and leaves also contain high levels of these nutrients. The byproducts of glucosinolates known as raphanin and raphasatin, are known to be bioactive in A. flavipinata. The hepatoprotective activity of Raphanus sativus has been pharmacologically proven to protect the liver against oxidative stress and damage due to pathogen infection, by regulating the levels of antioxidant enzymes and lipid peroxidation.[11,12]

Carica papaya

The plant *Carica papaya* (papaya or pawpaw) is a fast-growing perennial plant from the Caricaceae family. It is native to tropical America but is now widely grown worldwide in tropical and subtropical areas for its fruits, leaves and seeds which are valued for their nutritional and medicinal properties. The plant features a hollow, erect stem, large leaves with large lobes and fruit that looks like a melon but is loaded with bioactive chemicals.
Many secondary metabolites are phytochemically rich in C. papaya, such as alkaloids, flavonoids, tannins, saponins, phenolic acids, terpenoids and glucosinolates. The flavonoids, alkaloids like carpaine and the phenolic compounds are especially abundant in the leaves, and they have a great antioxidant potential. Papaya fruits contain high levels of vitamins A, C, E, folate and dietary fiber while the seeds contain benzyl isothiocyanate and fatty acids. Proteolytic enzyme papain present in the latex of unripe fruit is used in food, medicine and to heal wounds. *Carica papaya* has antioxidant, hepatoprotective, anti-inflammatory, anti-bacterial, anti-malarial, and anti-cancer pharmacological properties.[13,14]

2. MATERIAL AND METHODS

2.1 Collection of the selected herbal drugs

2.1.1 Plant material:

The fresh leaves of Carica papaya and Raphanus sativus were excised and meticulously rinsed with distilled water to eliminate surface impurities. Shade drying and fine mechanical grinding were employed for the leaves. The powdered components were preserved in an airtight container for subsequent analysis.

2.2 Pharmacognostical evaluation of drugs

2.2.1 Macroscopical study:

All chosen pharmacological samples underwent macroscopic assessment. Attention was paid to the physical character of the samples, and their results were thoroughly reported. The assessment was carried out with natural sunlight and under artificial light that mimics daylight to ensure accurate assessment of samples.

2.2.2 Microscopical study:

The selected medicines samples were all examined microscopically. The samples were carefully studied and results recorded systematically. Thin sections of the materials were prepared using a microtome, mounted on slides and then stained with chemicals to highlight and identify some of the microscopic characteristics.

2.3 Physicochemical Evaluation of Drug:

2.3.1 Determination of individual extractive values:

Extractive value is the amount of soluble active or inactive substances which can be recovered from a given amount of medicinal plant with solvent such as water or alcohol. This is often used as a quality control measurement of crude pharmaceuticals where there is no suitable biological or chemical evaluation method.

2.3.2 Soxhlet extraction:

The granules of the drug are individually packed in the different components of Soxhlet, chloroform, methanol, petroleum ether and water (10g). Each concentration is then evaporated to dryness and an extractive rating is given.

2.3.3 Determination of Ash values:

It is used in the identification of inorganic (crude medicines) complex such as carbonates, silica, oxalates, and phosphates. During the heating, all biological matter is completely removed in the form of carbon dioxide and thus inorganic residues are left behind, which are called ash. Ash value is an important quality control parameter and indicates the purity, quality and contamination of the medicine sample. The range of ash value varies from various medications, but generally for a given drug there is a range of ash values. Silica, which represents contamination with terrestrial material, such as sand and soil, is the main component of acid-insoluble ash. The determination of water-soluble ash is carried out to determine the amount of water-soluble inorganic constituents in the sample.

2.3.3.1 Determination of total ash values:

Total ash is obtained from the whole burning process of medicinal plant materials, which is a combination of physiological ash from plant tissues and non-physiological ash from external pollutants. For determining complete ash the powdered material was burnt in a silica crucible to the complete absence of carbon at a temperature not exceeding 450°C. The crucible was then cooled and weighed to see the amount of ash remaining in the sample.

2.3.3.2 Determination of Acid insoluble ash values:

Acid-insoluble ash is primarily an indicator of silica, sand and other earthy materials. To determine the composition of the ash, 25 mL of weak hydrochloric acid was added to it and heated for 5 minutes. The residue on the filter paper was then collected and carefully washed with hot water and burned to a constant weight at a temperature not exceeding 450°C.

2.3.3.3 Determination of Water-soluble ash values:

The ash obtained was washed with deionized water and the washed ash was collected on ashless filter paper. The rest was then burned to a constant weight at 450°C. The amount of water-soluble ash was calculated as the difference between the weight of ash and the weight of ash after removal of the insoluble ash.

2.4 Phytochemical screening

Appropriate solvents were used in the Soxhlet apparatus to extract the powdered medicines in increasing order of polarity: petroleum ether, chloroform, methanol and water. About 6 hours were allowed for each extraction procedure with sufficient amount of solvent. Extracts were concentrated through the process of being dried under reduced pressure at a controlled temperature of 40–50°C.

The various extracts were then subjected to the preliminary phytochemical screening to screen the principal secondary metabolites. Several chemical tests for alkaloids, carbohydrates, phenolic compounds, flavonoids, proteins, saponins, mucilage, resins, lipids and fats were carried out in the investigation.

Test for alkaloid

Mayer's, Dragendorff's, Wagner's and Hager's assays were used to identify the alkaloids. The precipitates formed in the plant extracts were cream, orange-brown, reddish-brown or yellow, confirming the presence of alkaloids.

Test for carbohydrates

The molisch's, Benedict's, Fehling's test and iodine test were used to identify carbohydrates. The extracts obtained from the plant were tested for carbohydrates and reducing sugar, as indicated by the different changes in colour / precipitate.

Test for proteins

Biuret, Ninhydrin and Xanthoproteic assays were used for the identification of proteins. Violet, purple or yellow colour development confirmed the presence of proteins and amino acids in the plant extracts.

Test for steroids

Steroids were found by using the Salkowski's and Liebermann-Burchard assays. Reddish brown, green or bluish-green colors indicated the presence of steroidal chemicals in the plant extracts.

Test for saponins

The foam test and hemolysis assay were used to identify saponins. The persistent froth and hemolytic activity were formed which confirmed the presence of saponins in the plant extracts.

Test for flavonoids

Shinoda's and alkaline reagent and lead acetate tests were used for the detection of flavonoids. The formation of pink, red or yellow colored hue or yellow precipitate confirmed the presence of flavonoids in the plant extracts.

Test for phenolic compounds

Ferric chloride and Lead acetate tests were used to detect the presence of phenolic chemicals. Color change to blue, green or dark precipitate is an indication of phenolic compound present in the plant extracts.

Test for fixed oils

Fixed oils were identified using the spot test and saponification test. Formation of permanent oily stains and soap-like froth confirmed the presence of fixed oils in the plant extracts.

2.5 Determination of Total Phenolic Content:

The total phenolic content was determined by the Folin–Ciocalteu colorimetric method. Five milliliters of the diluted plant extract solution (0.5 mL in 10 mL freshly distilled water) or the diluted gallic acid solution was mixed with 5 mL of the diluted Folin–Ciocalteu reagent (1:10 ratio with freshly distilled water). Then 4 mL sodium carbonate (Na_2CO_3) solution was added to the mixture. The reaction mixture was allowed to stand for 15 minutes to ensure good colour development. The absorbance was measured at 765 nm by UV-Visible spectrophotometer (Shimadzu UV-1601). Calibration curve was prepared with varying concentration of gallic acid in methanol and then the total phenolic content (TPC) was calculated.

2.6 Determination of Total Flavonoid Content:

Aluminum chloride colorimetric method was used to determine the total flavonoids. The solution of diluted plant extract (0.5 mL, 10 mg/mL) in methanol was mixed with 0.1 mL of 10% aluminum chloride, 1.5 mL of methanol, 0.1 mL of 1 M potassium acetate and 2.8 mL of deionized water. The reaction mixture was then incubated for 30 minutes at room temperature to ensure that the colour was developed. The resulting solution was then measured at 415 nm on a UV–Visible Spectrophotometer. A calibration curve was prepared using different concentrations (10–100 µg/mL) of a methanolic standard solution of the flavonoid and the amount of flavonoids was calculated accordingly.

2.7 Quantitative estimation of quercetin and rutin by using HPTLC

2.7.1 Reagents and other materials: Pre-coated silica gel F254 aluminum plates were utilized in the analysis of HPTLC. The solvents used in mobile phase were analytical grade solvents (E. Merck) namely ethyl acetate, formic acid, methanol and toluene. The reference markers used for the study were quercetin and rutin (both from Sigma, USA).

2.7.2 Preparation of the extracts:

The drying in shade and coarse grinding of plant materials was done separately for Raphanus sativus and Carica papaya. Soxhlet extraction was performed on the powdered materials with methanol as the extraction solvent for about 6 hours at 40-50°C. Carefully filtered, the filtrates were concentrated further under reduced pressure in Rotary Evaporator to obtain the concentrated extracts.

Chromatographic conditions:

Adsorbent: TLC aluminum plates were pre-coated with silica gel 60 F25.

Mode of TLC for development: Ascending mode

Solvent run up to: 80 mm

Standard preparation: 100 ng/µl solution of standard quercetin and rutin in methanol.

Volume of standard application: 1-10 µl

Scanning wavelength: 297 nm

Solvent system:

For HPTLC analysis, ethyl acetate, toluene and formic acid were used in the ratio 40:70:10, v/v. An HPTLC system (CAMAG, Linomat) consisted of a CAMAG TLC Scanner and a computer running the software program winCATS under Microsoft Windows, and a Linomat IV sample applicator with a nitrogen gas supply. The chromatographic separation was done on precoated silica gel HPTLC plates. The chromatographic development was carried out in a glass chamber which was filled with the vapor of the mobile phase for 30 minutes. Solvent front was allowed to travel 80 mm from the bottom edge of the plate. All chemicals and solvents used were analytical reagent grade. The optimum parameters for sample application were a band width of 5 mm, a spacing between bands of 4 mm, an application volume from 1–10 µL and a nitrogen gas flow rate of 10 s/L. The slit dimension for the densitometric scanning was set to 4.00 mm × 0.30 mm (micro); the scanning speed was 20 mm/s; the data resolution was set to 100 µm/step. Regresional analysis and statistical assessment were conducted using winCATS software of the acquired chromatograms.

2.7.3 Preparation of standard solutions:

The standard quercetin and rutin were accurately weighed as 10 mg and added to each 10 mL volumetric flask separately. All chemicals were then dissolved in 5 mL of methanol and later brought up to 10 mL with methanol to make the standard stock solutions of 1 mg/mL.

2.7.4 Sample preparation

About 100 mg of plant extract was accurately weighed and then transferred to a 10 mL volumetric flask and dissolved in the methanol. The solution obtained was filtered through Whatman filter paper No. 42 and the volume of the filtrate was adjusted with methanol to obtain the final sample solution.

2.8 Calibration curve for Quercetin and Rutin

Using a CAMAG sample applicator, various amounts of the standard solutions were applied in triplicate to precoated silica gel 60 F254 thin layer chromatography (TLC) plates.

2.8.1 The quantification of Rutin and Quercetin

A 6.0 µL sample solution of each sample was applied on the same TLC plate using the CAMAG applicator with the same application speed used for the reference standards. These plates were then fabricated and investigated with the enhanced chromatographic conditions. The peak areas corresponding to the peak regions identified were used to determine the concentration of quercetin and rutin using their calibration curves.

2.9 Anti-oxidant activity of the extracts

2.9.1 DPPH scavenging activity

DPPH test was used to evaluate the antioxidant activity of the herbal extracts. DPPH solution was prepared with a concentration of 0.004% w/v in 95% methanol. Stock solutions of the plant extracts (1mg/mL) were prepared using suitable solvents. The extracts were serially diluted in test tubes and 3 mL of freshly prepared DPPH solution added to each test tube to give different concentrations of extracts ranging from 25µg – 200µg.

The reaction mixtures were allowed to react and the absorbance was measured at 515 nm using a Shimadzu UV–Visible spectrophotometer after 10 minutes. The reference chemical was ascorbic acid, which was purchased and used as a stock solution of 1 mg/mL in distilled water. To compare the results, an equivalent volume of DPPH solution without extract or standard was also prepared which served as the blank solution.

2.9.2 Nitric oxide scavenging activity

The nitric oxide scavenging activity was evaluated with sodium nitroprusside in the phosphate buffer solution. The various concentrations of the plant extracts (25 – 200 µg/mL) were prepared in standard phosphate buffer (pH 7.4) and were placed in a 5 hour incubation with sodium nitroprusside at 25°C. After incubation, 0.5 mL of the reaction mixture was taken and mixed with 0.5 mL of Griess reagent. The UV–Visible spectrophotometer was used to determine the absorbance of the resulting chromophore at 546 nm.

2.9.3 Reducing Power Assay

Reducing power of extracts from the plants was determined by the technique of reduction of potassium ferricyanide. Different concentrations of plant extracts (25 to 200 µg/mL) and ascorbic acid solutions (2 to 16 µg/mL) were mixed with 1 mL of distilled water, 2.5 mL of potassium ferricyanide (1% w/v) and 2.5 mL of phosphate buffer (0.2 M, pH 6.6). The reaction mixture was allowed to incubate for 20 minutes at 50°C. After the incubation, 2.5 mL of 10% w/v of trichloroacetic acid was added, after which the mixture is centrifuged at 1000 rpm for 10 minutes. Supernatant was collected and added to 2.5 mL of deionised water and 0.5 mL of ferric chloride ($FeCl_3$ 0.1% w/v). The absorbance was measured on a spectrophotometer at 700 nm. Increased absorbance of the reaction media indicated increased reducing power and antioxidant activity of reaction medium.

3. RESULT

3.1 Pharmacognostical Evaluation of *Raphanus sativus*

3.1.1 Macroscopical evaluation

Figure 1. Leaves of *Raphanus sativus*

Table 1. Macroscopical characters of *Raphanus sativus*

Description of the macroscopic characters	Observation
External Color	Green
Size	18-30 cm in length
Shape	Pinnate
Surface	Smooth
Odour	Characteristic
Taste	Slightly bitter

International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 5, May 2026 ijppr.humanjournals.com ISSN: 2349-7203

3.1.2 Microscopical evaluation

Figure 2.view of mid rib

Figure 3. view of trichomes

Figure 4. view of stomata

3.2 Physicochemical evaluation of *Raphanus sativus*

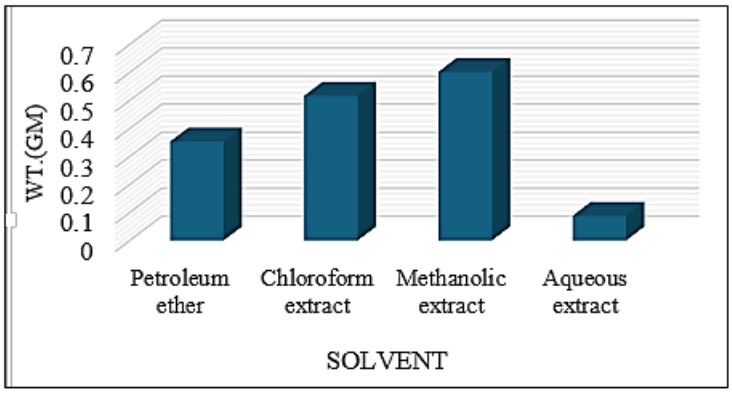

Figure 2. Determination of extractive values

3.3 Determination of ash values of *Raphanus sativus*

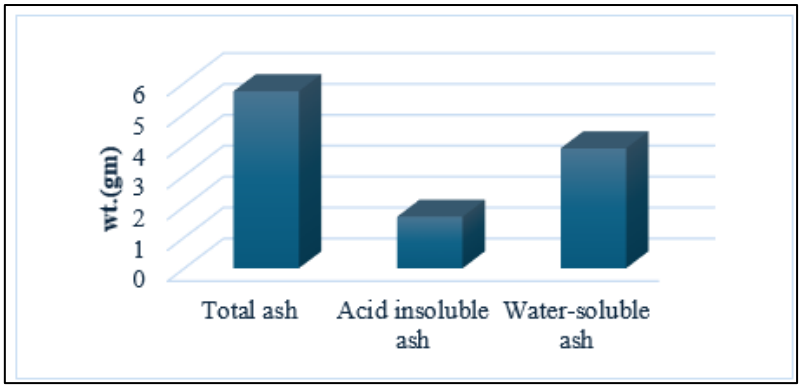

Figure 3. Determination of ash values

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3.4 Phytochemical screening of *Raphanus sativus*

Table 2. Phytochemical screening of *Raphanus sativus*

Constituents	Extract			
	Petroleum ether	Chloroform	Methanol	Aqueous
Carbohydrates	-	-	-	+
Phenolic compounds	-	-	+	-
Alkaloids	+	-	+	+
Flavonoid	-	+	+	-
Lipids	-	-	-	-
Saponins	+	+	+	-
Steroids	-	-	+	+
Amino acid	-	-	-	-
Proteins	-	+	+	+
Terpenoids	-	-	+	+

3.5 Pharmacognostical Evaluation of *Carica Papaya leaves*

3.5.1 Macroscopical evaluation

Figure 4. Leaves of *Carica Papaya*

Table 3. Macroscopical characters of leaves of *Carica Papaya*

Description of the macroscopic structure	Observation
External Colour	Green
Size	50- 70 cm in diameter
Shape	Simple, lobed
Surface	Smooth
Margin	Entire
Odour	Characteristic
Taste	Bitter

3.5.2 Microscopical evaluation

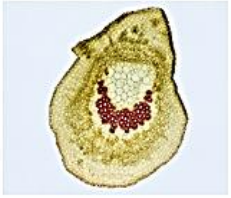

Figure 8. view of mid rib

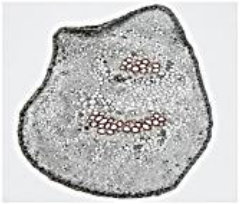

Figure 9. view of vascular bundle

Figure 10. view of crystal

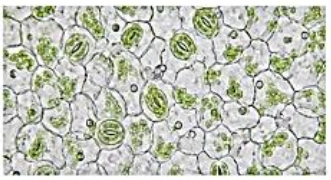

Figure 11.view of stomata

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3.6 Physicochemical of *Carica Papaya* leaves

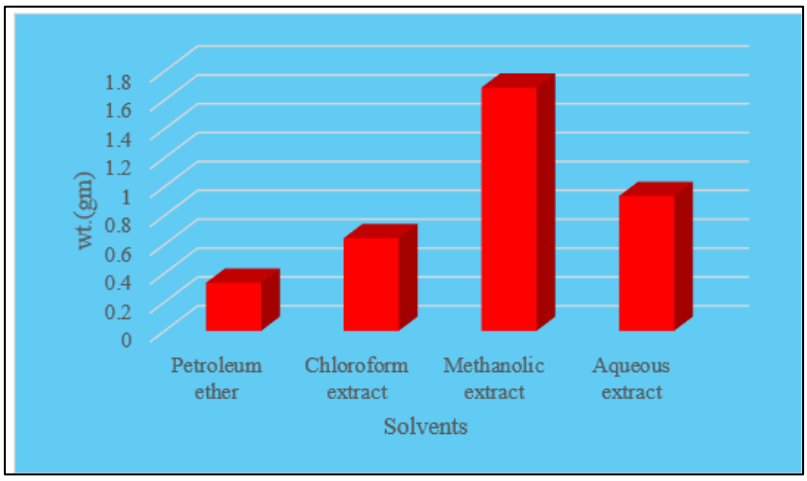

Figure 5. determination of extractive values

3.7 Determination of ash values of *Carica Papaya*

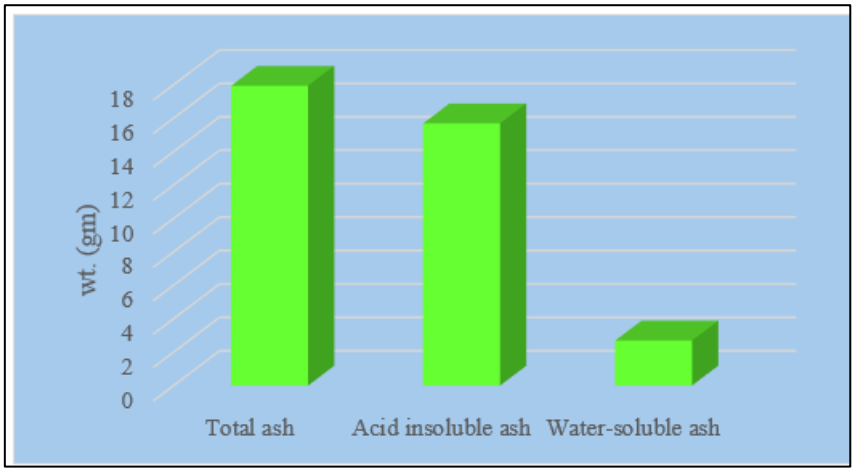

Figure 6. Determination of ash values of *Carica Papaya*

3.8 Phytochemical screening of *Carica Papaya*

Table 4. Phytochemical screening of *Carica Papaya*

Constituents	Extract			
	Petroleum ether	Chloroform	Methanol	Aqueous
Carbohydrates	-	-	+	+
Phenolic compounds	+	+	+	+
Alkaloids	+	+	+	+
Flavonoid	-	-	+	-
Lipids	-	-	-	-
Saponins	-	-	+	-
Steroids	+	+	+	-
Amino acid	-	-	+	-
Proteins	-	+	+	-
Terpenoids	-	-	+	+

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3.9 Analytical Evaluation of the Drugs

3.9.1 Determination of Total Phenolic Contents

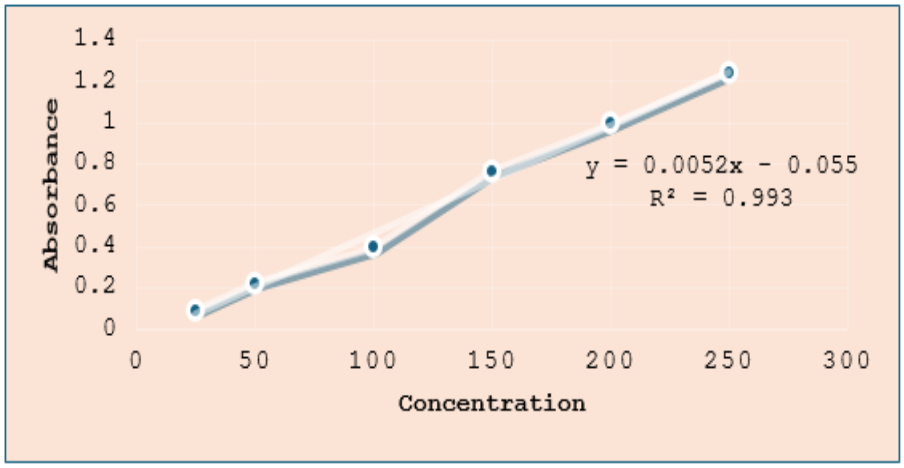

Figure 7. Standard calibration curve for determination of total phenolic contents

Table 5. Total phenolic contents of tested plants

S. No.	Name of plants	Total phenolic content (mg GAE/gm)
1	*Carica Papaya* leaves	46.9
2	*Raphanus sativus* leaves	57.8

3.9.2 Determination of Total Flavonoids Contents:

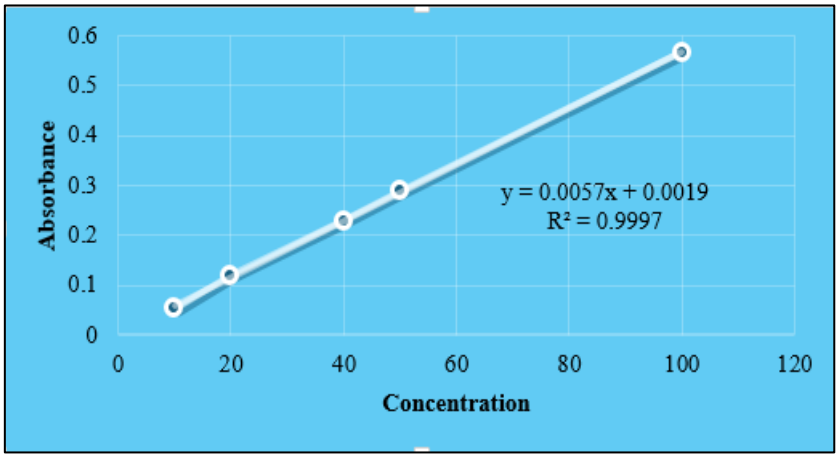

Figure 8. Standard calibration curve for determination of total flavonoid contents

Table 6. Total flavonoids content of tested plants

S. No.	Name of Plant	Total flavonoids Contents (mg QE/g)
1	*Carica Papaya*	4.85
2	*Raphanus sativus*	5.62

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3.10 Quantitative estimation of quercetin and rutin by using HPTLC

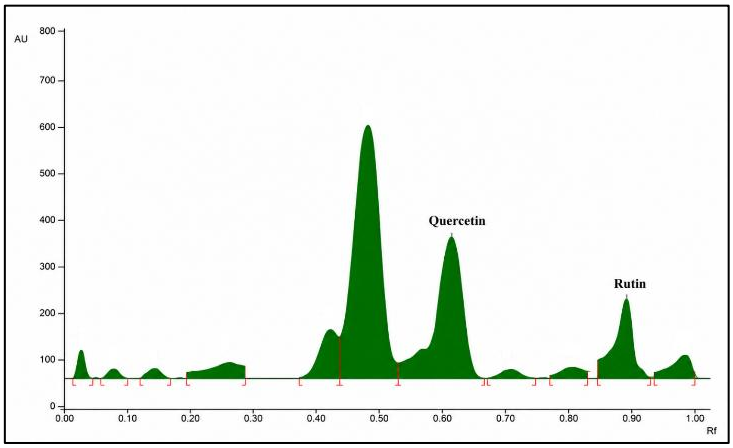

Figure 9. HPTLC chromatogram of Raphanus sativus showing peaks of Quercetin and Rutin

Table 7. Content of Quercetin and Rutin

Marker	λmax	Rf value	*Raphanus sativus* (RS)
Quercetin	297	0.56	0.883%w/w
Rutin	297	0.88	0.355%w/w

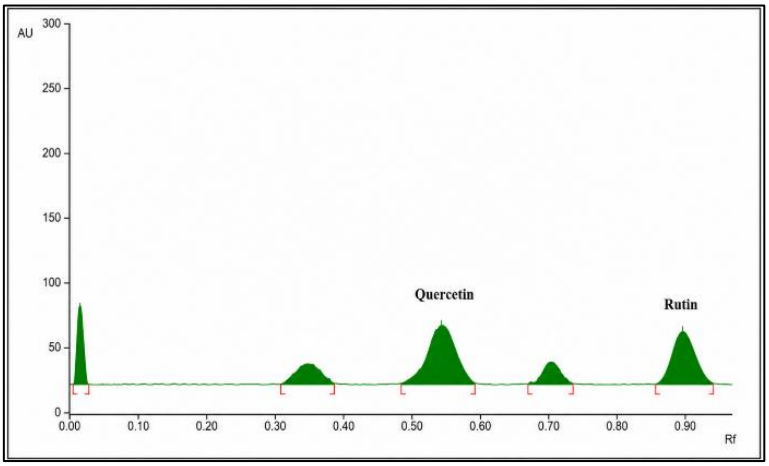

Figure 10. HPTLC chromatogram of *Carica Papaya* showing peaks of Quercetin and rutin

Table 8. Content of Quercetin and Rutin

Marker (SIGMA)	λ max	Rf value	*Carica Papaya* (CP)
Quercetin	297	0.55	0.125%w/w
Rutin	297	0.88	0.098%w/w

3.11 Anti-oxidant activity of Raphanus sativus

3.11.1 DPPH scavenging activity

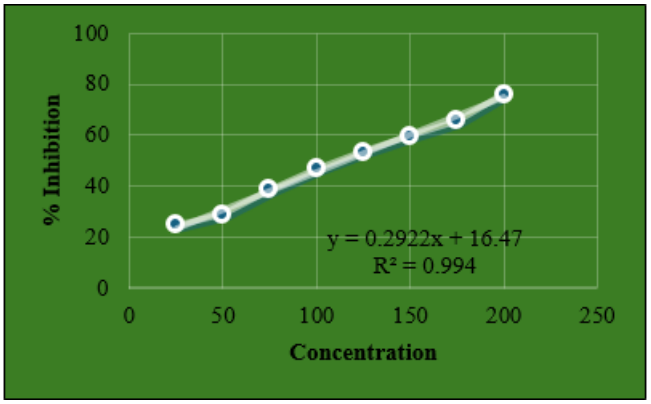

Figure 11. DPPH scavenging activity of methanolic extract of Raphanus sativus

3.11.2 Antioxidant activity by Nitric Oxide Scavenging method

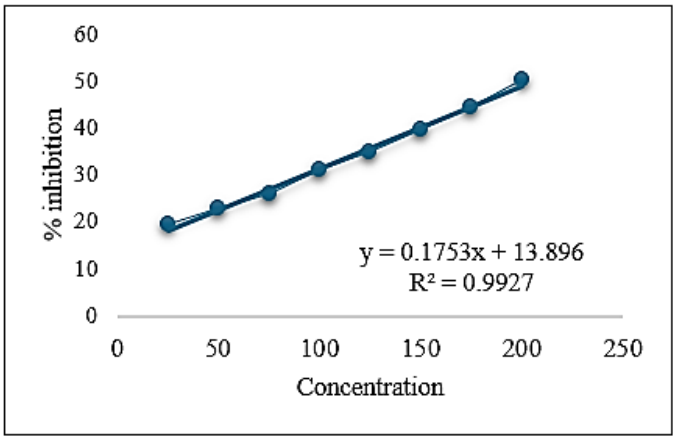

Figure 12. Nitric oxide scavenging activity for Methanolic extract of *Raphanus sativus*

3.11.3 Antioxidant activity by Reducing Power Determination method

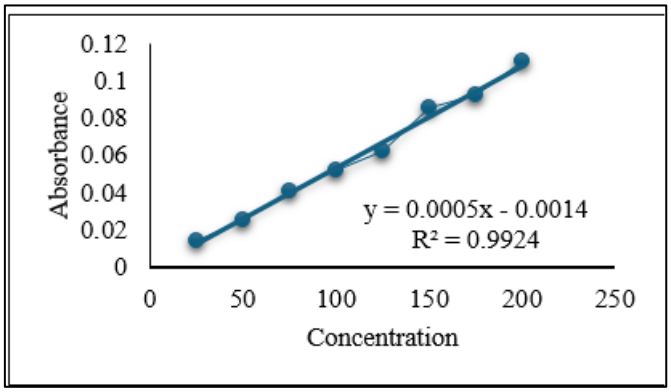

Figure 13. Reducing Power determination for Methanolic extract of *Raphanus sativus*

3.12 Antioxidant activity of *Carica Papaya* leaves

3.12.1 DPPH radical scavenging activity

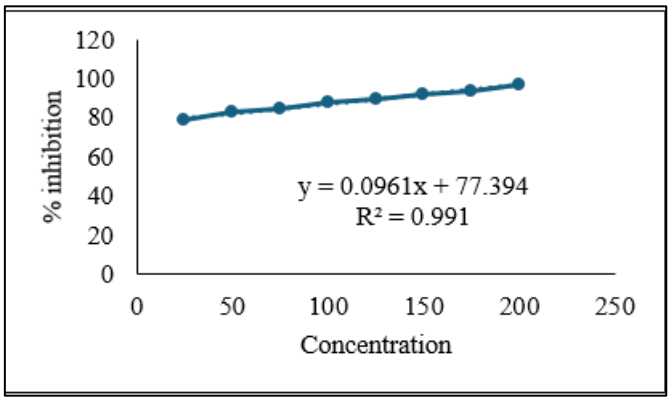

Figure 14. DPPH scavenging activity of methanolic extract of *Carica Papaya*

3.12.2 Antioxidant activity by Nitric Oxide Scavenging method

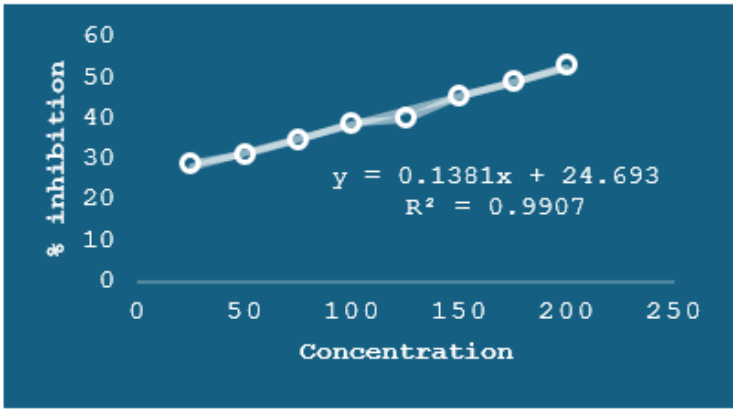

Figure 15. Nitric oxide scavenging activity for Methanolic extract of *Carica Papaya*

3.12.3 Antioxidant activity by Reducing Power Determination method

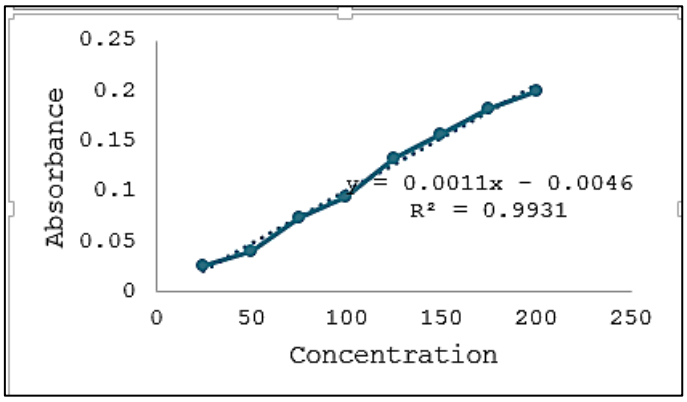

Figure 16. Reducing Power determination for Methanolic extract of *Carica Papaya*

4. SUMMARY AND CONCLUSION

The objective of this study was to evaluate the pharmacognostic, physicochemical, phytochemical and antioxidant potentials of leaves of Raphanus sativus and Carica papaya. The pharmacognostical study included the macroscopic and microscopic analysis that confirmed the distinguishing morphological and anatomical features of both plants. Under the microscope, epidermal layers, palisade parenchyma, spongy parenchyma, vascular bundles, calcium oxalate crystals and supporting tissues were observed, which are necessary to confirm the authenticity and identification of crude pharmaceuticals. Soxhlet extraction and ash content were the methods used for physicochemical assessment. Different solvents such as petroleum ether, chloroform, methanol and aqueous solutions were used for extraction. Both plants had the highest methanolic extractable value indicating efficient extraction of phytoconstituents. The ash content study confirmed the purity and quality of the botanicals studied. The total ash content exceeds that of acid-insoluble ash and water-soluble ash which indicates inorganic material content and minimal presence of siliceous materials. Based on the results of the initial phytochemical analysis, the significant presence of secondary metabolites: alkaloids, flavonoids, phenolic compounds, carbohydrates, saponins, proteins, steroids, and fixed oils, was confirmed. The quantitative evaluation of total phenolic and total flavonoid showed that the Raphanus sativus has significantly higher contents of polyphenols and flavonoids than Carica papaya, suggesting its higher antioxidant potential. Important constituents such as rutin and quercetin were detected in both the plant extracts by HPTLC analysis. The phytochemical richness of the extract was confirmed by the presence of Quercetin at λmax 297 nm and in significant quantity, as well as rutin. Methanolic extracts were evaluated for their antioxidant activity with DPPH radical scavenging assay, nitric oxide scavenging and reducing power determination method. Both plant extracts had concentration-dependent antioxidant properties. The antioxidant potential of Raphanus sativus was also higher than Carica papaya as shown by % inhibition and reducing power values. The higher correlation coefficient values confirmed the good linear relationship and reliability of the antioxidant assays.

The findings of the present study show that both species possess high phytochemical and antioxidant activity, although R. sativus shows comparatively higher activity. The findings support the application of medicinal plants for therapeutic purposes as natural antioxidants associated with oxidative stress disease, which makes them a promising approach.

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How to cite this article:
Kalpana Maurya et al. Ijppr.Human, 2026; Vol. 32 (5): 404-418.

Conflict of Interest Statement: All authors have nothing else to disclose.