Phytochemical and Antifungal Analysis of Root Bark, Inner Seed and Seed Coat Crude Extracts of *Jatropa curcas*

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Abstract

Background: Jatropha curcas, a member of the Euphorbiaceae family, is known for its medicinal and biofuel applications. Rich in phytochemicals such as alkaloids, flavonoids, tannins, and phenolics, it has shown antimicrobial properties, though its antifungal potential remains underexplored. This study evaluated the antifungal activity of Jatropha curcas root bark, inner seed, and seed coat crude extracts while identifying bioactive compounds responsible for this activity.

Materials and methods: Samples were harvested from Kitui, Kenya, authenticated at the National Museums of Kenya, air-dried, ground, and extracted using hexane and methanol in a Soxhlet apparatus. Phytochemical screening employed standard qualitative tests to detect alkaloids, glycosides, phenols, tannins, flavonoids, terpenoids, and coumarins. Antifungal activity was assessed by measuring inhibition zones of different extract concentrations (50 µl/ml and 100 µl/ml) against *Candida auris*.

Results: Phytochemical screening revealed that glycosides and terpenoids were present in all extracts, while phenols, flavonoids, tannins, and coumarins were mainly detected in methanol extracts. Alkaloids were absent in root bark and seed coat but moderately present in inner seed extracts. Antifungal tests showed that hexane extracts exhibited higher inhibition zones than methanol extracts. Among the plant parts, root bark hexane extract at 100 µl/ml demonstrated the highest antifungal activity, with a 15 mm inhibition zone.

Conclusion: These findings confirm the presence of bioactive compounds in Jatropha curcas with potential antifungal properties. The hexane-extracted root bark exhibited the strongest antifungal activity, suggesting its possible use in developing natural antifungal agents. Further research is recommended to isolate specific active compounds and understand their mechanisms.

Key words: Phytochemical screening, *Jatropha curcas*, antifungal activity, bioactive compounds

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Introduction

Jatropha curcas, commonly known as the physic nut, is a member of the Euphorbiaceae family and is indigenous to Central and South America. Over time, it has been cultivated in various tropical and subtropical regions due to its adaptability and diverse applications (Heller, 1996). Traditionally, *J. curcas* has been utilized for medicinal purposes, serving as a remedy for ailments such as infections and skin diseases (Fagbenro-Beyioku et al., 1998). In recent years, there has been a resurgence of interest in *J. curcas* owing to its potential as a biofuel source. The seeds contain approximately 30–40% oil, which can be processed into biodiesel, offering a renewable energy alternative (Heller, 1996). However, beyond its energy applications, *J. curcas* has garnered attention for its rich phytochemical composition, which includes compounds such as alkaloids, flavonoids, tannins, saponins, and phenolics (Tiwari et al., 2011). These bioactive constituents are believed to contribute to the plant's antimicrobial properties. Several studies have investigated the antimicrobial efficacy of *J. curcas*. For instance, ethanol extracts from the stem have demonstrated significant antibacterial activity against *Klebsiella pneumoniae*, with inhibition zones measuring up to 40 mm (Igbinosa et al., 2009). Additionally, the plant's latex has exhibited antifungal properties, inhibiting the growth of pathogens like *Candida albicans* (Suhaili et al., 2011). Despite these findings, there remains a need for comprehensive studies focusing on the antifungal potential of *J. curcas* against a broader spectrum of fungal pathogens, particularly those affecting agricultural crops.

This study aims to evaluate the antifungal activity of various solvent extracts from different parts of *Jatropha curcas* specifically the root bark, inner seed, and seed coat against selected phytopathogenic fungi. Additionally, the research seeks to identify and characterize the phytochemical constituents present in these extracts that may contribute to their antifungal properties. Fungal diseases pose a significant threat to global agriculture, leading to substantial yield losses and economic hardships for farmers. The reliance on synthetic fungicides has been the conventional approach to managing these diseases. There is a pressing need to explore and develop eco-friendly and sustainable alternatives for fungal disease management. Plants have been a rich source of bioactive compounds with antimicrobial properties. In this context, *Jatropha curcas* emerges as a promising candidate due to its diverse phytochemical profile and demonstrated antimicrobial activities. Exploring the medicinal properties of *J. curcas* can lead to value addition, providing farmers with alternative income sources through the cultivation of this multipurpose plant. This study seeks to bridge the knowledge gap concerning the antifungal properties of *Jatropha curcas*,

aiming to contribute to the development of natural and effective solutions for managing fungal diseases in agriculture.

Material and Methods

Plant material

The *Jatropha curcas* plan root bark, inner seed, and seed coat were harvested from Kitui at the farms of local farmers and identified at National Museums of Kenya Herbarium department, under voucher specimen No. MUT/KKM-20. The plan parts were tansported to Murang'a University of Technology for processing.

Preparation of extract

Sufficient amount of freshly young *Jatropha curcas* root bark, inner seed, and seed coat were harvested from Kitui at the farms of local farmers. The plant parts were air dried in the analytical chemistry laboratory at room temperature for 4 weeks. Air dried root bark, inner seed, and seed coat were pre-crushed and later pulverized into fine powder using electric blender, then soaked in respective solutions for 72 hours in the ratio of 1:2 by weight volume (w/v) respectively in analytical grade of hexane and methanol reagents. The crude extract were extracted in a Soxhlet apparatus using hexane and methanol as solvents for the root bark, inner seed, and seed coat. A small portion of each extract was subjected to chemical analysis to identify the presence of various chemical constituents. Further, phytoconstituents were isolated from the extracts.

Qualitative phytochemical screening of the various *Jatropha curcas* crude extracts preparations

Alkaloids Testing (Mayer's Test)

In separate test tubes containing 5ml of 10% sulphuric acid, 1ml of crude extract samples of root bark, inner seed and seed coat extracted in hexane and methanol was added respectively. They were then incubated in water bath for 2 minutes followed by introduction of 1ml Mayer's reagent and 1 ml of chloroform. The formation of a turbid-colored precipitate showed the presence of alkaloids.

Glycosides Testing (Keller-Killiani Test)

This procedure involved addition of 10ml of 10% ethanol to 2ml of crude extract samples of root bark, inner seed and seed coat extracted in hexane and methanol in separate test tubes then brought to boiling. Adding 2ml 5% acetic acid and 1ml of 5% ferric chloride on to the samples, the mixtures were once more brought to boiling before addition of 10% sulphuric acid. A blue- green coloration of the formation indicated the presence of glycosides.

Tannins Testing (Ferric Chloride Test)

To 2ml of the crude extract samples of root bark, inner seed and seed coat extracted in hexane and methanol in separate test tubes, deionized water was added then brought to boiling. Adding three drops of 5% ferric chloride to each of the test tubes, the appearance of blue and green colors signified the presence of tannins, respectively.

Flavonoids Testing (Shinoda Test)

After observing a crimson red color in the crude extract samples of root bark, inner seed and seed coat extracted in hexane and methanol in separate test tubes, a mixture of 5 drops of 5% ferric chloride were added. The appearance of this color within a few minutes signified the presence of flavonoids.

Terpenoids Testing (Salkowski Test)

To 2ml of the crude extract samples of root bark, inner seed and seed coat extracted in hexane and methanol in separate test tubes, they underwent treatment with 2ml chloroform and 3ml concentrated sulfuric acid each. The appearance of reddish brown color at the interface layer indicated the presence terpenoids.

Coumarins Testing

On separate filter papers (whatman No.1), few drops of ammonia solution was added to the filter papers. Subsequently, a drop of the crude extract samples of root bark, inner seed and seed coat extracted in hexane and methanol respectively was added to the filter papers. The appearance of fluorescence was an indication of presence of coumarins.

Results

Phytochemical components

Alkaloids, bioactive compounds often possessing pharmacological activities, were tested across various parts of *Jatropha curcas*. Different extraction solvents, hexane and methanol for root bark, inner seed and seed coat, were employed. Interestingly, alkaloids were not detected in the root bark and seed coat when extracted with hexane and root bark when extracted with methanol, but a moderate presence was observed in the inner seed when extracted with Hexane and methanol. Glycosides, another significant class of bioactive compounds, were found abundantly throughout all parts of *Jatropha curcas*. This was evidenced by the positive results obtained from the Keller-kilani test, regardless of the extraction solvent used. Phenolic compounds, known for their antioxidant properties, showed varied presence across different parts of the plant. While the root bark and inner stem exhibited a positive reaction when extracted with methanol, the seed coat showed no reaction when extracted with hexane. Tannins, polyphenolic compounds with diverse biological activities, were detected primarily in the root bark and seed coat when extracted with methanol, showing a positive reaction. However, no reaction was observed in other parts of the plant when extracted with hexane. Flavonoids, another class of polyphenolic compounds, were found to be present in the root bark, inner seed and seed coat when extracted with methanol, as indicated by positive reactions. No reaction was observed in the seed coat when extracted with either solvent. Terpenoids, known for their diverse biological activities, were

found abundantly across all parts of *Jatropha curcas*. This was evidenced by the positive reactions obtained from the extraction process involving chloroform and concentrated sulphuric acid H_2SO_4. Coumarins, aromatic compounds with various pharmacological activities, were detected in the root bark, inner seed and seed coat, particularly when extracted with methanol, as indicated by positive reactions. No reaction was observed in the seed coat when extracted with either solvent. Additionally there was no reaction for inner seed extracted in hexane. The phytochemical analysis of *Jatropha curcas* revealed a diverse array of bioactive compounds, including glycosides, phenols, flavonoids, terpenoids, and coumarins, distributed across its various parts (Table 1)

Table 1: Phytochemical components found in various *Jatropha curcas* crude extracts preparations

Sn.	Constituents	Tests	RBN	RBM	ISN	ISM	SCN	SCM
1	Alkaloids	Mayer's reagent	-	-	+	+	-	+
2	Glycoside	Keller-kilani test	+	+	+	+	+	+
3	Phenols	Extract+70% Ethylacetate+5% $FeCl_3$	-	+	-	+	-	+
4	Tannins	Extract+5% FeCl3	-	+	-	-	-	+
5	Flavanoids	Residue+5% $FeCl_3$	-	+	-	+	-	+
6	Terpenoids	Extracts + Chloroform + Conc H_2SO_4	+	+	+	+	+	+
7	Coumarins	Salkowski test	+	+	-	+	-	-

Key: + means presence, and –means absence

Antifungal activities

The hexane extracts generally showed higher zones of inhibition compared to the methanol extracts. This indicates that hexane might be a better solvent for extracting antifungal compounds from *Jatropha curcas.* In terms of concentration Impact, the hexane extract, an increase in concentration from 50 µl/ml to 100 µl/ml generally increased the zone of inhibition, especially noticeable in the inner seed and root bark. For the methanol extract, the results were mixed. The seed coat showed a decrease in inhibition with higher concentration, while the inner seed and root bark show inconsistent patterns. Comparison for Plant Part, the root bark extract (hexane) at 100 µl/ml showed the highest zone of inhibition (average: 15 mm), suggesting that the root bark contains potent antifungal compounds effective against *Candida auris*. The inner seed also showed significant activity, especially with the hexane extract at higher concentration (average: 12.3 mm). Hexane extract of the root bark at 100 µl/ml demonstrated the best antifungal activity with the largest zone of inhibition, averaging 15 mm. This suggests that for antifungal applications targeting *Candida auris*, the hexane extract of the root bark is most effective.

Table 2: Antifungal activities (zone of inhibition) of the different extracts of *Jatropha curcas*

	Zone of inhibition (in mm)			
Extracting Solvents	**Hexane**		**Methanol**	
Concentration of Extract	**50ul/ml**	**100ul/ml**	**50ul/ml**	**100ul/ml**
Seed coat	12±2.26	11.7±0.65	11.7±0.65	10±1.13
Inner Seed	11.3±1.31	12.3±2.84	12±2.30	8.7±1.3
Root Back	10.7±0.65	15.3±0.65	19.7±0.65	10.3±0.65

Discussion

The exploration of bioactive compounds within *Jatropha curcas* unveils a complex interplay of chemical constituents distributed across its various parts. This research presents intriguing findings regarding alkaloids, glycosides, phenolic compounds, tannins, flavonoids, terpenoids, and coumarins, each contributing to the plant's pharmacological potential.

The absence of alkaloids in the root bark and seed coat when extracted with hexane is an interesting discovery, suggesting either a low concentration of alkaloids in these parts or an inefficient extraction method. In contrast, the moderate presence of alkaloids in the inner seed underscores its potential pharmacological significance. Comparable studies on alkaloid distribution in plant species, such as those by Harborne and Baxter (1999), emphasize the importance of extraction solvents in determining alkaloid yields. The ubiquitous presence of glycosides throughout all parts of *Jatropha curcas* indicates their fundamental role in the plant's bioactivity. The positive results obtained from the Keller-kilani test across different extraction solvents reaffirm the widespread distribution of glycosides. Similar observations have been reported in studies investigating glycoside content in medicinal plants like Digitalis species (Kapoor, 2017).

The varied presence of phenolic compounds across different parts of the plant highlights the influence of both the plant's physiology and the choice of extraction solvent. The positive reaction observed in the root bark and inner stem when extracted with methanol aligns with the antioxidant properties attributed to phenolic compounds. These findings resonate with studies on phenolic content in medicinal plants like

Eucalyptus species (Vuong et al., 2017). The detection of tannins primarily in the root bark and seed coat underscores their localization within protective tissues. The positive reaction observed when extracted with methanol suggests a higher affinity of this solvent for tannin extraction. Similar observations have been reported in studies on tannin distribution in plants like Acacia species (Kong, 2012).

The presence of flavonoids in the root bark, inner seed, and seed coat underscores their widespread distribution across different parts of the plant. The positive reactions obtained with methanol extraction highlight its efficacy in extracting flavonoids. These findings align with studies on flavonoid content in medicinal plants like Ginkgo biloba (Mahady et al., 2001). The abundant presence of terpenoids across all parts of *Jatropha curcas* suggests their integral role in the plant's biochemistry. The positive reactions obtained with chloroform and concentrated H_2SO_4 extraction underscore the importance of solvent selection in maximizing terpenoid yield. Similar findings have been reported in studies on terpenoid distribution in plants like Mentha species (Burt, 2004). The detection of coumarins in the root bark, inner seed, and seed coat highlights their potential pharmacological significance. The preference for methanol extraction aligns with its efficacy in extracting coumarins from plant tissues. These findings resonate with studies on coumarin content in medicinal plants like Melilotus species (Oprea et al., 2005).

Conclusion

The ongoing research on bioactive compounds in Jatropha curcas offers valuable insights into its pharmacological potential. The distribution of alkaloids, glycosides, phenolic compounds, tannins, flavonoids, terpenoids, and coumarins across different plant parts underscores the importance of solvent selection in maximizing compound yield. These findings pave the way for further investigations into the therapeutic applications of Jatropha curcas and its bioactive constituents. The study also highlights the potential of *Jatropha curcas* extracts, particularly hexane extracts, in combating *Candida auris*. The root bark, when extracted with hexane and used at higher concentrations, shows promising antifungal properties, suggesting its potential use in developing antifungal treatments. Further research could explore the specific compounds responsible for this activity and their mechanism of action.

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Competing Interests

The author declares that there is no conflict of interest.

Availability of Data Statement

The corresponding author can provide the datasets used and/or analyzed in the current study upon reasonable request.

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