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Antioxidant Capacity and Phytochemical Profile of *Jatropha curcas* L. Leaf Extracts and Fractions Using the FRAP Method

Amran Nur[1], Fahmi Sadik[2*], Nur Asma S. Somadayo[1], Sitti Hartina[3]

[1]Pharmacy Study Program, Department of Pharmacology, Faculty of Medicine and Health Sciences, Khairun University, Ternate, Indonesia
[2]Pharmacy Study Program, Department of Pharmaceutical Biology, Faculty of Medicine and Health Sciences, Khairun University, Ternate, Indonesia
[3]Pharmacy Study Program, Department of Clinical Pharmacy, Faculty of Medicine and Health Sciences, Khairun University, Ternate, Indonesia
[1]amran.nur@unkhair.ac.id; [2]fahmisadik@unkhair.ac.id*; [1]asma@unkhair.ac.id; [3]thinaalmaimun@gmail.com
*corresponding author

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ABSTRACT

This study explored the free radical scavenging potential of *Jatropha curcas* L. leaves, focusing on identifying active biochemical constituents and evaluating antioxidant efficacy through a series of extraction and chemical analysis procedures. Phytochemical screening confirmed the presence of several bioactive secondary metabolites, including alkaloids, saponins, triterpenoids, tannins, and flavonoids. In the Ferric Reducing Antioxidant Power (FRAP) assay, the ethyl-acetate fraction exhibited the highest level of antioxidant activity (9.19 ± 0.38 µmol TE/g), significantly higher than that of the ethanol extract (3.52 ± 0.71 µmol TE/g), as well as the aqueous and n-hexane fractions. This value corresponds to strong activity according to the TEAC classification, indicating that *Jatropha curcas* leaves are a promising source of natural antioxidants. The total phenolic content (TPC) was determined to be 6.78 ± 0.27% GAE, while the total flavonoid content (TFC) measured 1.35 ± 0.07% QE. These findings indicate that phenolic and flavonoid constituents play a primary role in the antioxidant activity of the extract. Variations significantly influenced the observed outcomes in the extraction methodology and solvent polarity. In summary, Jatropha curcas leaves possess substantial antioxidant potential, particularly within the ethyl-acetate fraction, supporting further development for nutraceutical and pharmaceutical applications.

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1. Introduction

The increasing prevalence of medical conditions caused by oxidative stress has prompted significant attention to the protective role of antioxidants in human well-being. Free radicals, highly reactive molecules, are known to accelerate cellular damage, becoming a significant contributing factor in the onset of various clinical disorders, including cardiovascular diseases, cancer, and neurological problems (Holt, 2023). Therefore, antioxidant compounds, especially those obtained from natural sources, are needed to neutralize free radicals and alleviate the adverse effects of oxidation (Oluyele et al., 2022). Substances extracted from vegetation are now increasingly valued given their rich composition of bioactive phytochemical compounds such as flavonoids and phenolic acids, which have well-documented antioxidant effects (Grujić et al., 2020; Shalini et al., 2018).

Despite the general acceptance of the benefits of antioxidant compounds, there is a significant lack of information regarding the specific antioxidant capabilities possessed by botanical extracts, especially from less explored species. *Jatropha curcas* L., for example, has a broad pharmacological profile; however, research on its antioxidant capacity is minimal compared to other medicinal plants

(Tandoro et al., 2020). This plant's leaves are rich in phenolic content, which acts as a potent free radical scavenger (Oyeleke et al., 2021). Nevertheless, explaining how their antioxidant activity works, mainly when evaluated using the Ferric Reducing Antioxidant Power (FRAP) test, requires in-depth investigation to reveal its effectiveness and mechanism.

This study aims to evaluate the free radical scavenging capacity of ethanol extracts from *Jatropha curcas* leaves using the FRAP (Ferric Reducing Antioxidant Power) technique. This scientific inquiry focuses on measuring the antioxidant potential of these compounds and tracking the plant derivatives that trigger this activity. The principle of FRAP testing is to measure the antioxidant capacity based on its ability to convert ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}), thereby clarifying the characteristics of electron donors and the antioxidant action pathway of *Jatropha curcas* (Ouahdani et al., 2021). The findings of this research are anticipated to provide scientific insights to validate the potential application of *Jatropha curcas* extract in supporting nutrition and health as a natural antioxidant.

This scientific work has special value because it solves the existing knowledge gap regarding the antioxidant potential of *Jatropha curcas*. By conducting planned testing of ethanol extracts obtained from its leaves, this research aims to reveal the antioxidant properties of this rarely studied species and contribute to developing studies on antioxidants derived from nature. The data generated is expected further to explore the health benefits of *Jatropha curcas* leaves, while validating the possibility of its use in the manufacture of functional foods and nutraceutical formulations (Poongodi & Nazeema, 2019). In addition, mastery of this antioxidant system can be a foundation for creating innovative therapeutic approaches in treating disorders triggered by oxidative stress.

In conclusion, research activities that test the ability to eliminate free radicals in *Jatropha curcas* leaf extract using the FRAP method are considered scientifically significant. The primary focus of this initiative is to gather concrete evidence that reinforces the possibility of using *Jatropha curcas* leaves in contemporary and traditional pharmaceutical practices. This aligns with the scientific community's growing interest in natural antioxidants and their positive impact on health. It is estimated that the results of this analysis will sharpen insights regarding the botanical antioxidant system and its more significant role in preventing health disorders and managing well-being in general (Behera, 2019).

2. Methods

This research applies a quantitative experimental design to measure the strength of free radical inhibition possessed by ethanol extracts from *Jatropha curcas* leaves, using *Ferric Reducing Antioxidant Power* (FRAP) analysis. This work was carried out systematically, covering the stages of collecting botanical material, making ethanol extracts, and determining antioxidant effectiveness using the FRAP procedure. The FRAP technique was specifically chosen for its ability to measure antioxidant reduction capacity; this method calculates antioxidant activity based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) under acidic conditions (Héritier, 2023). Another reason for using FRAP is its widespread recognition for its efficiency and reproducibility of analytical outcomes, which makes it a reliable technique for assessing antioxidant potential in diverse plant extracts. (Héritier, 2023; Kubilienė et al., 2020). The test material used was fresh leaves of *Jatropha curcas* L. obtained from the coast of Ternate, Indonesia. The leaves were cleaned, dried in the open air, and ground into a fine powder to facilitate extraction. The sample volume was adequate to ensure the statistical data's reliability in assessing the ethanol extract's antioxidant potential (Calvindi et al., 2020).

2.1. Preparation of Extracts

Ethanol extracts from *Jatropha curcas* leaves were prepared using the maceration method. A total of 400 g of dried and powdered material was soaked in 96% ethanol at a ratio of 1:10 (w/v) for 72 hours at room temperature (27 ± 2 °C) with occasional stirring to ensure optimal solvent penetration. Ethanol was selected because it is widely recognized for its efficiency in dissolving a broad range of phytochemical constituents, particularly phenolic and flavonoid compounds associated with antioxidant activity (Sahid et al., 2021; Fall et al., 2019). The use of ethanol as an extraction solvent is further supported by numerous studies demonstrating its effectiveness in separating bioactive components from plant matrices (Zhao et al., 2022).

After filtration, the ethanol extract was concentrated using a rotary evaporator at 45 °C under reduced pressure. The crude extract was then subjected to liquid–liquid fractionation using *n*-hexane, ethyl acetate, and water to separate compounds based on polarity, allowing a more detailed evaluation of the antioxidant potential of each fraction. All extraction and fractionation steps were conducted in triplicate to ensure reproducibility and analytical reliability.

2.2. Identification of Phytochemical Constituents

The extract produced previously was examined chemically using a series of standard qualitative protocols (Sadik & Disi, 2023). Alkaloids were indicated by the formation of precipitates after the filtrate reacted with Mayer's, Dragendorff's, and Bouchardat's reagents. Meanwhile, flavonoids were confirmed by the appearance of a yellow or red coloration when the extract was treated with 10% NaOH or with magnesium powder and concentrated HCl. To determine saponins, the extract was shaken together with distilled water, with the benchmark for its presence being foam that did not disappear for at least ten minutes. Tannins are identified through the ability of the filtrate to form a dark green or blue-black color when $FeCl_3$ is mixed. Finally, the identification of terpenoids is completed through the Liebermann-Burchard reaction; a positive indication is achieved when the addition of acetic anhydride and concentrated sulfuric acid causes a gradation of color ranging from red, blue, purple, to green.

2.3. Antioxidant Activity Assessment

The antioxidant capacity of the extract was determined using the Ferric Reducing Antioxidant Power (FRAP) assay. All reagents were freshly prepared before each analysis to ensure data accuracy, and all readings were standardized against a known antioxidant reference (Akdogan et al., 2019).

An aliquot (3.3 mL) of the stock extract solution (600 µM) was transferred into a measuring flask and diluted with methanol (p.a.) to a final volume of 5 mL, producing a working concentration of 400 µM. Subsequently, 3 mL of the newly prepared FRAP reagent was added to the test solution and mixed thoroughly.

The mixture was incubated at 37 °C for 30 minutes to allow the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. After incubation, the absorbance of the resulting solution was measured at a wavelength of 596 nm using a UV–Vis spectrophotometer (Syarif et al., 2015). All analyses were performed in triplicate, and results were expressed as µmol Trolox equivalents per gram of extract (µmol TE/g).

2.4. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the ethanol extract was determined spectrophotometrically using the Folin-Ciocalteu method, with gallic acid as a reference standard. Gallic acid was selected because it represents a well-characterized phenolic compound that exhibits a strong and stable response to the Folin-Ciocalteu reagent, thus providing a reliable calibration curve for quantifying total phenolics.

In this procedure, the extract was mixed with the Folin-Ciocalteu reagent, producing a characteristic blue–violet coloration due to the reduction of the reagent by phenolic hydroxyl groups. The absorbance of the mixture was measured at 765 nm using a UV–Vis spectrophotometer, and results were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g) (Mahfudh et al., 2024).

2.5. Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of the extract was determined spectrophotometrically using the aluminum chloride ($AlCl_3$) colorimetric method. This technique is based on the formation of a yellow complex that results from the reaction between $AlCl_3$ and the keto or hydroxyl groups of flavonoids, forming a stable flavonoid-$AlCl_3$ complex. The absorbance of the resulting solution was measured at 510 nm using a UV-Vis spectrophotometer, and the TFC values were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g) (Mahfudh et al., 2024).

2.6. Data Analysis

Quantitative analysis of the antioxidant capacity of plant extracts was performed using the GraphPad Prism program. The values obtained from the FRAP analysis were then standardized and reported in units equivalent to micromoles of Trolox per gram of sample (µmol TE/g) (Liwanda, 2024). Descriptive statistical calculations, such as standard deviations and mean values, were performed to

characterize the distribution of antioxidant activity. Furthermore, the correlation between total phenolic content (TPC) and antioxidant activity was evaluated using Pearson's correlation analysis, providing an indication of the association between phenolic concentration and antioxidant performance (Nowak et al., 2019). At the final stage, the differences between the samples were evaluated for statistical significance using the Kruskal-Wallis test; a threshold of < 0.05 was set as the measure of statistical significance (Gomez-Urios, 2024).

3. Results and Discussion

3.1. Extraction Yield

The ethanolic extraction of powdered *Jatropha curcas* leaves yielded 18.52% (74.08 g) of crude extract from 400 g of dried material using a 1:10 (w/v) solvent-to-sample ratio. This yield figure exceeds the 4.98% published by Bachri et al., 2024 who only obtained 84.7% of extract from 1,700 g of initial material. The discrepancy in these results is likely due to changes in the solvent-to-sample ratio and the level of isolation success, particularly the lower solvent ratio (1:4) that was applied in previous studies, even though the type of solvent and extraction procedure used were the same.

Table 1. Yield of *Jatropha curcas* leaf ethanolic extract

Sample mass (g)	Solvent volume (L)	Extract weight (g)	Extraction yield (%)
400	4	74.08	18.52

The higher yield observed in this study can be attributed to more favorable extraction conditions, particularly the increased solvent-to-sample ratio and the resulting improvement in process efficiency. In addition, factors such as the initial quality of the plant material, moisture content, and particle size may also significantly influence extraction performance. These findings highlight the importance of optimizing extraction parameters, as such optimization can markedly enhance the recovery of bioactive compounds from *Jatropha curcas* leaves and support their potential applications in medicinal and functional food formulations.

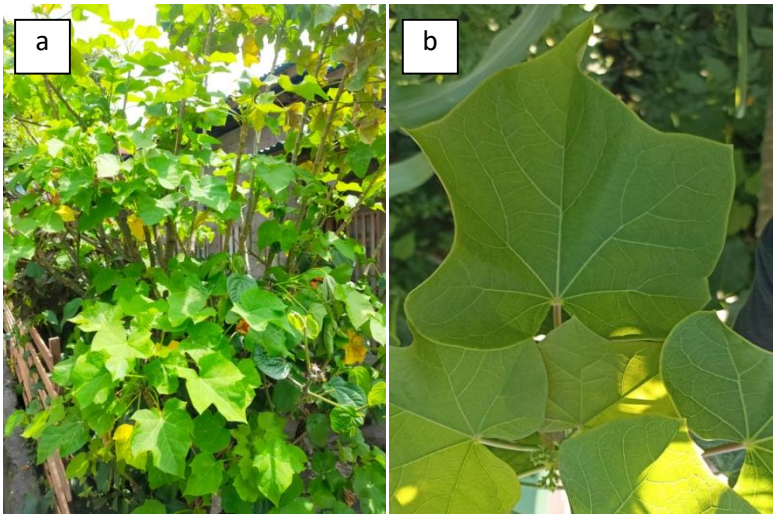

Figure 1. (a) Morphological structure of *Jatropha curcas* plant; (b) Leaf morphology showing the palmate venation pattern used for ethanolic extraction.

3.2. Phytochemical Composition

Examination of the plant's chemical components has confirmed that the ethanol extract taken from the leaves of *Jatropha curcas* L. is rich in various classes of bioactive substances. The presence of alkaloids was confirmed after the formation of white precipitate in the Mayer test and the appearance of orange gradations in the Bouchardat protocol. Flavonoids also showed a positive response, marked by a dense red color, while triterpenoids were identified through the appearance of a color between red and purple. Meanwhile, saponins were proven to be present thanks to the production of stable foam for a minimum duration of ten minutes (≥10 min), and tannins were confirmed through a color change to dark blue. All of this data validates the presence of vital secondary metabolites that support the efficacy of *Jatropha curcas* L, especially its antioxidant power. Our results are consistent with

the report by Krisdiyanto 2023, who also found positive results in all observed phytochemical categories.

Table 2. Results of phytochemical screening of *Jatropha curcas* leaf ethanolic extract

Phytochemical	Reagent/Test Used	Interpretation	Observation
Alkaloids	Mayer's reagent	+	White precipitate
	Bouchardat's reagent	+	Orange precipitate
Flavonoids	Mg powder + conc. HCl	+	Dark-red color
Saponins	2 N HCl + distilled water	+	Stable foam > 10 min
Triterpenoids	EtOH + acetic acid + H_2SO_4	+	Red/purple color
Tannins	EtOH + $FeCl_3$	+	Dark-blue color

The positive reactions observed during phytochemical testing are attributed to the result of specific chemical encounters between bioactive substances and relevant detection solutions. Using Mayer's reagent results in white precipitation for alkaloids, which occurs when metal ions react with the functional nitrogen groups of alkaloid compounds to create insoluble alkaloid salts. The change to red observed in the flavonoid analysis is obtained from a reduction reaction triggered by magnesium in an acidic state, which confirms the presence of flavone or flavonol derivatives. Meanwhile, the ability of saponins to reduce surface tension contributes to the formation of durable foam in specific saponin tests. Similarly, triterpenoids cause a gradation of red to purple colors when reacted with concentrated sulfuric acid, thus creating a characteristic dye complex. The appearance of a dark blue color in the tannin test is ultimately explained by the process of complex formation between ferric ions (Fe^{3+}) originating from $FeCl_3$ with the polyphenolic framework of tannin compounds.

3.3. Antioxidant Activity

The antioxidant capacity of the ethanol extract and solvent fractions of *Jatropha curcas* leaves was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay, and the results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) in µmol TE/g. According to the TEAC classification system proposed by Leccese et al., 2008, the antioxidant capacity can be categorized as presented in Table 3.

Table 3. Classification of Antioxidant Capacity Based on TEAC Values (Leccese et al., 2008)

Category	TEAC Value Range (µmol TE/g)	Description of Antioxidant Capacity
Very Weak	< 1	Minimal antioxidant activity; negligible radical-scavenging effect
Low	1 - 2	Low antioxidant capacity; limited ability to neutralize free radicals
Medium	2 - 5	Moderate antioxidant potential; indicative of phenolic contribution
High	> 5	Strong antioxidant capacity; significant radical-scavenging and reducing power

Based on this classification, the ethanol extract showed medium antioxidant capacity (3.52 ± 0.71 µmol TE/g). In contrast, the n-hexane fraction exhibited very weak activity (0.68 ± 0.16 µmol TE/g), while the aqueous fraction demonstrated low antioxidant capacity (1.84 ± 0.30 µmol TE/g). Remarkably, the ethyl acetate fraction displayed the highest antioxidant activity (9.19 ± 0.38 µmol TE/g), which falls into the high category according to the TEAC system.

This result indicates that the ethyl acetate fraction possesses the greatest antioxidant potential among all tested samples. Its superior activity is likely associated with its strong ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), reflecting a higher concentration of phenolic and flavonoid compounds. The variation in antioxidant strength among the fractions can be attributed to differences in solvent polarity, suggesting that semi-polar solvents such as ethyl acetate are more effective in extracting antioxidant-active constituents.

This study used Trolox, a water-soluble analog of vitamin E, as the reference standard due to its well-established radical-scavenging activity and consistent reactivity in antioxidant assays (Anwar et al., 2022). The observed TEAC values, therefore, represent the relative antioxidant efficiency of each extract and fraction compared with Trolox.

Table 4. Antioxidant capacity of *Jatropha curcas* leaf extract and fractions

Sample/Fraction	Antioxidant Activity (µmol TE/g)	Category
Ethanolic extract	3.52 ± 0.710	Moderate
n-Hexane fraction	0,68 ± 0.164	Very low
Ethyl-acetate fraction	9.19 ± 0.380	High
Aqueous fraction	1.84 ± 0.297	Low

The Kruskal-Wallis statistical test revealed a significant difference in antioxidant capacity among the four sample groups: the ethanolic extract, *n*-hexane fraction, ethyl acetate fraction, and aqueous fraction. The resulting p-value < 0.0001 was substantially lower than the significance threshold (0.05), indicating that the median antioxidant activities of these groups were not identical.

These statistical findings corroborate the FRAP assay data, confirming that fractionation using solvents of varying polarity significantly influenced the overall antioxidant activity. Specifically, the ethyl acetate fraction was statistically identified as the most effective in enhancing antioxidant capacity compared with the other fractions. This outcome strongly suggests that the ethyl acetate fraction is a reservoir of bioactive compounds with potent antioxidant potential.

The superior efficacy of this fraction is likely due to the nature of ethyl acetate as a semi-polar solvent with high efficiency in dissolving phenolic and flavonoid compounds widely distributed in plant tissues. These compounds exhibit strong antioxidant potential, acting as electron or hydrogen donors to neutralize free radicals (Ummah, 2019).

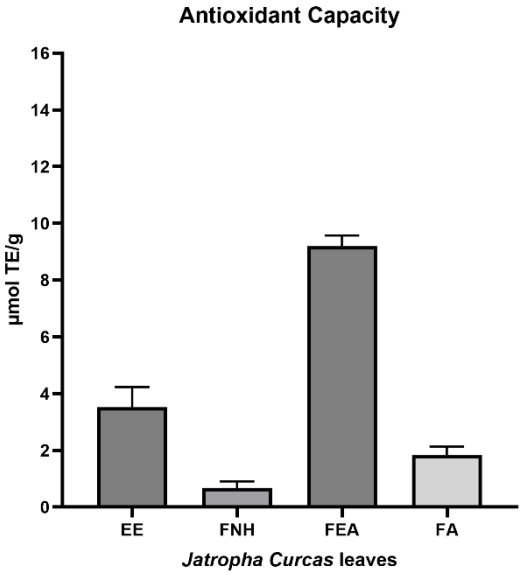

Figure 2. Comparative graph of the antioxidant capacities of the ethanolic extract (EE), *n*-hexane fraction (FNH), ethyl acetate fraction (FEA), and aqueous fraction (FA) of *Jatropha curcas* leaves.

3.4. Total Phenolic Content

The total phenolic content (TPC) assessment results show that the tested sample contains 6.78% gallic acid equivalent (GAE). This relatively high concentration emphasizes the essential role of phenolic components in supporting the antioxidant power of the extract. Their substantial contribution to the antioxidant function stems from the presence of hydroxyl groups (-OH), which can donate electrons to neutralize free radicals. The high levels of TPC are closely correlated with the data on antioxidant capacity, especially the results of the 9.19 ± 0.38 µmol TE/g activity recorded in the ethyl acetate fraction. This strong positive association is expected to come from the efficiency of ethyl acetate, a semi-polar solvent, in selectively attracting phenolic compounds, which are the leading providers of antioxidant potential. In short, these findings confirm the vital impact of phenolic constituents on the overall antioxidant power of the extract. Adjusting the isolation parameters and selecting a better solvent can potentially increase the number of compounds that can be obtained, thereby enhancing the value of the extract as a valuable source of natural antioxidants.

3.5. Total Flavonoid Content

The total flavonoid content (TFC) analysis shows a value of 1.35% equivalent to quercetin (QE) in the studied extract. This moderate concentration indicates that the contribution of flavonoids to the total antioxidant power is not as significant as that provided by other phenolic components. As polyphenolic compounds, flavonoids are characterized by having aromatic rings and hydroxyl (-OH) groups that enable them to stabilize free radicals through the donation of electrons or hydrogen atoms. Although the amount of flavonoids is not high, their presence is still important because of the possibility of synergistic effects with other phenolic compounds when capturing free radicals. Consequently, this data indicates that the antioxidant activity of the extract arises from the combination of non-flavonoid phenolics such as tannins and phenolic acids, which are more abundant in fractions based on their polarity, together with flavonoids.

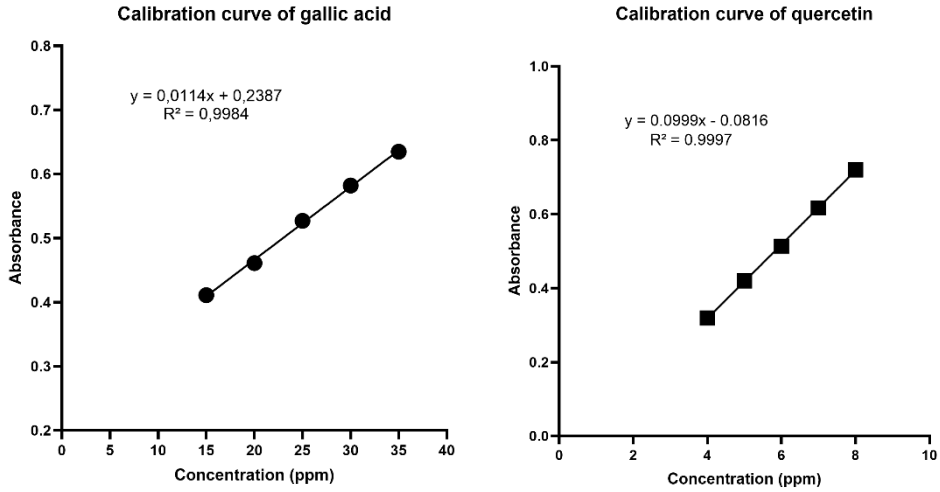

Figure 3. Standard curves for gallic acid and quercetin

The calibration curves for gallic acid and quercetin demonstrated excellent linearity, with coefficients of determination (R^2) of 0.9984 and 0.9997, respectively. These values confirm a strong linear relationship between standard concentration and absorbance, validating the regression equations used for quantifying total phenolic and flavonoid contents.

The slope of the quercetin curve was steeper than that of gallic acid, indicating higher absorbance sensitivity and suggesting that even small concentration changes in quercetin resulted in more pronounced absorbance variations. This reflects the stronger chromophoric response of flavonoids compared with phenolic acids.

Such high linearity ($R^2 > 0.99$) demonstrates excellent precision and reliability in the quantification of bioactive compounds. Moreover, the strong correlation between standard concentration and absorbance supports the robustness of the analytical method, ensuring accurate estimation of phenolic and flavonoid levels that contribute significantly to the overall antioxidant performance of *Jatropha curcas* extracts.

4. Conclusion

The findings of this study demonstrate that the leaves of *Jatropha curcas* L. possess strong potential as a natural source of antioxidant compounds. Among the tested fractions, the ethyl acetate fraction exhibited the highest antioxidant activity (9.19 ± 0.38 µmol TE/g), followed by the ethanolic extract (3.52 ± 0.71 µmol TE/g), aqueous fraction (1.84 ± 0.30 µmol TE/g), and n-hexane fraction (0.68 ± 0.16 µmol TE/g). Phenolic compounds were identified as the primary contributors to antioxidant capacity, supported by the presence of flavonoids that act as complementary radical scavengers. Variations compared with previous studies may be attributed to differences in extraction methods, solvent polarity, and the physicochemical characteristics of the plant materials used.

These results underscore the importance of selecting an appropriate extraction solvent to maximize antioxidant potential. Furthermore, the high antioxidant capacity of the ethyl acetate fraction suggests its possible application in the development of natural antioxidant formulations, such as

phytopharmaceuticals, nutraceuticals, or cosmetic antioxidants. Future studies should focus on isolation, structural characterization, and mechanistic evaluation of the active compounds to better understand their roles in redox regulation and biological activity.

RECOMMENDATIONS

Further research should focus on isolating and characterizing individual bioactive compounds with strong antioxidant potential from *Jatropha curcas* leaves, particularly phenolic and flavonoid constituents. Subsequent in silico and in vitro analyses are recommended to clarify their molecular mechanisms and interactions with redox-related biological targets. These approaches will enhance understanding of the structure–activity relationships and support the future development of *Jatropha curcas*-based antioxidant agents for pharmaceutical, nutraceutical, or cosmetic applications.

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