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Phytochemical screening and antioxidant activity of leaf extracts from Jatropha curcas collected in the Diourbel region of Senegal.

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Abstract
*The main objective of this study was to evaluate the secondary metabolite composition and antioxidant activity of methanolic extracts from the leaves of Jatropha curcas, a plant used in traditional medicine to treat various ailments. Extraction was performed by methanol maceration, followed by qualitative phytochemical screening, assessment of antioxidant activity using the DPPH assay, and thin-layer chromatography (TLC). The extraction yield obtained was 4.26%. Phytochemical screening revealed the presence of polyphenols, **saponins**, catechins, leucoanthocyanins, catechols, as well as polyterpenes and sterols. Antioxidant activity was found to be dose-dependent, with an IC50 of 1.09 mg/mL, indicating moderate antioxidant activity compared to gallic acid (0.0676 mg/mL). These results suggest that Jatropha curcas is a potential source of natural antioxidant compounds, thus justifying further investigations to identify its active principles.*

Keywords: *Jatropha curcas , antioxidant activity and secondary metabolites*

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I. Introduction

For thousands of years, medicinal plants have been an essential source of therapeutic substances. Even today, a large number of medicines are derived directly from natural plant products **[1] .** Medicinal plants can be used whole or only by part of the plant (leaves, stems, roots, bark, or fruit) in various pharmaceutical forms. However, excessive harvesting of certain parts, especially the roots, can compromise the plant's survival. Traditional medicine remains less expensive **[2]** than modern medicine, accessible to the majority of the population, and closely integrated into the socio-cultural lifestyles in which it is deeply rooted. Much research has focused on the use of natural molecules to counteract oxidation and its harmful effects on health **[3]** . Oxidative stress occurs when the production of free radicals exceeds the body's antioxidant defense capacity. Cardiovascular diseases, cancer, diabetes, ischemia, and aging are among the conditions linked to oxidative stress **[4].** Many plants, rich in phenolic compounds (such as flavonoids, phenolic acids, cinnamic acid derivatives, and tocopherols), nitrogenous compounds (such as alkaloids), and carotenoids, are important sources of antioxidants **[5]** . Today, plant-derived antioxidants are recognized as essential protectors of human health. Among the plants with strong potential, *Jatropha curcas* , widely distributed in tropical regions and belonging to the Euphorbiaceae family, is of particular interest. Different parts of the plant have been traditionally used in the treatment of wounds, burns, and muscle cuts due to its antimicrobial and antifungal properties **[6**] . Phytochemical analyses have revealed the presence of phenolic compounds, flavonoids, saponins, and alkaloids, known for their diverse biological effects. In particular, flavonoids and saponosides contribute to the antioxidant and anti-inflammatory activities observed in various plant extracts **[7]** , which justifies the evaluation of the antioxidant activity of leaf extracts of this species. In this context, the objective of this study is to evaluate the chemical composition in secondary metabolites as well as the antioxidant activity of methanolic extracts of *Jatropha curcas leaves* , in order to justify their traditional use within Senegalese society, particularly for medicinal and food purposes.

II. Materials and methods

2.1. Plant material

The plant was harvested in October 2025 in the commune of Ndondol, located in the department of Bambey, Diourbel region. The leaves were kept with the stems, and the entire assembly was then dried at room temperature

(25 to 30 °C), protected from light and without ventilation, for a period of four weeks. After drying, the plant material was mechanically ground into a powder, which was stored in paper bags until use.

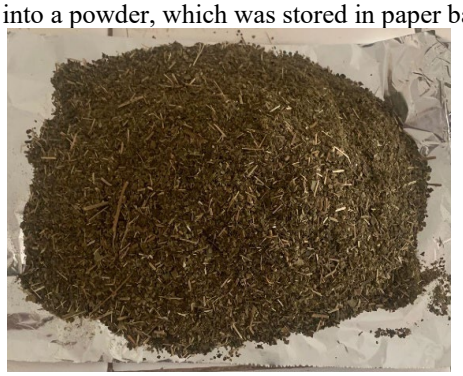


Figure 1 : Jatropha curcas leaf powder

2.2. Extraction and calculation of extraction rates

The solid/liquid extraction method was adopted. Twenty-five g of extract were macerated in 200 mL of methanol in an Erlenmeyer flask placed on a magnetic stirrer with a magnetic stir bar for 24 hours to promote the extraction of bioactive compounds. After filtration, the process was repeated a second time using 100 mL of methanol for an additional 24 hours, followed by further filtration. The resulting methanolic filtrates were then combined and concentrated by evaporating the solvent under reduced pressure using a rotary evaporator until a dry extract was obtained. This step ensured the complete removal of methanol while preserving the integrity of the compounds of interest. The resulting dry extract was then used to evaluate antioxidant activity.

2.3. Chemical composition of metabolites

2.3.1. Phytochemical screening

To obtain an initial chemical characterization of *Jatropha curcas* , a phytochemical screening was performed. This preliminary step involved conducting various chemical tests to identify the main groups of compounds before any in-depth analysis. A 5% aqueous infusion (5 g of plant material in 100 mL of boiling water) was prepared for 30 minutes. Following this step, the preparation was filtered to remove solid residues, and the resulting filtrate was used for the various phytochemical tests.

2.3.2. Determination of polyphenols

The presence of polyphenols was detected using the ferric chloride ($FeCl_3$) test. Two test tubes were used, each containing 2 mL of the extract. One tube served as a control, while two drops of $FeCl_3$ solution were added to the other. The appearance of a blue-black color indicates the presence of polyphenols in the extract.

2.3.3. Determination of flavonoids

The search for flavonoids was carried out using two test tubes. One mL of the extract was introduced into each tube. One tube served as a control **[8]** , while in the other, 1 mL of 50% hydrochloric acid and a few magnesium shavings were added. Although a slight release of heat was observed, no characteristic color change was noted, suggesting the absence of flavonoids.

2.3.4. Determination of saponins

The search for saponins was performed using the foam index method. One gram of powder was added to 100 mL of water. The mixture was gently boiled for 15 minutes, and the suspension was then filtered. The filtrate was introduced into 10 tubes at 1 mL increments (ranging from 1 to 10 mL), and each tube was then filled to the 10 mL mark with water. All tubes were shaken vigorously for 30 seconds and then left to stand for 15 minutes. After this stand time, foam was observed in all tubes. However, tube number 4, with a foam height of exactly 1 cm, was selected for calculating the foam index, confirming the presence of saponins in the extract.

2.3.5. Determination of catecholic tannins and gallic tannins

The detection of catechins was performed using Stiasny's reagent **[9].** For this test, two test tubes were used. One mL of the extract was introduced into each tube. One tube contained 0.5 mL of Stiasny's reagent, while the other served as a control. Both tubes were then placed in a water bath at 90 °C for 15 minutes. After incubation, the formation of a precipitate in the tube containing Stiasny's reagent indicated the presence of catechins in the extract. The presence of gallic tannins was detected in the filtrate obtained after filtering the solution from the tube containing the precipitate in the catechin tannin test. The filtrate was then saturated with sodium acetate, followed

by the addition of 0.1 mL of ferric chloride ($FeCl_3$). After the reagent was added, the expected characteristic color was not observed, indicating the absence of gallic tannins (hydrolyzable tannins) in the extract.

2.4. Evaluation of antioxidant capacity using the DPPH method

The antioxidant activity of the methanolic extract of *Jatropha curcas* was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging test **[10, 11]** . In ethanolic solution, the DPPH $^{\bullet}$ radical exhibits a characteristic violet color with a maximum absorbance at 517 nm. In the presence of an antioxidant compound capable of donating a hydrogen atom or an electron, DPPH $^{\bullet}$ is reduced to diphenylpicrylhydrazine, resulting in decolorization of the solution. The decrease in absorbance at 517 nm is proportional to the antioxidant capacity of the extract.

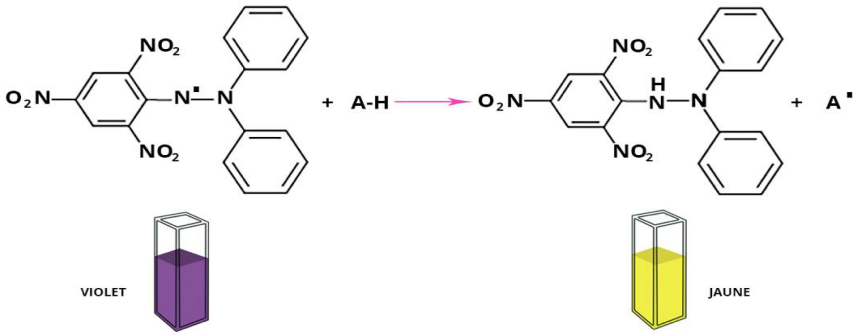


Figure 2 : Reduction of the DPPH free radical $^{\bullet}$ by an antioxidant (AH) leading to the formation of DPPH-H.

III. Results and Discussion

3.1. Results

3.1.1. Extraction rate

The extraction yield was determined to assess the quantity of extract obtained from the plant material used. It is expressed as a percentage and calculated according to the following formula:

$$\textbf{Rendement} = \frac{\mathbf{M_1}}{\mathbf{M_0}} \times \mathbf{100}$$

M1: Mass in grams of the dry extract and M0 : Mass in grams of the plant powder. Methanol extraction of *Jatropha curcas* yielded a yield of 4.26%.

3.1.2. Phytochemical screening

The photographs below illustrate some of the reactions observed during the various tests carried out during the phytochemical screening of the methanolic extract of *Jatropha curcas* .

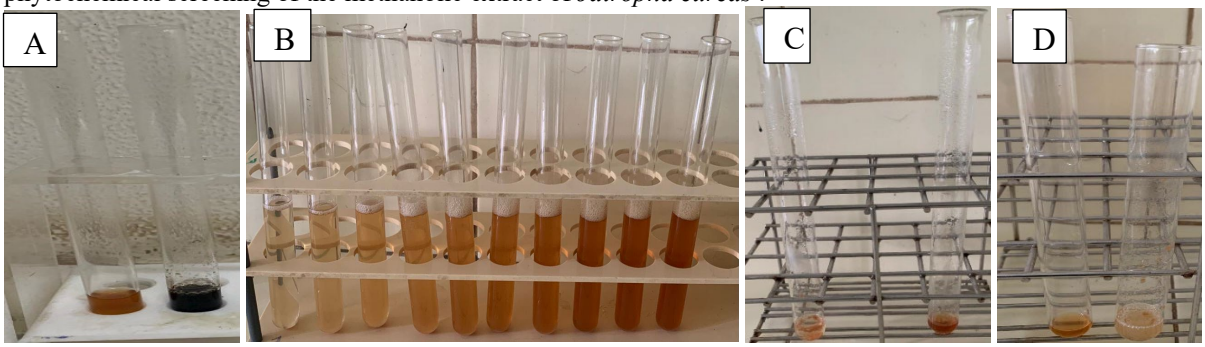


Figure 3 : Photographs illustrating the reactions observed for different secondary metabolites in the methanolic extract of Jatropha curcas.

A: Polyphenols, B: Saponins, C: Catecholic tannins, D: Flavonoids

The following table summarizes the results obtained for all tests carried out during the phytochemical screening.

Table 1 : Overall results of phytochemical screening

Secondary metabolites	Observations	Results

Polyphenols	Blue-black coloration	+
Saponosides	Im = 250	+
Catechetical tannins	Presence of precipitate	+
Gallic tannins	Absence of color	_
Flavonoids	Absence of orange coloration	_

+; Presence -; Absence

These results are consistent with those of other studies on the organs of *Jatropha curcas* **[12].** These various metabolites are recognized for their remarkable medicinal and physiological activities **[13, 14].**

3.1.3. Antioxidant capacity by the DPPH test

The absorbances measured by UV-visible spectrophotometry were used to determine the percentage of inhibition of the DPPH radical. This percentage was calculated using the following formula:

$$\textbf{Pourcentage d'inhibition (PI)} = \frac{\textbf{Absorbance blanc} - \textbf{Absorbance échantillon}}{\textbf{Absorbance blanc}} \times 100$$

The percentages of inhibition obtained for the different concentrations are grouped in the following table.

Table 2 : Percentages of DPPH radical inhibition ˙ calculated from absorbances measured for different concentrations of the methanolic extract.

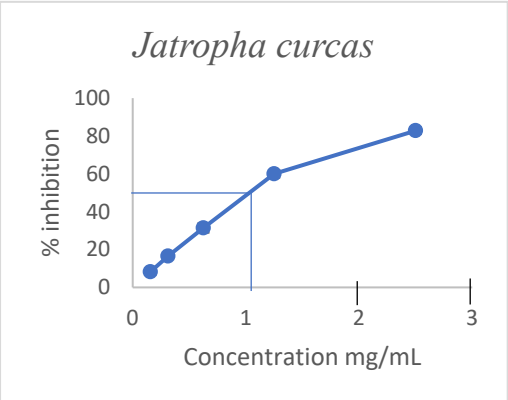
Concentrations (mg/mL)	Absorbance 1	Absorbance 2	PI1(%)	PI2(%)	Average PI	Standard deviation
0.15625	0.986	1,012	7,156	9,319	8,237	1,529
0.3125	0.886	0.932	16,572	16,487	16,529	0.060
0.625	0.747	0.746	29,661	33,154	31,407	2,469
1.25	0.440	0.430	58,568	61,469	60,018	2,051
2.5	0.196	0.178	81,544	84.05	82,797	1,772

DPPH radical scavenging test was also performed using gallic acid as a reference molecule. The results are presented in the table below.

Table 3 : Percentages of DPPH radical inhibition ˙ calculated from absorbances measured for different concentrations of gallic acid.

Concentrations (mg/mL)	Absorbance 1	Absorbance 2	PI1 (%)	PI2 (%)	Average PI (%)	Standard deviation
0.0097	1.044	1.068	8.66	6.56	7.61	1.48
0.019	0.917	0.935	19.77	18.2	18.98	1.11
0.039	0.714	0,721	37,57	36,92	37,22	0,43
0,078	0,342	0,335	70,08	70,69	70,38	0,43
0,156	0,06	0,063	94,74	94,49	94,62	0,18

The curves shown below illustrate the variation in percentage inhibition as a function of the concentration of *Jatropha curcas extract, compared to gallic acid used as a reference. These curves allowed* us to determine the IC50 values, corresponding to the concentration required to inhibit 50% of DPPH radicals



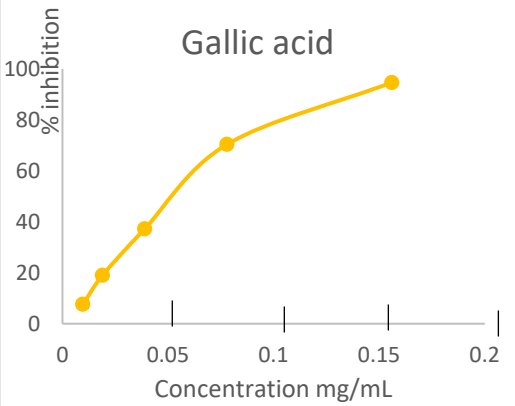


Figure 4 : Percentage of inhibition as a function of the concentration of Jatropha curcas extract and gallic acid.

The 50% inhibitory concentration (IC_{50}) of the methanolic extract of *Jatropha curcas leaves* , evaluated for DPPH scavenging activity ، is 1.09 mg/mL, while that of gallic acid, used as a reference, is 0.0676 mg/mL.

3.2. Discussion

The beneficial pharmacological effects observed when using plant materials are primarily linked to a set of chemical compounds called plant secondary metabolites. These metabolites play a crucial role in the plant's defense mechanisms, contributing to its protection against herbivores and microorganisms **[15] . The yield of the methanolic extract of** *Jatropha curcas* leaves obtained in this study was 4.26%, indicating moderate recovery of secondary metabolites after extraction by maceration in methanol. This yield is lower than those reported in the literature, with 8.36% obtained for maceration and 9.86% when ethanol is used as the solvent. **[16]** This observation highlights that, even if the extraction method is identical, the nature and polarity of the solvent influence the solubility of phytochemicals and, consequently, the yield. Furthermore, the observed differences can be attributed to the quality and quantity of plant material, the experimental conditions (temperature, duration, and solvent/plant material ratio), as well as the stage of leaf harvest, which can affect the content of secondary metabolites. Despite this relatively lower yield, the extraction performed in this study provided a sufficient quantity of extract for evaluating antioxidant activity. Phytochemical screening of *Jatropha curcas leaves* revealed the presence of polyphenols, saponins, and catechins, while certain metabolites, such as gallic tannins, flavonoids, alkaloids, and some steroids, were not detected. Previous studies using water as a solvent on the plant have shown that certain compounds, including tannins and phenols **[17]** , are regular components of aqueous extracts, although the exact profile can vary depending on the extraction method and the geographical origin of the leaves. In other studies using solvents other than water, different phytochemical profiles were observed. Using methanol, alkaloids and flavonoids were detected, while using ethanol, additional steroids and phenols were identified. These results highlight that solvent polarity plays a crucial role in the extraction of secondary metabolites, with each solvent favoring the solubilization of specific compounds. Furthermore, the variations observed between different studies may also result from the extraction method used, as well as the ecological (local climate, soil type, etc.) and geographical conditions that influence the biosynthesis of secondary metabolites in Jatropha *curcas.* The antioxidant capacity of the methanolic extract of *Jatropha curcas* was evaluated by determining its IC50 and compared to that of gallic acid. The results show that the IC50 of the extract is 1.09 mg/mL, while that of gallic acid is significantly lower (0.0676 mg/mL), indicating that the extract possesses significant antioxidant activity, albeit lower than that of the standard. This difference can be explained by the fact that gallic acid is a pure compound, known for its strong reducing power, while the plant extract is a complex mixture of secondary metabolites with varying concentrations and efficiencies. Nevertheless, the extract demonstrates a notable ability to neutralize free radicals. Furthermore, the antioxidant activity was assessed by thin-layer chromatography. revealed a pronounced antioxidant activity of the plant extract. This result can be correlated with the polyphenols previously detected during phytochemical screening. These results showed that plant extracts rich in polyphenols exhibit concentration-dependent **DPPH radical scavenging activity.** Furthermore, the difference observed with certain studies, such as that of JU Imah-Harry et al. **[18**] which reports an IC50 of 0.22 mg/mL, could be explained by variations related to experimental conditions, the extraction solvent used, and the chemical composition of the plant. Thus, the methanolic extract of *Jatropha curcas*, although less active than gallic acid, constitutes a promising source of natural compounds with antioxidant properties.

Thin-layer chromatography (TLC) analysis of the extract revealed the presence of several spots, indicating the diversity of compounds present. Under UV light at 366 nm, fluorescent pink-violet spots, as well as blue spots, were observed, suggesting the presence of different classes of secondary metabolites. These results are consistent with previous observations reported by H. Hasan et al. **[19**] for *Jatropha curcas* and by PN Nzuzi *et al* **[20]** for a plant belonging to the same genus *Jatropha* . The spots revealed by chromatography would be due to the presence of compounds such as flavonoids, known for their variable fluorescence under UV, as well as other secondary metabolites, including alkaloids and phenolic acids.

IV. Conclusion

Phytochemical screening revealed the presence of several families of bioactive compounds, including polyphenols, saponins, and catechins, known for their biological effects. Evaluation of antioxidant activity using the DPPH method showed that the extract possesses the capacity to neutralize free radicals, with an IC50 of 1.09 mg/mL. Compared to gallic acid, used as a reference, this activity remains lower, indicating moderate antioxidant power. The study of natural compounds in plants represents an important field for the valorization of local resources, the conservation of medicinal plants, and the development of traditional medicine. These compounds, generally safe at low doses, are recognized for their diverse biological properties, particularly their antioxidant

activities. In this context, *Jatropha curcas* appears as a plant of growing interest due to its therapeutic, nutritional, and economic potential.

ABBREVIATIONS

%: Percentage
$A^{\bullet}$: Radical derived from the antioxidant
AH: Antioxidant (hydrogen donor)
CI_{50}: Inhibitory concentration 50%
TLC: Thin-layer chromatography
$DPPH^{\bullet}$: 2,2-diphenyl-1-picrylhydrazyl
$FeCl_3$: Iron (III) chloride
mg/mL: Milligram per milliliter
$O_2^{\bullet -}$: Superoxide anion
WHO: World Health Organization
pH: Potential of hydrogen
$R^{\bullet}$: Free radical
RH: Organic substrate (target molecule)
$ROO^{\bullet}$: Peroxyl radical
UV: Ultraviolet
$^{\bullet}OH$: Hydroxyl radical

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