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# Antibacterial Activity and Toxicity of the Sap and Aqueous Extract of the Leaves of *Jatropha multifida* Linn

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## Abstract

**Introduction:** *Jatropha multifida* Lin was a plant of traditional Beninese medicine used as an antibiotic. This study aimed to evaluate the antibacterial activity and the toxicity of the sap and the aqueous extract of *Jatropha multifida* leaves. **Methods:** Phytochemical screening of *Jatropha multifida* leaves was carried out. The extract was obtained by maceration. The antimicrobial activity of sap and leaves was evaluated on the five strains of hospital germs. Acute oral toxicity by forced gavage in a single dose of 2000 mg/kg body weight was performed on female Wistar rats. Biochemical and hematological parameters were determined. **Results:** The presence of flavonoids, tannins, alkaloids, anthocyanins, mucilages, leuco-anthocyanins and saponosides was noted in the leaves of *Jatropha multifida*. The aqueous extracts of the leaves inhibited two strains of *Staphylococcus aureus* out of three, while the sap of *Jatropha multifida* was 100% bactericidal against the strains of *Staphylococcus aureus* and *Streptococcus* D. The sap and aqueous leaf extract were not bactericidal on strains of *Escherichia coli*, *Klebsiella oxytoca* and *Pseudomonas aeruginosa*. For the toxicity, there was no death of rats and the aqueous extract of the leaves did not significantly vary the weight of the rats, the crea-

tinine, the ALAT transaminase, the hemoglobin level, the number of white blood cells and blood platelets. **Conclusion:** The sap of *Jatropha multifida* exerted a more effective antibacterial activity than the aqueous extract of its leaves. The leaves were not acutely toxic.

## Keywords

*Jatropha multifida*, Antibacterial Activity, Toxicity, Benin

## 1. Introduction

The use of plants was very old and is currently experiencing a resurgence of interest among populations. Traditional medicine referred to health practices, methods and knowledge that involve the use of plants for the purpose of treating, diagnosing and preventing diseases or preserving health [1]. Phytotherapy was enjoying considerable success in many parts of the world. The World Health Organization estimated that approximately 80% of the world's inhabitants use traditional herbal medicine as primary health care [2] [3]. The increased use of medicinal plants was explained by their accessibility and the availability of traditional medicine in developing countries, on the one hand, as well as the high cost of synthetic drugs and the harmfulness of the side effects they caused on the other hand [4]. In the world there were serious and deadly diseases caused by microorganisms. These microbes could be bacteria, viruses, fungi and parasites. Among health problems, particular interest was taken in infectious diseases because these diseases were the cause of a third of global mortality and were more frequent in poor countries where one person in two died of them prematurely [5]. Treatments were available but it was observed that the pathogenic microorganisms became resistant to most of the existing conventional anti-infectious agents. This situation made it difficult to care for people with infectious diseases, especially with the expansion of new resistance mechanisms, which hindered the effectiveness of treatments for common infectious diseases [6]. Resistance phenomena were on the rise worldwide and constituted a real public health problem in developing countries [7]. Ethnobotanical investigations showed that several plants were used in traditional medicine for the treatment of infectious diseases [8]. One of the plants used to treat infectious diseases was *Jatropha multifida* [9] [10].

*Jatropha multifida* Linn was known as alovi aton in the local Fon language of Benin and has widely recognized medicinal properties [11]. It was used to treat various diseases of the human population in Africa, Asia, or Latin America. The antibacterial effect of it leaves, stems, roots were reported [12]. It anti-ulcer and healing properties were reported [11]. African healers used it against fungal infections [9]. In Chinese medicine, the bark and leaves were used against itchy skin and eczema [10].

However, despite these uses in traditional medicine, few studies scientifically confirmed the biological activities of this plant. Also the main problem of tradi-

tional treatments, especially those based on plants, was the lack of scientific knowledge in relation to the effectiveness, the mode of action, the doses to be used, the indications, the harmlessness and the control of the quality. This study was part of the search for new bioactive molecules through the valorization of *Jatropha multifida* L from the traditional Beninese pharmacopoeia. This study aimed to evaluate the antibacterial activity of the sap and the aqueous extract of the leaves of *Jatropha multifida* on the one hand and the toxicity of the leaves of *Jatropha multifida* on the other hand.

## 2. Material and Methods

### 2.1. Plant Material and Aqueous Extraction

The plant material consisted of the leaves and sap of *Jatropha multifida*. The samples were collected in May 2021 in Dassa-Zoumè in central Benin from local traditional healers and brought back to the laboratory the same day. The experiments were carried out from May to July 2021 in the Laboratory of Experimental and Clinical Biology (LaBEC), National School of Applied Biosciences and Biotechnologies (ENSBBA), National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM), R. Benin.

**Treatment of plant material**

The harvested leaves were washed and then cut into small pieces and dried in a cold drying room (22˚C) for about 30 days, after which time the leaves were brittle and were practically anhydrous. Then the dry leaves were reduced to powder using an electric grinder. The powder thus obtained was sieved with a sieve with a diameter of 710 µm. Thus the powder obtained was used for the extraction [13] [14].

The sap was carefully collected directly into the eppendorfs tubes after cutting the branches of *Jatropha multifida* Linn without any external contamination. The sample was brought immediately to the laboratory and chilled at 4˚C in the refrigerator.

### 2.2. Microbial Strains

Strains of Gram-positive cocci such as *Staphylococcus aureus*, *Streptococcus* D and Gram-negative bacilli such as *Escherichia coli*, *Klebsiella oxytoca* and *Pseudomonas aeruginos* isolated from skin infections and abscesses in the clinical biology laboratory of the Saint Jean de Dieu Zone Hospital of Boko in Benin were used for antimicrobial testing. They were stored in agar-based nutrient media at 4˚C before use.

### 2.3. Culture Media

Culture media Mueller-Hinton (MH) broth was used for the culture of bacteria. EMB (Eosin Methylene Blue) culture medium was used to isolate Gram-negative bacilli and Chapman medium for Staphylococcus aureus. The culture media used came from the companies Biomerieux® and Biorad®.

### 2.4. Animal Material

For this study we used 120-day-old Wistar strain rats with an average body weight of 140 grams. The rats were acclimatized for a period of one week in the animal facility before the start of the experiments.

### 2.5. Phytochemical Analysis

Phytochemical screening was based on the differential reactions (coloration and precipitation) of the main groups of chemical compounds contained in plants according to the classic method of Houghton and Raman [15] adapted to laboratory conditions [13]. This analysis included: catechic tannins, gallic tannins, flavonoids, leuco-Anthocyanins, anthocyanins, alkaloids, reducing compounds, mucilage, saponoside, cyanogenic derivatives, triterpenes, steroids, coumarins, quinone derivatives, free antracenes, c-geosides, o-heterosides and cardiotonic derivatives.

### 2.6. Preparation of Crude Extracts

The extraction of the total chemical principles was made by maceration in accordance with the traditional use of this plant.

50 g of powder was dissolved in 500 mL of distilled water. It was left with continuous stirring for 48 hours. Then the mixture obtained was filtered (3 times in a row) on absorbent cotton and the filtrate was transferred to a 1000 mL flask then subjected to evaporation until dryness at 40˚C. Using a rotavapor (Heidolph Laborota 4000 efficient) coupled to a water chiller (Julabo FL 300). The extraction was repeated twice on the same quantity of 50 g powder [13].

Finally, the dry residue obtained (macerated) was weighed and the yield was calculated according to the expression:

$$\text{Yield}(\%) = \frac{\text{Mass of dry extract}}{\text{Initial mass of powder}} \times 100 .$$

### 2.7. Acute Oral Toxicity Test

The acute oral toxicity test was performed according to the guidelines of the Organization for Economic Cooperation and Development [16]. Two batches of rats were formed, namely batch 1 (control), batch 2 for *J. multifida*. Each batch consists of three female wistar rats. Each animal of batch 1 received by force gavage and a single dose of distilled water, the animals of batch 2 received by force gavage and a single dose 2000 mg/kg of body weight of the aqueous extract of *J. multifida*. Animals were carefully observed for four (4) hours and then daily for 14 days. They were weighed and blood was collected by orbital puncture at the start of the experiment and then after 14 days [17] [18]. We performed the following blood tests: serum creatinine, ALT transaminases and hemoglobin level.

### 2.8. Antimicrobial Activity

The aqueous extract of the leaves or the sap of *Jatropha multifida* was incubated

for 24 hours at a dose of 50 mg/ml with bacterial colonies. After incubation, the bacteria were cultured to assess the effect of the extract [19].

### 2.9. Statistical Analysis

The graphs were drawn using Graghpad software. The results were expressed as mean ± 2 times the standard error of the mean (mean ± 2 SEM). Means were compared using the Mann Whitney test. The significance level was set at 5%.

## 3. Results

### 3.1. Phytochemicals from the Aqueous Extract of *Jatropha multifida* leaves

The results of the phytochemical screening carried out on the leaves are recorded in Table 1 below.

*Jatropha multifida* leaves were rich in secondary metabolites. This plant did not contain toxic chemical groups, namely cardiotonic, cyanogenic and quinonic derivatives, but there were alkaloids that can be toxic at high doses. It also did not contain anthocyanins, reducing compounds, triterpenes and steroids. In addition, it contained mucilages, saponosides and polyphenols (catechic and gallic tannins, flavonoids, leuco-anthocyanins).

Table 1. Results of phytochemical tests.

| Chemical Groups | Stainning |
|---|---|
| Catechic tannins | + |
| Gallic tannins | + |
| Flavonoids | + |
| Leuco-Anthocyanins | + |
| Anthocyanins | − |
| Alkaloids | + |
| Reducing compounds | − |
| Mucilages | + |
| Saponosides | + |
| Cyanogenic derivatives | − |
| Triterpenes | − |
| Steroids | − |
| Coumarins | − |
| Quinone derivatives | − |
| Free antracenes | − |
| C-Geosides | − |
| O-Heterosides | − |
| Cardiotonic derivatives | − |

+ = presence, − = absence.

### 3.2. Extraction Yields

The yield of the aqueous extraction of the leaves of *Jatropha multifida* was 8.22% ± 1.15%. *Jatropha multifida* was not very rich in polar compounds.

### 3.3. Safety of the Aqueous Extract of the Leaves of *Jatropha multifida*

Safety of the aqueous extract of the leaves of *Jatropha multifida*.

Figure 1 and Figure 2 presented the safety results of *Jatropha multifida* the leaves.

On days 0 (D0), the mean weight of the rats was 154 ± 11 g and increased to 174 ± 9 g on day 14 (D14), following treatment of rats with 2000 mg of *Jatropha multifida* extract/Kg/body weight. However, this increase was not statistically significant, an absence of physical disturbance of the rats.

The mean creatinine level was 10 ± 1 mg/mL on D0 and drops to 7 ± 1.1 mg/mL on D14, following treatment of rats with 2000 mg of *Jatropha multifida* extract/Kg/body weight. However, this decrease was not statistically significant, indicating an absence of disturbance of renal function.

The mean ALT transaminase level was 28 ± 7 U/L on D0 and 34 ± 7 U/L on D14, following treatment of rats with 2000 mg of *Jatropha multifida* extract/Kg/body weight. However, this increase was not statistically significant, indicating an absence of disturbance of liver function.

The hemoglobin level was 13.7 ± 0.76 g/dL on D0, and 14.6 ± 0.70 g/dL on D14, following treatment of rats with 2000 mg of *Jatropha multifida* extract/Kg/body weight. The mean hemoglobin level did not vary significantly, indicating an absence of anemia.

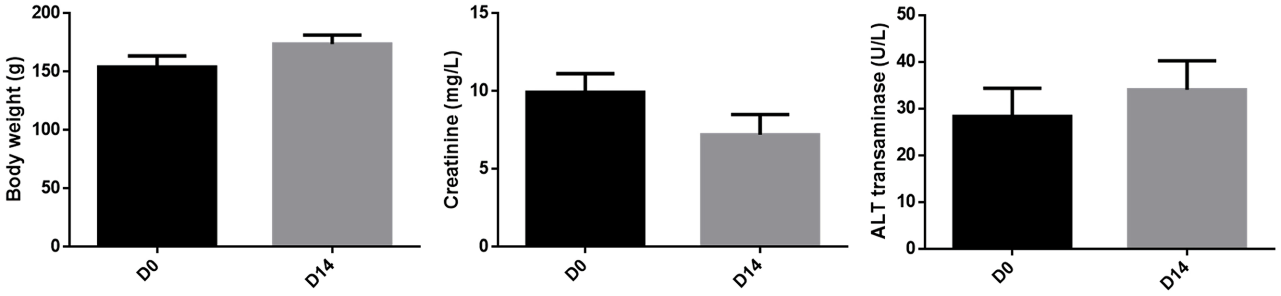


Figure 1. Weight and biochemical parameters.

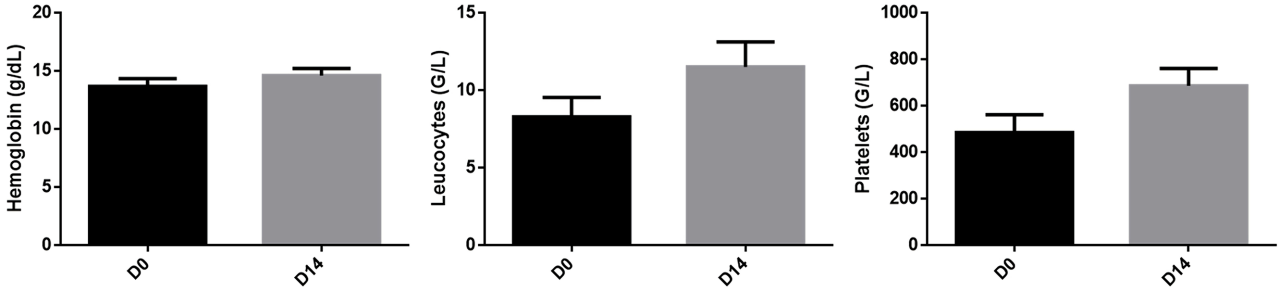


Figure 2. Hematological parameters.

The number of white blood cells was 8.3 ± 1.5 G/L on D0 and 11.5 ± 1.9 G/L on D14, following treatment of rats with 2000 mg of *Jatropha multifida* extract/Kg/body weight. The mean white blood cell number did not vary significantly, suggesting an absence of disturbance of immunity.

The mean number of blood platelets was 487 ± 88 G/L on D0 and 687 ± 88 G/L on D14, following treatment of rats with 2000 mg of *Jatropha multifida* extract/Kg/body weight. The mean platelets number did not vary significantly, suggesting an absence of disturbance of hemostasis.

For the control group, there was no variation in the parameters between D0 and D14 (data not shown).

### 3.4. Efficacy of *Jatropha multifida* Sap and Leaf Extract

Table 2 presented the antibacterial activity of the aqueous extracts of *Jatropha multifida* leaves.

On three strains of *Staphylococcus aureus*, *Jatropha multifida* leaf extract exhibited a total bactericidal effect on one strain, a partial bactericidal effect on one strain and no bacteridal effect on one strain. The extract was ineffective on other bacteria: *Streptococcus* D, *Escherichia coli*, *Klebsiella oxytoca* and *Pseudomonas aeruginosa*.

Table 3 presents the antibacterial activity of the raw sap of *Jatropha multifida.*

*Jatropha multifida* sap exhibited 100% bactericidal effect on strains of *Staphylococcus aureus* and *Streptococcus* D. It did not show bactericidal effect on strains of *Escherichia coli*, *Klebsiella oxytoca* and *Pseudomonas aeruginosa*.

## 4. Discussion

The evaluation of the antimicrobial activity of *Jatropha multifida* leaves and sap from the Benin pharmacopoeia was carried out on germs isolated from skin

Table 2. Antibacterial activities of aqueous extracts of *Jatropha multifida* leaves.

| Bacteria | Strain | Number of colonies | Bactericidal effect |
|---|---|---|---|
| ***Staphylococcus aureus*** | 1 | >100 | 0% |
| | 2 | 0 | 100% |
| | 3 | 43 | 50% |
| ***Streptocoque* D** | 1 | >100 | 0% |
| | 2 | >100 | 0% |
| ***Escherichia coli*** | 1 | >100 | 0% |
| | 2 | >100 | 0% |
| ***Klebsiella oxytoca*** | 1 | >100 | 0% |
| ***Pseudomonas aeruginosa*** | 1 | >100 | 0% |
| | 2 | >100 | 0% |
| | 3 | >100 | 0% |

**Table 3.** Antibacterial effect of the crude sap of *Jatropha multifida*.

| Bacteria | Strain | Number of colonies | Bactericidal effect |
|---|---|---|---|
| ***Staphylococcus aureus*** | 1 | 0 | 100% |
| | 2 | 0 | 100% |
| | 3 | 0 | 100% |
| ***Streptocoque D*** | 1 | 0 | 100% |
| | 2 | 0 | 100% |
| ***Escherichia coli*** | 1 | >100 | 0% |
| | 2 | >100 | 0% |
| ***Klebsiella oxytoca*** | 1 | >100 | 0% |
| ***Pseudomonas aeruginosa*** | 1 | >100 | 0% |
| | 2 | >100 | 0% |
| | 3 | >100 | 0% |

infections and abscesses. The choice of leaves and sap especially of this plant as study material was linked to their common traditional uses to treat infectious diseases [9] [10]. Antimicrobial tests were conducted on hospital germs, the most frequently isolated in the laboratory in human pathology. Phytochemical screening carried out with *Jatropha multifida* leaf powder revealed the presence of numerous secondary metabolites known to have therapeutic effects. These results were in agreement with those of [10] [20] who found the presence of tannins (gallic and catechic), flavonoids and alkaloids in the leaves of this plant. In addition to other secondary metabolites, the screening showed leuco-anthocyanins, mucilages and saponosides. This difference could be due to harvest location and handling conditions. Thus the presence of a chemical family or not within the same species differs from one region to another and could also be due to the influence of several factors such as the variation of the genetic constitution, the meteorological conditions, the part of the plant studied and the extraction method used [21]. These different secondary metabolites would be the basis of the therapeutic activity.

Antimicrobial tests shown that the sap of *Jatropha multifida* was inactive on strains of *Escherichia coli*, and active in strains of *Staphylococcus aureus*. These results were partially in agreement with those of [22] where the sap of *Jatropha multifida* completely inhibited *Staphylococcus aureus*, *Escherichia coli* and other bacteria.

These effects differed from those obtained by [23] who found that *Capsicum annuum* and *Capsicum frutescens* inhibit the proliferation of *Escherichia coli*. *Jatropha multifida* sap also inhibited the growth of strains of *Streptococcus* D, this opened up a great opportunity for the treatment of infections due to this germ.

For the extract of *Jatropha multifida* leaves, antimicrobial tests shown that it was inactive against strains of *Escherichia coli*, *Klebsiella oxytoca* and *Pseudo-*

*monas aeruginosa*. But on the other hand, the extract inhibited the growth of two strains of *Staphylococcus aureus*, one totally and the other partially. The [24] during their studies found that the aqueous extracts of the leaves of *Jatropha multifida* were active in *Escherichia coli* and on *Staphylococcus aureus*. A study on the leaves, stems, roots of *Jatropha multifida* disagrees with our results in relation to *Escherichia coli* [10] [12].

The extracts tested were active in Gram-positive microorganisms and inactive on Gram-negative ones. Previous studies shown that Gram-positive bacteria were generally the most sensitive to plant extracts [10] [25]. This could be explained by the difference in the composition of the cell wall of the bacteria. Indeed, Gram-positive bacteria wall were rich in peptidoglycan favoring the penetration of phytomolecules which could therefore reach their intracellular targets more easily. In contrast, Gram-negative bacteria with their outer membrane composed of phospholipids which would interfere with phytomolecules, makes their passage through the wall difficult, especially hydrophilic compounds [10] [26]. The sap extract exhibit a very high bactericidal effect on *Streptococcus* D while the leaves had no effect. This difference in activity observed between these two organs of *Jatropha multifida* could be explained by their variation in the secondary metabolite they contained.

Regarding the toxicological approach, no deaths were observed during the acute toxicity test. In fact, the tests shown a non-significant increase in weight of treated rats. These results contrasted those of [10] which found a weight loss. In addition, serum creatinine, serum ALT transaminase, hemoglobin, blood platelet and white blood cell counts did not vary significantly between the start and end of the experiment during acute oral toxicity. Thus, the extract of *Jatropha multifida* leaves was not toxic to the liver and kidneys, and did not create immune or thrombocytic damage. This would be explained by the presence of secondary metabolites which offers protection to these different organs.

Similar observations were made by other authors on *Psorospermum febrifugum* and *Cocos nucifera* whichweare anti-anemic plants [27] [28].

The sap showed more bactericidal activity than the aqueous leaf extract. We did not study the toxicity of the sap, which constitutes one of the limits of this work. As a perspective, it would be interesting to continue with acute and chronic toxicity tests on the sap.

## 5. Conclusion

The leaves and sap of *Jatropha multifida* showed antibacterial activity. Leaf extracts and sap of *Jatropha multifida* were active against strains of *Staphylococcus aureus*. In addition, the sap was active in strains of Stre*ptococcus* D. These results demonstrated the antimicrobial potential of the leaves and sap of *Jatropha multifida* and might justify the plant use in traditional medicine with the many secondary metabolites found in the leaves. The leaves were not acutely toxic.

In perspective, the study will be continued by testing the extract and the sap of

other bacteria and the chronic toxicity test.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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