

Phytochemical analysis of *Jatropha curcas* leaves: A study of bioactive compounds extracted from various solvents

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Abstract:

The increasing interest in herbal medicine as a safer alternative to conventional drugs motivates the investigation of bioactive compounds in *Jatropha curcas* leaves. This study aims to screen the phytochemical constituents of methanolic, aqueous, and hexane extracts from the leaves of *Jatropha curcas*. Methodologically, the leaves were taxonomically identified, collected, powdered, and extracted using three different solvents. Phytochemical screening was conducted to identify the presence of bioactive components. The results showed that all extracts contained alkaloids, steroids, and terpenoids, while the aqueous extract also included flavonoids and saponins, indicating a higher concentration of bioactive constituents. The aqueous extract exhibited an inhibition index of 0.65 relative to the standard antibiotic, Cifoxitin, suggesting potential antimicrobial efficacy. This discussion emphasizes the varied pharmacological potential of *Jatropha curcas*, advocating for further research into its medicinal applications and the importance of solvent selection in extracting bioactive compounds. In conclusion, the study highlights the therapeutic promise of *Jatropha curcas* in the pharmaceutical field.

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1. Introduction

The exploration of medicinal plants has gained significant momentum, primarily because of the growing perception that herbal remedies are safer and more dependable alternatives to expensive conventional medicines, which often come with undesirable side effects. In many African nations, medicinal plants are integral to the natural biodiversity and are increasingly used as raw materials for developing new pharmaceuticals, particularly in addressing drug resistance in parasitic infections [1]. The integration of traditional medicinal knowledge with modern healthcare practices is evident, as over 40% of widely prescribed medications originate from plant or animal sources [2]. Additionally, the trend towards utilizing medicinal plants in developed countries can be linked to the successful extraction and formulation of various drugs and therapeutic agents derived from these botanical sources, alongside traditional herbal practices [3]. The therapeutic benefits of plants often stem from their phytochemicals, which exhibit antioxidant, antimicrobial, and antipyretic properties. For many people, especially in developing nations like Nigeria, plants continue to be a primary source of medicinal treatments [4].

Jatropha curcas is one of the popular natural antimicrobial substances used for its medicinal properties to treat a wide variety of ailments since ancient times. Extracts from the leaf, fruits, latex and bark of *J. Curcas* exhibit wide range of medicinal properties and antimicrobial activity such that it is used in folklore remedies for

treatment of various ailments such as skin infections and fever. However, the efficacy of these plants as claimed, have not been subjected to modern scientific methods for verification. For example, several studies have shown that intake of plant extracts in high dosage could lead to various complications such as injury to the kidney, induce acute renal failure and interfere with renal tubular function [4]. Furthermore, despite the advent of pharmaceutical chemistry during the early twentieth century which brought with it the ability to synthesize an enormous variety of medicinal drugs, allowing treatment of a wide range of diseases, chemically derived drugs are now plagued by recurrent side effects, toxicity (leading to proliferation of disorders) and resistance by the parasites they are intended to eliminate. Also, the truth remains that development, research and production of synthetic drugs (also known as orthodox) remain very costly, time consuming and even sometimes unsuccessful in actual use [5]. These among many other factors have reawakened the need and urge to source for and develop alternative and effective pharmaceuticals. Though ancient medical documents hold a large reportage of medicinal plants, for example: the manuscript of "Eber papyrus" which contains 700 medical formulas dating back to 16th century B.C, others remained undocumented [6]. Although phyto-compounds are generally considered safe, they carry inherent risks that must be acknowledged. This underscores the importance of understanding potential side effects, determining appropriate dosages, identifying

bioactive phyto-compounds, and establishing effective extraction and conservation methods. This study aims to scientifically validate the medicinal properties of *Jatropha curcas* by screening the bioactive compounds found in its leaf extracts from various organic solvents.

1.1 *Jatropha Curcas* Applications

Jatropha belongs to the tribe *Jatropheae* in the *Euphorbiaceae* family and contains approximately 170 known species that manifest as succulents, shrubs and trees [6]. Zengin, *et al.* [7] show that *Jatropha* species are rich in secondary metabolites reported as follows:

1.1.1 Anti-bacterial activity

Acetone, chloroform, ethanol and methanol extracts of *J. curcas* root bark has been reported to inhibit the growth of both gram-positive (*Staphylococcus aureus*) and gram-negative bacteria like *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Escherichia coli* [7].

1.1.2 Anti-fungal activity

Various plant parts of *J. curcas* have antifungal activity against *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *B. subtilis*, *P. omnivora*, *C. albicans* etc. which are responsible for many diseases in human being and plants [8].

1.1.3 Antiviral activity

The water extract of the branches of *J. curcas* strongly inhibit the HIV-induced cytopathic effects with low cytotoxicity. Latex of *J. curcas* possesses inhibitory property against Water melon mosaic virus [9]

1.1.4 Anti-inflammatory activity

Topical application of *J. curcas* root powder in paste form in mice and rats has been reported to possess anti-inflammatory activity. Methanolic extract of *Jatropha curcas* obtained from the roots has showed anti-inflammatory activity against carrageenan induced rat paw odema [7].

1.1.5 Anti-oxidant activity

The root bark extract of *J. curcas* has been shown to scavenge hydroxyl radicals in a concentration-dependent manner, exhibiting stronger hydroxyl scavenging activity compared to ascorbic acid. Additionally, the shoots of *J. curcas* demonstrate antioxidant activity, containing higher concentrations of glutathione reductase, ascorbate peroxidase, and guaiacol peroxidase. This enzymatic protective system in the shoots plays a crucial role in managing oxidative stress and regulating enzymes that help reduce the accumulation of reactive oxygen species (ROS). Furthermore, acetic acid is significant in controlling lethal browning and disease incidence in tissue culture shoots. Elevated activity levels can increase concentrations within the tissue, triggering programmed cell death, which ultimately leads to browning and necrosis [10].

1.1.6 Coagulant and anticoagulant activities

Latex is a blood coagulant whereas dilute latex is anticoagulant. Curcin from seed produces deleterious effects to the blood [11].

1.1.7 Anti-diarrhoeal activity

The petroleum ether and methanol extract of *J. curcas* roots shows anti-diarrhoeal activity in various species of albino mice. The methanolic extract of roots of *Jatropha curcas* shows anti-diarrhoeal activity in various species of albino mice. The result indicates that methanolic extract of *Jatropha curcas* in combination of elevated prostaglandins biosynthesis can reduce propulsive movement of small intestine [12].

1.1.8 Anti-diabetic activity

Ethanol extract of leaves of *Jatropha curcas* L. shows antihyperglycemic effect was studied in normal oralloxon induced diabetic rats. It decreases the elevated level of serum glucose and also reverses the cholesterol, triglyceride values when compared with untreated diabetic rats [13].

1.1.9 Pregnancy terminating effect

The fruits of *Jatropha curcas* showed abortifacient activity, foetal resorption activity and pregnancy terminating effects. The antifertility effect of *Jatropha curcas* may be due to the presence of cytotoxic agent *Jatrophone* [11].

1.1.10 Wound healing activity

Wound healing activities of stem bark of *J. curcas* has been documented in Kaur and Kaur [14].

1.1.11 Insecticidal, larvicidal and anthelmintic activity

Various plant parts of *J. curcas* have been reported to possess insecticidal and larvicidal and anthelmintic activity. Insect pests of stored grains (*Sitophilus zeamais* and *Rhyzopertha dominica*) are susceptible to seeds and pericarps of *J. curcas* [9].

1.2 Phytoconstituents of *J. curcas*

Phytochemicals can be defined as naturally occurring plant substances that function in prevention and curing of diseases. Plants produce a wide variety of secondary metabolites, many of which have been reported to be of therapeutic value. The leaf, bark and latex of *Jatropha* species are rich sources of phytochemicals such as alkaloids (*jatrophine*, *jatropham*, *curcacycline A*), curcain, tannins, glycosides, flavonoids and sapogenins with anti-cancerous properties. However, different phytochemicals show various mechanisms of action, well documented in Musa [5].

1.2.1 Diterpenes

Diterpenes have been dominating the research area in *J. curcas* for their novel chemical structures and medicinal values. Diterpenes have a range of in vitro biological activities such as antihypertensive, anticancer, antiretroviral, anti-inflammatory, analgesic, antimicrobial, insecticidal and molluscicidal activities [10].

1.2.2 Phorbol esters

Phorbol esters are diterpenes with a tigliane skeletal structure and are believed to be the most toxic molecules

in *Jatropha* species. Phorbol esters cause skin-irritation and tumor promotion by stimulating protein kinase C (PKC) [15].

1.2.3 Alkaloids

Alkaloids are a large group of nitrogen-containing compounds with important medicinal uses. They are known as compounds with powerful narcotic analgesic, antimalarial, antibacterial, antineoplastic, and anticancer activities. The compounds have basic properties and are alkaline in reaction, turning red litmus paper blue [16].

1.2.4 Flavonoids

Flavonoids are secondary metabolites with many pharmacological activities such as anticancer, antiviral, antitoxic, and hepatoprotective activities. Two flavonoids were isolated from *J. curcas* by Antwi-Bediako, *et al.* [17].

1.2.5 Phenolics

Phenolic compounds are present in all plants and are considered to be biologically active constituents. These compounds have antithrombotic and anti-inflammatory actions because they inhibit and antagonize the platelet activating factor (PAF) which is a potent inflammatory phospholipid mediator [18].

1.2.6 Proteins

J. curcas proteins and peptides have been studied for their roles in plant metabolic activities, defense against predators and biological activities. Functional proteins such as aquaporins were isolated from different parts of *J. curcas*. These proteins play essential roles in plant adaptation to drought stress by controlling the transmembrane water movement [16].

1.3 Therapeutic Values of *J. curcas*

Most parts of this plant are used for the treatment of various human and veterinary ailments [7]. These traditional medicinal properties of *Jatropha curcas* need to be investigated in depth for the marketable therapeutic products [5].

1.3.1 Latex

The latex of *Jatropha* contains an alkaloid known as *Jatrophine* which is known for anticancerous properties and it also serves as a disinfectant. The latex has shown haemostatic and antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* and *Candida albicans*. It is also applied topically to get relief from bee and wasp stings, toothache, burns, hemorrhoids, ringworm and ulcers [18].

1.3.2 Oil

Fresh oil extracted from the seeds of *Jatropha curcas* appears yellowish and fairly viscous. The external application of the *J. curcas* oil is useful against various skin diseases and rheumatism. It is also considered as a hair growth stimulant, arborificient, emetic, and laxative.

Efficacy of oil has also been shown against dropsy, sciatica, paralysis and sores in animals [19].

1.3.3 Leaves

The leaves are large, green, alternate to subopposite, three to five-lobed with spiral phyllotaxy. Some people drink the leaf decoction to cure venereal diseases and heartburn, while some apply it for relief in arthritis pain and to cure scabies. The twig sap of *Jatropha* is also used to dress wounds and ulcers. An emulsion of the sap with benzyl benzoate is known to be effective against scabies, wet eczema and dermatitis [11].

1.3.4 Seeds

The seeds become mature when the capsule (fruit) changes from green to yellow over a short period. The seeds of *Jatropha* have been used to cure stomachache, antidote for poisoning, as a purgative and as a styptic [12].

1.3.5 Roots

The roots are known to have strong anthelmintic action. The aqueous extract of the root is used as an antidote for snake bites. Some people use root decoction against dysentery and as a mouth wash to take care of the bleeding gums and teeth. The sap flowing from the stem is used to arrest bleeding of wounds while its branches are used as a chewing stick in Nigeria [8].

1.3.6 Toxicity and precautions

Various parts of the *Jatropha* plant, particularly the fruits and seeds, possess attractive medicinal properties, but they may sometimes be detrimental when ingested [10]. Many cases of poisoning with the physic nut (*J. curcas*) are reported in the literature mainly due to presence of toxalbumin called curcin and cyanic acid [5].

2. Materials and Method

2.1 Collection of *J. Curcas* Leaves

Fresh mature leaves were collected from fully-grown plant of *J. Curcas* earlier identified and labelled at the ATBU Conservation Garden, Gubi Campus collected between the months of August to October were used. The botanical identification was obtained by comparing the samples collected with a voucher specimen at the Garden. The leaves were selectively collected, where matured leaves with noticeable colour changes, tender leaves and leaves which have patches on them were removed and discarded. The harvested leaves of *Jatropha curcas* were washed with distilled water so as to remove dust and other foreign particles then air-dried to constant weight on a clean surface under shade. The dried material was grinded to fine powder using laboratory mortar and pestle and stored in an air tight bottle as in Figures 1 and 2.

2.2 Soxhlet Extraction with Different Solvents

The powdered material was extracted by hot extraction method using Soxhlet apparatus with water, Methanol and Water. Typically, Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent.



Figure 1: Dried *Jatropha curcas* Leaves



Figure 2: Powdered *Jatropha curcas* Leaves

Forty grams air-dried and coarsely powdered plant material was kept in Soxhlet extraction unit and exhaustively extracted with methanol at 60°C for eight hours and same procedure was applied for Water and Hexane. All the solvents used were of analytical grade and each extract was separately recovered through distillation as demonstrated in Figures 3 - 6.

2.3 Screening for Bioactive Compounds

Medicinal plants represent a constituent part of the natural biodiversity of many countries in Africa. Their use as raw materials in the production of new drugs is ever increasing because of their potential in combating the problem of drug resistance to parasitic microorganisms [12].

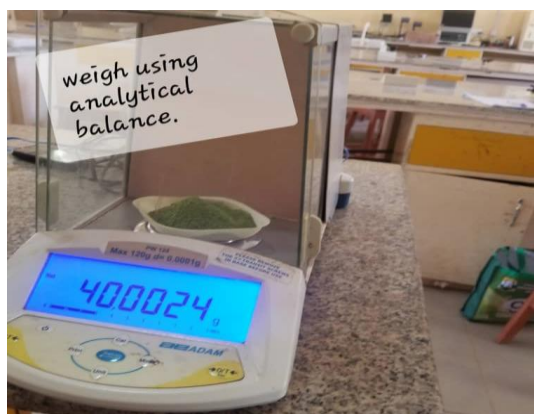


Figure 3: Powdered *Jatropha curcas* leaves being weighed for extraction



Figure 4: Soxhlet extraction unit being set up



Figure 5: Extraction in progress

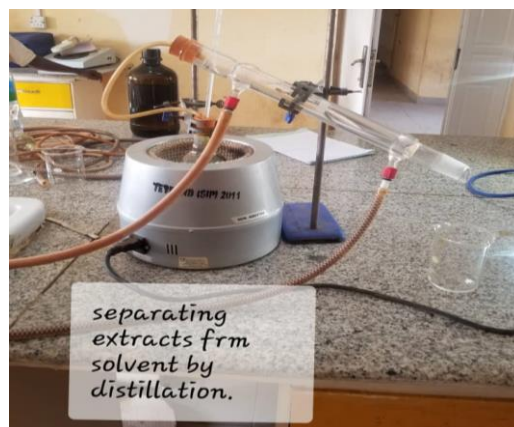


Figure 6: Separation by distillation

The use of medicinal plants through practices and knowledge has augmented modern medicine. This can be attested from the fact that more than 40% of commonly prescribed medicines throughout the world may have found their origins directly or indirectly in plants or animals. Furthermore, an increasing reliance on the use of medicinal plants in industrialized societies have been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as traditionally used herbal remedies [9]. The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effects of the phytochemical in them. Plants remain the main source of medicines for a large proportion of the world's population; particularly in

developing countries, of which Nigeria is on the front row of that list. Phytochemical tests of various plant parts of *Jatropha curcas* revealed variation in contents of total phenols, tannins, free amino acids and phytic acid. The presence of bioactive substances in the methanolic extract of *Jatropha curcas* leaves was adequately investigated at the Chemistry Laboratory, Federal University of Kashere. Each of the Aqueous, Methanolic and Hexane extract of *Jatropha curcas* leaves was qualitatively tested, according to the standard procedures as described by Momoh, *et al.* [13], for the detection of any kind of secondary metabolites including:

2.3.1 Test for Flavonoids

A few drops of 1% NH_3 solution was added to the aqueous extract of each plant sample in a test tube. A yellow coloration was observed which indicated that flavonoid compound is present.

2.3.2 Test for Tannins

0.5g of powdered sample of each plant is boiled in 20ml of distilled water in a test-tube and filtered. Three drops of 0.1% FeCl_3 was added to the filtered samples and a blue black colouration was observed which indicated the presence of tannins.

2.3.3 Test for Saponins

The powdered leaves (0.5 g) were placed in a test tube and 10 ml of distilled water was added and shaken vigorously for 30 s. It was then allowed to stand for 30 min and observed. Formation of honey comb froth indicated the presence of saponins.

2.3.4 Test for Phenols

The powdered leaves (0.5 g) were placed in a test tube and 10 ml of distilled water was added and shaken for 30 s. It was then allowed to stand for 30 min. Then 3 drops of FeCl_3 solution was added, production of a greenish-black colour indicated the presence of phenolic nucleus.

2.3.5 Test for Alkaloids

The powdered leaves (2 g) were boiled in a water bath with 20 ml of 5% sulphuric acid in 50% ethanol. The mixture was cooled and filtered. A portion of the filtrate was put in 100 ml of separating funnel and the solution was made alkaline by adding two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was run off into a second separating funnel. The ammoniacal layer was reserved. The chloroform layer was extracted with two quantities each of 5 ml of dilute sulphuric acid into test tubes. To the filtrate in the test tube, 1 ml of Mayer's reagent was added drop by drop. Formation of a greenish coloured or cream precipitate indicated the presence of alkaloids.

2.3.6 Test for Anthraquinones

Borntrager's test was used for free anthracene derivatives. The powdered leaves (0.5 g) were taken in a test tube and 5 ml of chloroform was added and shaken for

5min. The mixture was filtered and shaken with equal volume of 10% ammonia solution. A pink, red or violet colour in the aqueous layer after shaking indicated the presence of free anthraquinone.

2.4 Test for Phytosterols and Terpenes

Two qualitative chemical tests, the Salkowski's Test and the Liebermann-Burchard Test, are employed to identify sterols and triterpenes. Salkowski's Test, a colorimetric method primarily used for cholesterol detection, involves dissolving a sample extract in chloroform and adding concentrated sulfuric acid. A positive result for triterpenes or sterols is indicated by a golden yellow colour in the chloroform layer [20]. The Liebermann-Burchard Test, also colorimetric, detects cholesterol and phytosterols. This test involves dissolving the extract in chloroform, treating it with acetic anhydride, boiling, cooling, and then adding concentrated sulfuric acid. A brown ring at the interface signifies the presence of phytosterols [21].

2.4.1 Salkowski's Test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenes.

2.4.2 Liebermann-Burchard's test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicated the presence of phytosterols.

3. Results and Discussion

Aqueous, Methanolic, and Hexane extracts of the leaves of the plant was prepared. The cake from each solvent was weighted and the supernatant was stored in sterile McCartney bottles and kept in the refrigerator at 4°C and later used for phytochemical tests. Similar *in vivo* studies on Methanolic extract of *Jatropha curcas* leaves reported the presence of Alkaloid, Steroid, Terpenoid, Flavonoid and Saponins [13]. This implies that the plant contains mixtures of different chemical compounds that may act individually, additively or in synergy. The following results have sufficiently established the presence of bioactive constituents including Alkaloid, Steroid and Terpenoid from each of the three extracts. While Flavonoid and Saponins were present only in the Aqueous extract, as presented in Table 1.

Table 1: Phytochemical Constituents of *J. curcas*

Phytochemical Test	Aqueous Extract	Methanolic Extract	Hexane Extract
Alkaloid	+Ve	+Ve	+Ve
Steroid	+Ve	+Ve	+Ve
Terpenoid	+Ve	+Ve	+Ve
Flavonoid	+Ve	-Ve	-Ve
Saponins	+Ve	+Ve	-Ve

+Ve indicates the presence of the constituent; -Ve indicates the absence of the constituent

3.1 Testing for saponins

Each extract (0.5g) was mixed with water in test tube. Foaming which persisted on warming was taken as an evidence for the presence of saponins.

3.2 Testing for tannins and phenolics

Each extract (0.5g) was separately stirred with 10mL of distilled water and then filtered. Few drops of 5% FeCl₃ reagent was added to the filtrate. Blue-black or blue-green colouration or precipitation was taken as an indication of the presence of phenolics and tannins.

3.3 Testing for alkaloids

Each extract (0.5g) was stirred with 5mL of 1% HCl on a steam bath. The solution obtained was filtered and one 1mL of the filtrate was treated with a few drops of Mayer's reagent. Its turbidity was evidence of the presence of alkaloids.

3.4 Testing for steroids

0.5g of each extract was separately added with 5 drops of acetic anhydride and then a drop of concentrated H₂SO₄. The mixture was steamed for 1 hour and neutralized with sodium hydroxide (NaOH), followed by the addition of chloroform. The appearance of a blue-green colour indicated the presence of steroid.

3.5 Testing for glycosides

0.5g of each extract was dissolved in 2mL of chloroform. Tetraoxosulphate VI acid (H₂SO₄) was carefully added to form a lower layer. A reddish-brown colour at the interface indicated the presence of glycosides

4. Conclusion

Jatropha curcas product exhibits wide range of medicinal properties and antimicrobial activity such that it is used in folklore remedies for treatment of various ailments such as skin infections and fever. Solvents used for extraction played an important role in the extraction of the phytochemicals. Their selection is based on their polarity. Water has the highest extraction yield compared to methanol and Hexane. This is because polar compounds are easier to extract compared to non-polar compounds.

Recommendations

- The role of extraction methods and solvents used in bioactive compounds yields should be taken into consideration as it shows promising future in the pharmaceutical field.
- The economics of extraction and marketing bioactive compounds present in *Jatropha curcas* for medicinal uses should be further explored and encouraged.

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