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Phytochemical Profiling and Antioxidant Potential of Methanolic Extracts from Seed Kernel, Seed Coat, and Seed Husk of *Jatropha curcas*

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Abstract

Background: *Jatropha curcas* is a medicinally important plant possessing diverse bioactive compounds that contribute to its antioxidant and therapeutic properties. **Aim:** To evaluate and compare the total phenolic content, total flavonoid content, and antioxidant activity of methanolic extracts of the seed kernel, seed coat, and seed husk of *J. curcas*. **Methods:** Methanolic extracts were prepared using Soxhlet extraction. Total phenolic content was estimated by the Folin-Ciocalteu method, total flavonoid content by the aluminium chloride colorimetric method, and antioxidant activity by the DPPH free radical scavenging assay using UV-Visible spectrophotometry. **Results:** The seed kernel showed the highest total phenolic content (9.32 ± 0.21 % w/w) and total flavonoid content (1.2 ± 0.02 % w/w). It also exhibited the lowest IC_{50} value in the DPPH assay, indicating superior antioxidant activity. The antioxidant activity followed the order: Seed kernel > Seed coat > Seed husk. **Conclusion:** The study demonstrates a positive correlation between phenolic and flavonoid content and antioxidant activity, highlighting the seed kernel of *J. curcas* as a potent natural source of antioxidants with potential pharmaceutical and nutraceutical applications.

Major Findings: All extracts exhibited concentration-dependent DPPH free radical scavenging activity. The antioxidant activity followed the order: Seed kernel > Seed coat > Seed husk.

Keywords: Antioxidant Activity, DPPH Assay, *J. curcas,* Methanolic Extract, Total Flavonoid Content, Total Phenolic Content

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1. Introduction

Medicinal plants have served as the foundation of traditional healthcare systems since ancient times and continue to be an indispensable source of therapeutic agents in modern medicine. According to the World Health Organisation, a significant proportion of the global population relies on plant-based medicines for primary healthcare, particularly in developing countries[1]. The growing interest in herbal medicines is attributed to their perceived safety, affordability, cultural acceptability, and the presence of biologically active secondary metabolites[2]. These secondary metabolites play a crucial role in protecting plants against environmental stress and pathogens and are responsible for a wide range of pharmacological activities in humans[3].

Among various secondary metabolites, phenolic compounds and flavonoids have attracted considerable scientific attention due to their potent antioxidant properties. Phenolic compounds constitute a large group of phytochemicals characterised by one or more hydroxyl groups attached to aromatic rings, enabling them to act as effective hydrogen or electron donors[4].

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Flavonoids, a major subclass of phenolic compounds, are widely distributed in plants and are known for their antioxidant, anti-inflammatory, antimicrobial, anticancer, and cardioprotective activities[5]. The antioxidant activity of these compounds plays a vital role in neutralizing free radicals and Reactive Oxygen Species (ROS), thereby preventing oxidative damage to biomolecules such as lipids, proteins, and DNA[6].

Oxidative stress arises due to an imbalance between the production of free radicals and the antioxidant defense mechanisms of the body. It has been strongly implicated in the pathogenesis of numerous chronic and degenerative diseases, including cancer, cardiovascular disorders, diabetes mellitus, neurodegenerative diseases, inflammatory conditions, and aging-related complications[7,8]. Antioxidants obtained from natural sources are therefore of immense importance in reducing oxidative stress and maintaining cellular homeostasis[9]. Plant-derived antioxidants are increasingly preferred over synthetic antioxidants due to concerns regarding the toxicity and side effects associated with long-term use of synthetic compounds[10].

The genus *Jatropha*, belonging to the family *Euphorbiaceae*, comprises approximately 175 species and is widely distributed in tropical and subtropical regions of the world[11]. Originally native to tropical America, *Jatropha* species are now extensively cultivated and naturalised in Asia and Africa[12]. The name *Jatropha* is derived from the Greek words "*jatros*" meaning doctor and "*trophe*" meaning food, reflecting its traditional medicinal importance[13]. Various species of *Jatropha* have been extensively used in folk medicine for the treatment of wounds, skin diseases, inflammation, gastrointestinal disorders, infections, and fever[14].

Phytochemical investigations of different parts of *J. curcas* (Figure 1) have demonstrated that this plant is a rich source of diverse bioactive constituents, including phenolic compounds, flavonoids, alkaloids, lignans, coumarins, cyclic peptides, terpenoids, steroids, and phorbol esters[15,16]. These phytochemicals are distributed variably among the leaves, stems, roots, seeds, and latex, contributing to the broad spectrum of pharmacological activities reported for *J. curcas*. These activities include antimicrobial, antifungal, anti-inflammatory, insecticidal, cytotoxic, larvicidal, and antioxidant effects[17,18].

Among the various plant parts, the leaves and seeds of *J. curcas* have received considerable attention due to their high phytochemical content and medicinal relevance[19]. The antioxidant potential of different parts of *J. curcas* has been strongly correlated with their phenolic and flavonoid composition[20]. Phenolic compounds present in the leaves, bark, and seeds act as effective antioxidants by scavenging free radicals, chelating pro-oxidant metal ions, and inhibiting oxidative enzymes[21]. Flavonoids, particularly abundant in the leaves and aerial parts, further enhance antioxidant defense by stabilising reactive oxygen species and modulating endogenous antioxidant mechanisms[22].

The quantitative estimation of total phenolic content and total flavonoid content in different parts of *J. curcas* therefore serves as an important indicator of their antioxidant potential[23]. Among the various antioxidant evaluation methods, the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay is widely employed due to its simplicity, rapidity, and reproducibility[24]. This assay measures the ability of plant extracts to reduce the stable DPPH radical, leading to a decrease in absorbance that is directly proportional to their antioxidant capacity[25].

UV-Visible spectrophotometric techniques are commonly used for the estimation of phenolic compounds, flavonoids, and antioxidant activity in different parts of *J. curcas* because of their accuracy, cost-effectiveness, and suitability for routine laboratory analysis[26]. Despite numerous studies reporting the pharmacological properties of *J. curcas*, integrated comparative evaluations of total phenolic content, total flavonoid content, and



Figure 1. *J. curcas* seed coat, seed kernel and seed husk.

antioxidant activity among different plant parts remain limited. Variations in phytochemical composition between leaves, stems, roots, and seeds necessitate systematic investigation to identify the most potent sources of natural antioxidants[27].

Therefore, the present research work was undertaken to estimate and compare total phenolic compounds, total flavonoids, and antioxidant activity of methanolic extracts obtained from different parts of *J. curcas* using UV-Visible spectrophotometry and the DPPH free radical scavenging assay. This study aims to provide scientific validation for the traditional medicinal use of various parts of *J. curcas* and to explore their potential pharmaceutical and nutraceutical applications[28].

2. Materials and Methods

Seeds of *J. curcas* were collected from Sasya Kashi, GM Institute of Pharmaceutical Sciences and Research (GMIPSR), Davangere, Karnataka, India. The plant materials were authenticated by Dr. Haleshi, Davangere University, Davangere, and voucher specimens were preserved for future reference.

Folin-Ciocalteu reagent and sodium carbonate were used for the estimation of total phenolic content, and gallic acid was employed as the standard reference compound. Aluminium chloride, potassium acetate, and methanol were used for the determination of total flavonoid content, with quercetin taken as the standard compound. Antioxidant activity was evaluated using 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) as the free radical source, and ascorbic acid was used as the standard antioxidant.

All chemicals and reagents used in the study were of analytical reagent grade and procured from standard suppliers. Double-distilled water was used throughout the experimental procedures. Absorbance measurements were carried out using a UV-Visible spectrophotometer equipped with matched quartz cuvettes. A digital analytical balance and standard laboratory glassware were used for accurate weighing and experimental analysis.

2.1 Methods

2.1.1 Preparation of Methanolic Extracts of *J. curcas* Seed, Seed Coat, and Seed Kernel Using Soxhlet Extraction

Mature seeds of *J. curcas* were collected, cleaned, and shade dried. The dried seeds were manually separated into seed coat and seed kernel. Each sample, namely whole seed, seed coat, and seed kernel, was individually coarsely powdered using a mechanical grinder and passed through a 40-mesh sieve. About 25 g of each powdered material was subjected to continuous hot extraction using a Soxhlet apparatus with methanol (250 mL) as the solvent. The extraction was carried out at a temperature of 60-65° C for 6-8 hours or until the siphon solvent became colourless, indicating exhaustive extraction of phytoconstituents.

After extraction, the methanolic extracts were concentrated under reduced pressure using a rotary evaporator at 40-45° C and dried to obtain solid residues. The dried extracts of seed, seed coat, and seed kernel were stored in airtight containers in a desiccator until further analysis[29].

2.1.2 Estimation of Total Phenolic Content

The total phenolic content of the methanolic extracts of *J. curcas* seed husk, seed coat, and seed kernel was determined using the Folin-Ciocalteu spectrophotometric method. An aliquot of 0.5 mL of each extract (1 mg/mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and allowed to react for 5 minutes. Subsequently, 2.0 mL of 7.5% w/v sodium carbonate solution was added. The reaction mixture was incubated at room temperature for 30 minutes.

The absorbance was measured using a UV-Visible spectrophotometer at 765 nm. Gallic acid was used as the standard, and total phenolic content was expressed as mg Gallic Acid Equivalents (GAE)/g of extract[23,29].

2.1.3 Estimation of Total Flavonoid Content

Total flavonoid content was estimated by the aluminium chloride colorimetric method. An aliquot of 0.5 mL of each extract (1 mg/mL) was mixed with 1.5 mL methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The reaction mixture was incubated at room temperature for 30 minutes to allow complex formation.

The absorbance was measured using a UV-Visible spectrophotometer at 410 nm. Quercetin was used as the standard reference compound, and total flavonoid content was expressed as mg Quercetin Equivalents (QE)/g of extract[29].

2.1.4 Evaluation of Antioxidant Activity (DPPH Assay)

The antioxidant activity of the methanolic extracts of *J. curcas* seed coat, seed husk and seed kernel was evaluated using the DPPH free radical scavenging assay. A 0.1 mM DPPH solution was prepared in methanol. Different concentrations of the extracts (20-100 µg/mL) were mixed with 3.0 mL of DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 30 minutes.

The decrease in absorbance was measured using a UV-Visible spectrophotometer at 517 nm. Ascorbic acid was used as the standard antioxidant. The percentage inhibition of DPPH free radicals was calculated using the standard formula, and IC_{50} values were determined[25,29].

3. Result and Discussion

The total polyphenol content of different parts of *J. curcas* is summarised in Figure 2. The results indicate a clear variation in polyphenol distribution among the evaluated plant parts. The seed kernel exhibited the highest polyphenol content (9.32 ± 0.21 % w/w), suggesting that it is a major reservoir of phenolic compounds. This elevated level may be attributed to the role of the seed kernel in protecting the embryo from oxidative damage and environmental stress during seed maturation and storage. Phenolic compounds present in the kernel are known to contribute significantly to antioxidant and protective functions.

The seed showed moderate polyphenol content (4.64 ± 0.15 % w/w), reflecting its active metabolic role and involvement in defense mechanisms such as scavenging reactive oxygen species generated during photosynthesis. The presence of appreciable phenolic levels in seed supports their traditional medicinal use and potential antioxidant activity.

The seed coat exhibited slightly lower polyphenol content (4.41 ± 0.09 % w/w) compared to the other parts. Although lower, the phenolic content of the seed coat indicates its protective function against microbial invasion and physical damage. The low standard deviation values observed for all plant parts demonstrate the reproducibility and reliability of the analytical method. Overall, the findings highlight the seed kernel of *J. curcas* as a promising source of natural polyphenols with potential pharmaceutical and antioxidant applications.

The total flavonoid content present in different parts of *J. curcas* (JC) is illustrated in Figure 3. Among the analysed plant parts, the seed kernel exhibited the highest flavonoid content (1.2 ± 0.02 % w/w), indicating a relatively greater accumulation of flavonoid compounds in the kernel. Flavonoids are known to play an important role in protecting plant tissues from oxidative stress and contribute significantly to antioxidant activity.

The seed coat and seed husk showed comparable flavonoid content (1.1 ± 0.04 and 1.1 ± 0.06 % w/w, respectively). Although slightly lower than the seed kernel, the presence of appreciable flavonoid levels in these outer seed components suggests their involvement in protective functions such as defense against environmental stress and microbial attack. The low standard deviation values observed for all samples indicate good reproducibility and reliability of the analytical method employed.

The antioxidant activity of methanolic extracts of *J. curcas* seed coat (Sample 1), seed husk (Sample 2), and seed kernel (Sample 3) was evaluated using the DPPH free radical scavenging assay, and the results are presented in Figures 4-6. All three extracts demonstrated concentration-dependent radical scavenging activity, as evidenced by a progressive decrease in percentage Radical Scavenging Activity (% RSA) with increasing sample absorbance.

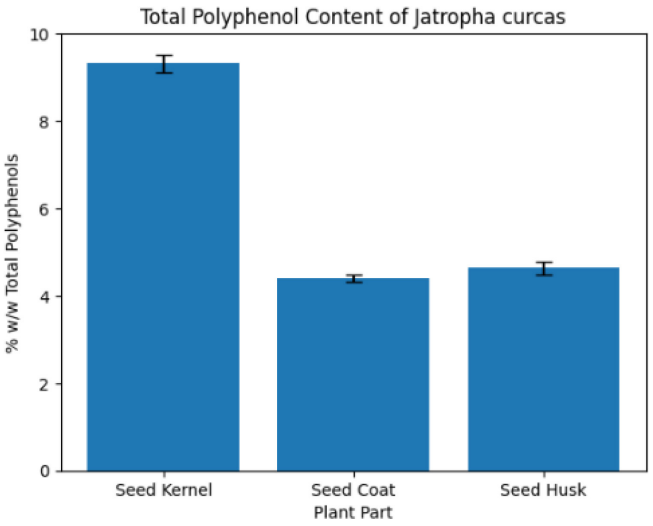


Figure 2. Total poly phenols present in different parts of JC.

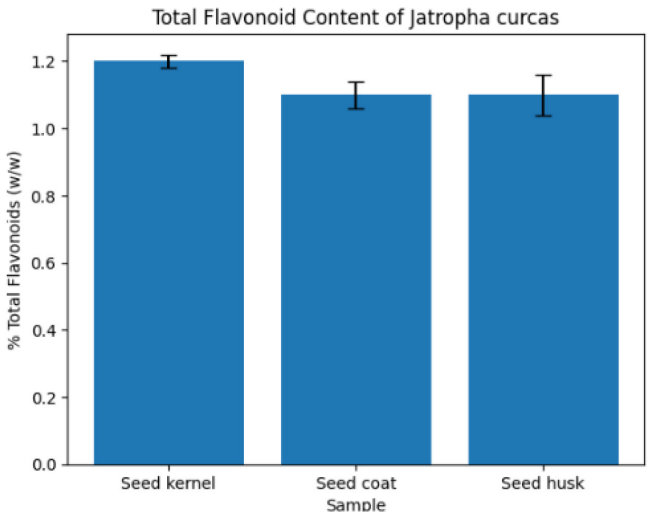


Figure 3. Total flavonoids present in different parts of JC.

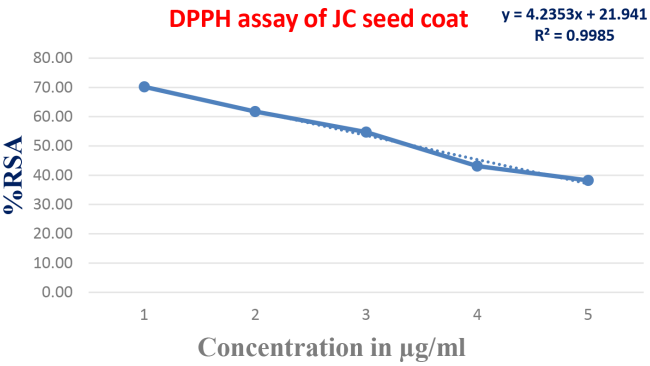


Figure 4. Methanolic extract of *J. curcas* seed coat (Sample 1).

Among the tested samples, the seed kernel extract exhibited the highest antioxidant potential, showing the maximum % RSA of 73.39% at 0.4 µg/mL and the lowest absorbance values at lower concentrations. The IC_{50} values obtained for the seed kernel were comparatively lower, indicating a stronger free radical scavenging efficiency. This enhanced activity may be attributed to the higher concentration of phenolic and flavonoid compounds previously observed in the seed kernel.

The seed coat extract showed moderate antioxidant activity, with a maximum % RSA of 70.22%, followed by a gradual decline at higher concentrations. The IC_{50} values indicated moderate scavenging efficiency, reflecting the presence of bioactive constituents involved in antioxidant defense.

The seed husk extract exhibited comparatively lower antioxidant activity, with % RSA values ranging from

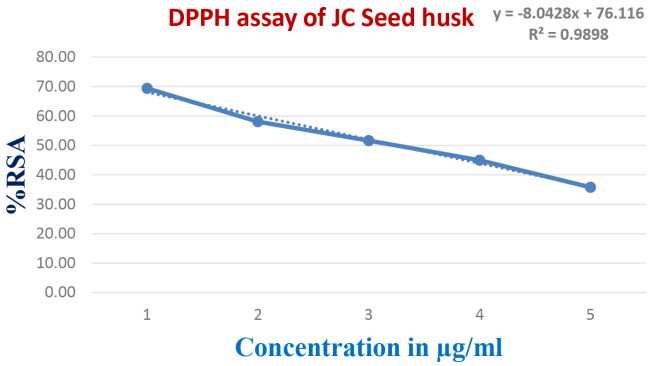


Figure 5. Methanolic extract of *J. curcas* seed husk (Sample 2).

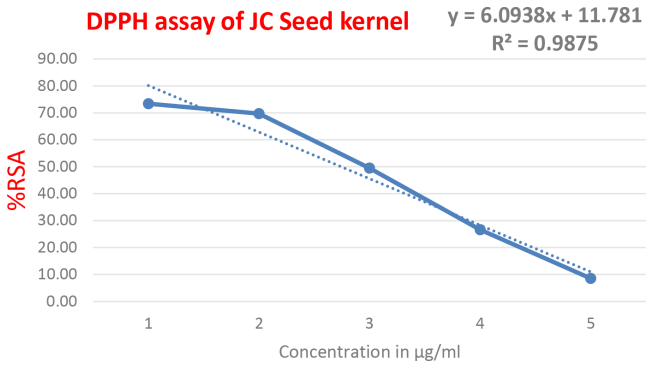


Figure 6. Methanolic extract of *J. curcas* seed kernel (Sample 3).

69.42% to 35.78%. Although antioxidant activity was evident, the higher IC_{50} values suggest reduced potency compared to the seed kernel and seed coat extracts.

Overall, the comparative analysis reveals that the seed kernel > seed coat > seed husk in terms of antioxidant efficacy. These findings support the role of phenolic and flavonoid constituents in governing antioxidant activity and highlight the seed kernel of *J. curcas* as a promising natural source of antioxidants with potential pharmaceutical applications.

4. Conclusion

The present investigation demonstrates that different parts of *J. curcas* possess appreciable amounts of phenolic compounds and flavonoids, which significantly contribute to their antioxidant activity. Among the evaluated plant parts, the seed kernel exhibited the highest total polyphenol and flavonoid contents, along with superior free radical scavenging activity in the DPPH assay. The seed coat and seed husk

also showed notable antioxidant potential, although to a lesser extent.

The observed variation in antioxidant activity among the plant parts correlates well with their phytochemical composition, emphasising the role of phenolic and flavonoid constituents in governing antioxidant efficacy. The concentration-dependent scavenging activity observed in all extracts further confirms their potential as natural antioxidants. Overall, the results suggest that the seed kernel of *J. curcas* is the most potent source of antioxidant compounds among the studied parts. This study provides scientific support for the traditional medicinal use of *J. curcas* and suggests its potential application in the development of natural antioxidant formulations for pharmaceutical and nutraceutical purposes. Further studies focusing on isolation and characterisation of individual bioactive compounds are warranted.

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Author Contribution

Mohammad Yosuf Malik Damani: Overall idea, formulation, manuscript write up, Jagdish Kakadiya: Analytics and interpretation, suggestions and corrections. All authors have read and agreed to the published version of the manuscript.