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Original Research

Comparative Study on the Phytoconstituents and Antimicrobial Analysis of *Jatropha curcas* Leaf and Stem Bark

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ABSTRACT: Most of the natural substances found in plants are used extensively for human well-being and the treatment of numerous diseases. Plants are a rich source of these products. The medicinal plant Jatropha curcas has been used to treat a number of diseases, including skin infections. This study compares the antibacterial activities of *Jatropha curcas* against some selected clinical isolates and the phytoconstituents present in the leaf and stem bark. The bark of the leaf and stem was macerated successively in n-hexane for 72 hours each. Six clinical isolates were subjected to the antibacterial susceptibility test by preparing discs with a standard dose of *Jatropha caurcas* extract in hexane. The result shows that the leaf and stem bark extract contains cardiac glycosides, steroids, alkaloids, tannins, flavonoids, triterpenes and saponins. The analysis shows that 20 phytochemicals were present in the leaf extract of *Jatropha curcas* and 18 in the n-hexane extract. The highest amounts of flavonoids were found in the leaf and stem bark of Jatropha curcas, with resveratrol reaching 39.200 g/g in the leaf and 39.586 g/g in the stem bark. The amounts of heart glycoside (4.120 g/g) and sapogenin (42.276 g/g) in leaves were greater than in stem bark, which contained cardiac glycoside (3.950 g/g) and sapogenin (42.136 g/g, respectively). Steroid concentrations in stem bark were likewise greater (26.003 g/g) than in leaves (25.650 g/g). At a minimum inhibitory concentration of 10mg/L, Pseudomonas aeruginosa and Salmonella enterica were more susceptible to the n-hexane extract of *Jatropha carcus* stem bark than Bacillus licheriformis, Micrococcus roseus, and Bacillus subtilis. The n-hexane extract of leaf and stem bark demonstrates equal sensitivity based on a 22.3mm zone of inhibition of Staphylococcus aureus and Citobacter murliniae. According to the findings of the study, active metabolites with considerable therapeutic advantages are present in both leaf and stem bark, which is critical for the pharmaceutical industries.

Keywords: *Jatropha curcas*, leaf, phytoconstituent, stem bark, antimicrobial

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INTRODUCTION

Medicinal plants have been used to treat human ailments for a very long time. In addition to their traditional role as sources of biologically active chemical compounds that are both antifungal and antibacterial, medicinal plants also have a long history of use (Das et al., 2010). According to Gaibimei et al. (2018), medicinal plants are the most prevalent bio-resources for drugs used in conventional medical systems, contemporary medications, dietary supplements, folk pharmaceuticals, medication, intermediates, and chemicals used in the production of synthetic medicines. Plants have been used by mankind for the treatment of a wide range of ailments for thousands of years. More than 80 percent of the world's population still relies on traditional medicine, which is mostly composed of plant extracts. This is particularly prevalent in less developed areas (Mercy and David, 2018).

According to Nna et al. (2019), plant medicine is still

significant in contemporary pharmacology and therapeutic use. The composition and chemistry of human bodies are influenced by plant substances such as carbohydrates, lipids, proteins, vitamins, and minerals (Nna et al., 2019). Today's drug research and development processes are heavily influenced by plants, and it is inevitable that new drugs will be derived from plants. Traditional medicine may provide both brand-new therapies and inexpensive raw materials for the manufacture of already available medications (Nna, 2020). Examples include the discovery of reserpine from the Rauvolfia species and a contraceptive in the zoapatle (Montanoa tomentosa). A huge shrub that may reach a height of 3 to 4 meters, *jatropha curcas* is almost ubiquitous. The fruits are 2.5 cm long, ovoid-oblong, dull brownish black, and yellowish in color (Odugbemi and Ayoola, 2008; Saturnino et al., 2010). The leaves are alternately arranged, 10-15 cm 7.5-12.5 cm in length, broadly elliptic, connate, acute, often palmately 3 to 5 lobed, and hairless. The seed resembles a castor seed in appearance, despite being smaller and darker brown in colour. In many tropical and subtropical nations, jatropha plants are cultivated as biofuel crops, and they can be seen growing as a fence around agricultural plants in many places of India (Carels, 2009; Divakara et al., 2010). They may also be grown easily on marginal soils to assist recover land. These seeds' non-edible oil is utilized as a feedstock in the production of biodiesel (Maes et al. 2009). Numerous indigenous groups use various varieties of Jatropha for medicinal reasons in tropical and subtropical areas. For instance, the purgative seed oil of the Jatropha species is widely recognized. According to Odugbemi and Ayoola (2008), this purgative action has been utilized to treat digestive symptoms such diarrhea, dysentery, vomiting, retching, and stomachaches. Various plant components from the Jatropha genus are also utilized to treat skin issues. Crushed leaves, stems, roots, or seed oils from the jatropha plant are used to treat swellings, dermatitis, mouth blisters, carbuncles, and other skin diseases. They are also believed to be beneficial in treating urine discharge and sex-related infections. Several Jatropha species' roots have also historically been used to treat gonorrhea and leprosy (Sabandar et al., 2013; Shad and Andrew, 2013). Due to their enormous potential as natural sources of bioactive molecules, studies of plant-based compounds and the genus Jatropha have increased recently. Jatropha plants have been used to isolate various alkaloids, cyclic peptides, terpenes, flavonoids, lignans, coumarins, coumarino-lignoids, a non-cyanogenic glucoside, phenolics, and fatty acids (Shad and Andrew, 2013). Additionally, cytotoxicity, antimicrobiological, anti-inflammatory, antioxidant, insecticidal, larvicidal, cholinesterase inhibition, and toxicity activities have been discovered in extracts and isolated chemicals from this plant (Satheesh and Pari, 2008; Pathania et al., 2020). Leaf and stem bark extracts included a dark blue pigment in addition to secondary metabolites such saponins, steroids, tannins, glycosides, alkaloids, and flavonoids (Igbinosa et al., 2009). These substances support plant antibacterial activity because they are physiologically active. According to a number of different processes (Igbinosa et al., 2009; Sabandar et al., 2013), secondary metabolites exhibit antibacterial action. Choudhury et al. (2012) looked at tannins' tendency to combine with proline-rich proteins to form irreversible chemicals that stop cell protein production. According to Ekundayo et al. (2011), photochemicals interact with proteins to provide the characteristic tanning effect, which is helpful for healing inflamed or ulcerated tissues. According to Maiyo et al. (2010), astringent herbs with active metabolites are utilized to treat digestive problems like diarrhea and dysentery. Plants have the potential to be a source of essential bioactive molecules for cancer therapy and prevention since the biological activities of phytochemicals have been shown to have anticancer activity and may be exploited in cancer prevention (Gadekar et al., 2010). Plant stem bark contains phytoconstituents, which supports the plant's long-standing usage as a medicine to cure a number of diseases. The search for active metabolites is important to adapt and expand the use of different plants in response to the expanding global population in order to provide simple access and reduce certain healthcare issues by meeting nutritional and medicinal demands. To evaluate plants' therapeutic and nutritional worth in light of few resources, it is crucial to prove their phytochemical and antibacterial activity. Based on the alleged therapeutic benefits of plants, this study concentrated on comparative research on phytoconstituents and antimicrobial studies of *Jatropha curcas* leaves and stem bark.

MATERIALS AND METHODS

Chemicals and Reagents

Glacial acetic acid, acetic anhydride, sulphuric acid, ethyl acetate, ferric chloride, phenols, and HCl were among the substances employed in the study.

Collection of the plant materials

In the Khana Local Government Area of Rivers State's Kaa Community, *J. curcas* leaf and stem bark were harvested. The Botany Department of the University of Port Harcourt handled the identification and authentication.

Processing of the plant materials

For use in the initial investigation of phytochemicals and

antimicrobial activity, The stem bark and leaf of J. curcas were gathered and allowed to air dry at room temperature in the Chemistry Laboratory of Ignatius Ajuru University of Education in Port Harcourt, River State, Nigeria for a period of fourteen days. Prior to conducting the investigation, the components were first ground into a powder using a pulverizer and then stored in an airtight container. After the stem bark and powdered leaves had been weighed out to 100 grams, they were dissolved in 500 milliliters of n-hexane and placed in the refrigerator for three days. The extract was filtered using a chess cloth and Whatman filter paper No. 1 (24 cm) in order to obtain filtrates of the n-hexane solvents. After that, phytochemical analysis was performed using these filtrates as the sample material**.**

Phyto-chemical profiling test

Test for steroids and triterpenoids (Liebermann-Burchard test)

After adding three drops of acetic anhydride to the extract and heating and cooling it, the mixture was finally analyzed. A brown ring was seen to form at the boundary between two layers when concentrated sulfuric acid was injected from the sides of the test tube. According to Sabri et al. (2012), a positive test for triterpenoids and steroids would be indicated by the appearance of a deep red hue in the lower layer, as well as a change in the color of the upper layer, which would become green**.**

Test for cardiac glycosides (Keller-Killiani Test)

About three drops of glacial acetic acid and a solution of diluted ferric chloride were added to about three milligrams of the extract before being mixed. After the very concentrated sulfuric acid had been introduced to the mixture, two layers were created. The upper acetic acid layer would become blue green in the event of a positive glycoside test, whereas the lower reddish brown layer would remain the same.

Fehling's solution test for reducing sugars

According to Satheesh and Pari (2008), the methodology that was employed to examine the declining sugar content was accurate. Following the addition of about one milligram of an extract to a dry test tube and five milliliters of ethyl acetate, the tube was agitated for a period of five minutes. The extract went through a filtration process. It was determined by mixing 2 milliliters of the ethyl acetate extract with 5 milliliters of Fehling's solutions A and B in a container designated for the experiment. After that, the mixture was cooked for a total of five minutes. The presence of reducing sugar would be indicated by the formation of a brick-red precipitate.

Test for phenolics and tannins (ferric chloride test)

After being dissolved in 2 mL of extraction solvent, each crude extract was subjected to further processing by being treated with 4 drops of ferric chloride solution. If the sample had a bluish-gray color, phenols would be present**-**A dark black color began to take shape. According to Nna et al. (2017), the construction of a bluish-green hue is an indication that cathechic tannins are present, while the creation of a bluish-black color is an indication that gallic tannins are present**.**

Test for flavonoids (alkaline test)

After diluting the sodium hydroxide solution, 5 milligrams of the extract was added to 5 milliliters of the liquid. The appearance of a yellow tint that would become colorless with the addition of a few drops of diluted hydrochloric acid is a telltale sign that flavonoids are present in the sample under investigation**.**

Test for saponins.

It was determined by using the ability of saponins to generate foam in aqueous solutions as a screening test (Prakash et al., 2009). In a test tube, the extract (five milligrams) and pure water (five milliliters) were mixed together, then vigorously agitated. The presence of saponins could be deduced from the fact that a significant volume of foam was produced that persisted for almost half an hour**.**

Test for alkaloids.

It took twenty minutes to heat up a test tube that contained three milliliters of an extract and one milliliter of 10% hydrochloric acid. After the filtrate had been allowed to cool and then cooled on its own, a minute quantity of Mayer's reagent was added to 1 milliliter of the filtrate. In the event that there were any creamy precipitates, alkaloids would be present.

Antimicrobial screening

To investigate the plant extracts' antibacterial efficacy, pathogenic isolates were employed. The Department of Microbiology at the University of Port Harcourt is where the microorganisms were gathered. The extract (0.1 g) was weighed and diluted in 10 mL of DMSO to a concentration of 10 mg/mL. This served as the foundation for figuring out the antibacterial action. With the use of the agar diffusion method, the extracts were screened. Mueller Hinton Agar (MHA) served as the medium for microbial growth. The medium was prepared in accordance with the manufacturer's instructions, sterilized at 121 °C for 15 minutes, added to sterile Petri

plates, and then allowed to cool and solidify. The standard test microbe inoculum was then planted with 0.1 mL into the sterilized medium. The inocula were evenly dispersed throughout the media surfaces using sterile swabs. Each contaminated medium had a well made out of it in the middle using a typical cork borer (6 mm). Then, each well on the medium received 0.1 mL of the concentrated 10 mg/mL extract solution. Each plate was then checked for growth-inhibitory zones after the infected mixture had been incubated for 24 hours at 37 °C for bacteria and 30 °C for yeast. The zone of inhibition was measured using a clear ruler, and the measurement was recorded in millimeters.

Quantitative phytochemical screening by GC-FID

The phytochemicals in the extract were measured using BUCK M910 Gas Chromatography, manufactured in the United States by BUCK Scientific. For the gas chromatography, we used a flame ionization detector in conjunction with a RESTEK 15-m MKT1 column that measured 15m x 20m x 0.15um. The sample was injected without splitting across a distance of 20 centimeters at a temperature of 280 degrees Celsius and a velocity of 30 centimeters per second. Helium at a pressure of 5.0 pascals and a flow rate of 40 milliliters per minute was used as the carrier gas. The initial setting for the oven will be set at 200 degrees Celsius. While the oven was being heated up to 330 degrees Celsius at a rate of 3 degrees Celsius per minute, the detector was kept at a temperature of 32 degrees Celsius. A comparison was made between the area of the internal standard and the area of the newly discovered phytochemicals, which allowed for the identification of the phytochemicals.

RESULTS AND DISCUSSIONS

Qualitative phytochemical screening of *jatropha carcus* leaf and stem bark extract

Table 1 displays the results of the qualitative phytochemical analysis of the *Jatropha carcus* leaf and stem bark extract. The current investigation found that, with the exception of tannin, which was missing in the leaf, the different hexane extracts of *Jatropha carcus*' leaf and stem bark included modest quantities of alkaloids, tannin, flavonoids, triterpenes, saponins, cardiac glycosides, and steroids. Tannins may also have physiological impacts on the body, including speeding blood coagulation, reducing blood pressure, and lowering serum cholesterol levels, according to Rebecca et al. (2016) and others. They may also function as anti-diarrhea, anti-cancer, anti-oxidant, anti-microbial, anti-inflammatory, and anti-diabetic substances. Because *Jatropha carcus* stem bark contains tannin, it is likely that the stem bark extract is more important in medicine than the leaf extract. According to Asuk et al. (2015) and Igbinosa et al. (2015), who explored the biomedical relevance of the phytochemical, proximate, and mineral compositions of the leaf and root of Jatropha curcas, the leaf and stem bark extracts of Jatropha curcas contain alkaloids. These researchers examined the biomedical significance of the phytochemical, proximate, and mineral compositions of the leaf and root of Jatropha curcas, tannins, flavonoids, triterpenes, saponins, cardiac glycosides, and steroids. These studies support each other.

Table 1: Preliminary phytochemical screening of *Jatropha carcus* leaf and Stem Bark extract.

Phytochemicals	leaf	Stem bark
Alkaloids	+	+
Tannins	-	+
Flavonoids	+	+
Triterpenes	+	+
Saponins	+	+
Cardiac glycoside	+	+
Steroids	+	+

+(low) and –(absent)

Quantitative Phytochemical screening of *Jatropha carcus* leaf and stem bark extract

Table 2 displays the quantitative phytochemical analysis of Jatropha curcas leaf and stem bark extracts. The analysis shows that 20 phytochemicals were present in the leaf extract of *Jatropha curcas* and 18 in the n-hexane extract. Twelve (12) flavonoids and three (3) alkaloids were detected in the phytochemicals of the hexane extract, while cardiac glycosides, saponins, steroids, saponins, tannins, oxalates, phytates, and cyanates were all present and determined to be one each. The flavonoids flavan-3-ol and rutin, as well as the secondary metabolite phytate, were not present in the *Jatropha curcas* stem bark n-hexane extract, although catechin and tannin were. Proanthocyanin (0.166 g/g), naringin (2.390 g/g), cardiac glycoside (4.120 g/g), anthocyanin (0.740 g/g), cyanogenic glycoside (20.313 g/g), flavonones (22.760 g/g), epicatechin (29.86 g/g), flavone (34.60 g/g), and sapogenin (44.170 g/g)

The amount of metabolites identified in both the leaf and stem bark of *Jatropha curcas* were greatest in flavonoids; the highest concentration of resveratrol was found in the leaf, at 39.200 g/g, while the maximum concentration was found in the stem bark, at 39.586 g/g. Heart glycoside (4.120 g/g) and sapogenin (42.276 g/g) concentrations were higher in the leaf than in the stem bark, which contained cardiac glycoside (3.950 g/g) and sapogenin (42.136 g/g), respectively. Steroid concentrations were also higher in the stem bark (26.003

Table 2: Quantitative phytochemical screening of *Jatropha carcus* leaf and stem bark.

Compounds	Leaf (µg/ml)	Stem bark(µg/ml)	Class
Proanthocyanin	0.166	0.150	Flavonoid
Naringin	2.390	2.220	Flavonoid
Cardiac glycoside	4.120	3.950	Cardiac glycoside
Flavan-3-ol	6.016	-	Flavonoid
Anthocyanin	7.470	6.8930	Flavonoid
Ribalinidine	10.366	10.590	Alkaloid
Naringenin	12.970	13.300	Flavonoid
Spartein	15.460	15.783	Alkaloid
Rutin	17.96	-	Flavonoid
Cyanogenic glycoside	20.313	19.573	Glycoside
Flavonones	22.730	22.290	Flavonoid
Steroids	25.650	26.003	Steroids
Kaempferol	27.536	28.583	Flavonoid
Epicatechin	29.860	29.456	Flavonoid
Phytate	32.996	-	-
Flavone	34.600	34.093	Flavonoid
Oxalate	36.870	37.273	-
Resveratol	39.200	39.586	Flavonoid
Sapogenin	42.276	42.136	Saponins
Epihedrine	44.170	-	Alkaloid
Catechin	-	38.320	Flavonoid
Tannin	-	40.933	Tannins

Table 3: Priliminary antimicrobial susceptibility screening of *Jatropha carcus* leaf and stem bark.

Test organisms	Leaf Extract(mm)				Stem bark Extract(mm)				Control (Ampiclox)(mm)			
	X	Y	Z	M	X	Y	Z	M	X	y	Z	M
Pseudomonas aeruginosa	18	19	22	19.7	20	22	20	20.7	50	49	46	48.0
Staphylococcus aureus	25	23	22	23.3	23	22	25	23.3	52	56	53	53.7
Salmonella enterica	23	21	20	21.3	21	24	22	22.3	43	44	46	44.3
Citobacter murliniae	23	20	24	22.3	21	23	23	22.3	40	35	37	37.3
Bacillus licheriformis	22	21	23	22.0	20	22	21	21.0	40	44	40	41.3
Micrococcus roseus	19	22	24	21.7	18	20	22	20.0	40	39	37	38.7
Bacillus subtilis	22	21	22	21.7	21	19	22	20.7	41	39	45	41.7

X,Y and Z = triplicate measure; M= average

g/g) than in the leaf (25.650 g/g). The results of Sharma et al. (2012), who investigated the phytochemical assessment of methanolic extracts of the root, stem, and leaf of *Jatropha curcas*, supported the findings, and also a similar research on the phytoconstituents and antibacterial properties of an ethyl acetate extract of Carica papaya seed reported by Nna et al. (2018). The high resveratrol concentration of Jatropha curcas leaves and stem bark implies that it may be able to protect against a range of chronic illnesses. These disorders include heart disease, cancer, liver disease, obesity, diabetes, and Alzheimer's disease. This is as a result of the fact that it has antioxidant, anti-inflammatory, immunomodulatory, glycemic and lipid regulating, neuroprotective, and cardiovascular protective properties (Nna et al., 2020). This is consistent with Rebecca et al. (2016) investigation into the phytochemistry and antibacterial resistance pattern of extracts from Jatropha curcas leaves and stem bark, as well as with previous studies on the phytochemical examination of seeds and stem barks.

Another phytochemical that is abundant in the stem bark but missing from the leaf of Jatropha curcas is tannins. This is consistent with Nna (2020), who investigated tannins for use in pharmaceutical, medical, and allied fields. According to Nna et al. (2017), the presence of substantial concentrations of tannins in stem bark implies that it may be utilized as an anticancer agent, virucide, antioxidant, antibacterial and antiinflammatory agent, anti-diabetic, wound healing, cardiovascular protection, antidiarrheal agent, and for a variety of other medical purposes.

Other types of phytochemicals, such as steroids, oxalate, phytate, and sapogenin (saponin), were found during this inquiry and are included in Table 2. Because sapogenin has been used as an antibiotic and a molecule that protects plants, the presence of phytochemicals in both the leaf and the stem bark of Jatropha curcas implies that

it may serve as an anti-microbial (Carels, 2009). Jatropha curcas is a tree that is native to South America.

Oxyalate is a prominent secondary metabolite that can be found in both the leaf and the stem bark of this plant. In contrast to phytate, which could only be found in the Jatropha curcas plant's leaves and not its stem bark, oxyalate can be found in both locations. It was determined that the concentration of oxalate in the leaf was 36.870 g/g, whereas the concentration in the stem bark was 37.273 g/g. Oxalates are most often used in the process of metal detoxification as well as calcium regulation in tissue. Phytate is a chemical that the body utilizes as a source of sustenance. It has been found to have antilipidemic and antiinflammatory characteristics, and the body consumes it (Nna, 2020).

Antimicrobial Activity of Leaf and Stem Bark Extract of*Jatropha carcus*

Table 3 displays the results of an antibacterial test on *Jatropha carcus* leaf and stem extract. Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella enterica, Citobacter murliniae, Bacillus licheriformis, Micrococcus roseus, and Bacillus subtilis were a few clinical pathogens taken into consideration. Table 3 shows the antimicrobial profile for the standard ampiclox solution, stem bark extract, and leaf extract. Bacillus licheriformis, Micrococcus roseus, and Bacillus subtilis were more susceptible to the leaf n-hexane extract of *Jatropha carcus*, but Pseudomonas aeruginosa and Salmonella enterica were more susceptible to the stem bark at a minimum inhibitory dose of 10mg/L. Based on a 22.3mm zone of inhibition of Staphylococcus aureus and Citobacter murliniae (Table 3), the n-hexane extract of leaf and stem bark exhibits equivalent sensitivity (Odoki et al., 2019).

The outcome of the antimicrobial profile revealed that for all of the identified pathogens, ampicillin, the common antimicrobial agent, was more sensitive than leaf and stem bark extracts. This is due to the fact that the zone of inhibition for the common antibacterial agent was greater than that of the *Jatropha carcus* fruit and seed extracts. This finding is in line with Prasad et al. (2012), who described the *Jatropha curcas* plant's medicinal properties, and Sharma et al. (2012), who investigated the *Jatropha curcas* leaf's in vitro antibacterial and phytochemical properties.

Conclusion

The results of this study showed that the amounts of comparable phytochemicals in the Jatropha curcas leaf and stem bark n-hexane extract were very consistent. The stem bark contains 18 phytochemicals in the n-hexane extract, compared to 20 in the leaf. The flavonoids flavan-3-ol and rutin, as well as the secondary metabolite phytate, were not present in the n-hexane extract of the stem bark of Jatropha curcas; on the other hand, catechin and tannin were not present in the n-hexane extract of the leaf. In general, the findings of this study have shown that the leaf and stem bark of Jatropha curcas contain certain phytochemicals. These phytochemicals have the potential to bacteriostatically restrict the growth of the pathogenic organisms that were researched. The n-hexane extract had a substantial inhibiting effect on Bacillus licheriformis, Micrococcus roseus, and Bacillus subtilis; however Pseudomonas aeruginosa and Salmonella enterica were not. At 10 mg/L, Shigella dysenteriae, Escherichia coli, and Pseudomonas aeruginosa performed better for stem bark. This suggests that the *Jatropha curcas* leaf and stem bark extract might be a source of powerful antibacterial compounds. But additional clinical infections need to be evaluated; J. curcas could be more responsive than the typical antibiotic.

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