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ORIGINAL ARTICLE

Antimicrobial, Anti-inflammatory and Antioxidant Activities of *Jatropha multifida* L. (Euphorbiaceae)

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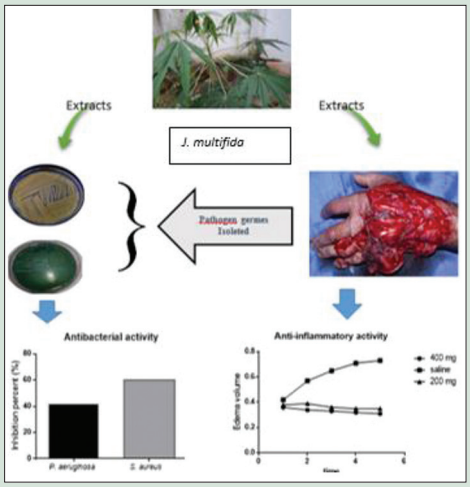
ABSTRACT

Background: *Jatropha multifida* is used in Togolease folk medicine for the healing of chronic wounds. **Objective:** This study aims to investigate antibacterial, anti-inflammatory and antioxidant activities of the leaves ethanolic extract. **Materials and Methods:** The antimicrobial activity was assayed by National Committee for Clinical Laboratory Standards broth microdilution method on strains of *Staphylococcus aureus* and *Pseudomoas aeruginosa* isolated from wounds, whereas the anti-inflammatory activity was performed by carrageenan and histamine induced paw edema method in rat modele. The 2, 2-diphenyl-1picrylhydrazyl (DPPH) free radical scavenging and ferric reducing antioxidant power (FRAP) were used for the antioxidant activity. **Results:** The antibacterial assay showed an *in vitro* growth inhibition of *P. aeruginosa* and *S. aureus* in dose-dependent manner, with minimum inhibitory concentration values ranging from 2.5 to 3.12 mg/mL for *S. aureus* and from 6.25 to 12.5 mg/mL for *P. aeruginosa*. The maximum paw anti-inflammatory effect occurred after 3 and 5 h administration of histamine and carrageenan, respectively. The DPPH radical scavenging and the FRAP assays yielded weak antioxidant activity. **Conclusion:** *J. multifida* possesses antibacterial and anti-inflammatory activities that could justify the use of the plant for the treatment of wounds in the folk medicine.

Key words: Antibacterial, Anti-inflammatory, Antioxidant, *Jatropha multifida*, wound

SUMMARY

- Antibacterial on germs isolated from wound, anti-inflammatory and antioxidant activities of *Jatropha multifida* were assayed by NCCLS broth method, carrageenan and histamine, DPPH and FRAP respectively. The results indicated that *Jatropha multifida* possesses antibacterial and anti-infl ammatory and weak antioxidant activities that could justify its use for the treatment of wounds in the folk medicine.



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INTRODUCTION

The increasing prevalence of multidrug resistant strains of pathogen microorganisms constitutes an important and growing threat to the public health.[1] Uncontrolled use and availability of effective antimicrobial agents against bacterial infections are often the causes of those resistances.[2] The current spread of multi-resistant bacteria isolated from wounds has further reinforced the need to find plants that inhibit the growth of germs involved.[3] The germs most frequently isolated from wounds are *Pseudomonas aeruginosa* and *Staphylococcus aureus*.[4] Chronic wounds seem to be arrested in a stage dominated by inflammatory process in which, there is continuing influx of polymorphonuclear leukocytes (PMNLS). The activated PMNLS release cytolytic enzymes, free oxygen radicals and inflammatory mediators that cause extensive collateral damages to the host tissues. The presence of bacteria is most likely to influence this imbalance.[5] Medicinal plants have become the focus of intense study in terms to constitute alternatives matching antimicrobial resistance challenge. Indeed, herbal remedies are used in the treatment of many diseases either by habit or for economic reasons, or following failure of conventional medicine against certain chronic diseases.[6] The plant kingdom is a source of a vast array of natural products that have been exploited as medicaments for a variety of disease conditions, because of many interest bioactive compounds.[7] Many pharmacological studies have been conducted to investigate the properties of *Jatropha multifida* multi-purpose medicinal agent. The leaves, the latex and the fruits of this plant are used in the treatment of infected wounds, skin infection

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and as a cicatrizing, ulcers, oral thrush, constipation and fever.[8] Roots and stem contained lathyrane (diterpenoid), responsible of anticancer, cytotoxic, antitumor, antimalarial, antileshmanial, antimicrobial, insecticidal and molluscidal activities of *J. multifida*.[9] Considering the vast potentiality of *J. multifida* to cure ailments, much work has been done on the root and the stem. We undertook this study to evaluate the antimicrobial, anti-inflammatory and antioxidant activities of crude extracts of this plant.

MATERIALS AND METHODS

Plant materials and extracts

The leaves of *J. multifida* L. (*Euphorbiaceae*) were harvested at Agomé-Séva: Approximately, 100 km in the north of Lomé, in March 2013. Authentication was performed at the Department of Botanic and Plant Ecology of the University of Lomé where voucher specimen was deposited (voucher number: Togo 12737).

Two hundred grams of fresh leaves of *J. multifida* were ground and added to 1 L ethanol. The mixture was set under continuous stirring for 48 h. The solution was decanted and then filtered through Whatman filter paper No.1 before being evaporated to dryness with an evaporator (Buchi). The dry extracts were then frozen and lyophilized.

Culture media and reagents

The following medium: Nutrient agar, Mueller-Hinton (agar and broth) and antibiotics discs were from Oxoid (Basingstoke, Hampshire, England). Folin-Ciocalteu reagent, quercetin, 2, 2-diphenyl-1picrylhydrazyl (DPPH), 2, 4, 6- tripyridyl-s-trazine (TPTZ) and gallic acid were obtained from Sigma Chemical (Saint-Louis, USA). All the other chemicals were analytical grade.

Antioxidant activity

DPPH assay

The antioxidant activity was assessed using the 2, 2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging with quercetin as standard.[9] To perform each test, 250 µL of the extracts or standard (50–100 mg/mL) dissolved in methanol were added to 1500 µL of 100 µmol/mL methanolic DPPH.[10] A blank was constituted with methanol. The mixtures were then incubated at room temperature for 30 min and the absorbance was determined at 517 nm using spectrophotometer Thermo Scientic Genesys 10S UV-VIS. The assay was conducted in triplicate. The lowest absorbance represented the highest DPPH scavenging activity (SA). This effect was expressed as a percentage (%) calculated as follows:

$$SA(\%) = \frac{Ac - Ae}{Ac} \times 100$$

Where Ac was the absorbance of the control at 517 nm and Ae was the absorbance of the extracts at 517 nm. IC50 value was the concentration of the sample required to scavenge 50% DPPH free radicals during the incubation period. This was determined using linear regression analysis.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was performed according to Nair *et al.*[11] The blank was FRAP solution daily prepared as follow: 25 mL of acetate buffer at 300 mmol/L, 2.5 mL of TPTZ at 10 mmol/L in 40 mmol/L HCL and 2.5 mL of $FeCl_3.6H_2O$ at 20 mmol/L. Ten microliters of plant extracts (12.5–100 mg) in 30 µL of distilled water were mixed with the FRAP solution. The mixture was incubated in the dark for 10 min. Different concentrations of $FeSO_4.7H_2O$ in methanol were used as standard. Absorbance was read at 593 nm. The FRAP activity was calculated as follow:

$$FA(\%) = (1 - \frac{Ac - Ae}{Ac}) \times 100$$

Where Ac was the absorbance of the control and Ae was the absorbance of the extracts at 593 nm. FA (%) was the inhibition percentage.

Determination of total phenolic content

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu procedure with gallic acid as standard.[12] A mixture of 10 mg of the lyophilized extracts dissolved in 10 mL of methanol to obtain the most concentrated solution (1 mg/mL) and 750 mL of Folin-Ciocalteu reagent were diluted 10 times in distilled water. The two solutions were allowed to stand for 5 min before to add 750 µL of Na_2CO_3 (60%). After 90 min of incubation at room temperature in the dark, the absorbance against the prepared reagent blank was determined at 765 nm. The phenolic content was calculated as gallic acid equivalents (GAE)/g on the basis of standard curve of gallic acid.

Isolation of bacteria and antibacterial activity

Strains of *P. aeruginosa* and *S. aureus* have been isolated from wounds and pus at "Hospital Sylvanus Olympio" and "Institut National d'Hygiène" of Lomé (Togo) according to National Committee for Clinical Laboratory Standard (NCCLS).[13] *P. aeruginosa* and *S. aureus* were identified using standard microbiological methods.[14] The following parameters were taken into account: Gram stain, colonial morphology, motility, carbohydrate fermentation tests, nitrate reduction, catalase, hydrogen sulfide and indole production, coagulase, hemolysis on blood agar and eosin-methylene blue agar. Forty-five multidrug resistant strains of *P. aeruginosa* and *S. aureus* were isolated.[15]

Antibacterial potencies of the hydroethanolic extracts of *J. multifida* was assessed *in vitro* by determining the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs). Tests were performed in 96-wells plates using the NCCLS.[13] Twenty-four hours colonies were used to inoculate broth Mueller-Hinton. Suspensions were adjusted to 0.5 McFarland standard and subjected to dilution in Mueller-Hinton broth to get about 10^3 CFU/well. Negative control wells consisted of extracts and medium while positive ones are made of medium and bacteria only. Subcultures were made from clear wells, which did not show any growth on nutrient agar to appreciate MBC. Thus, the lowest concentration that yielded no growth after the subculturing was taken as the MBC.

Anti-inflammatory activity

Anti-inflammatory activity was performed by carrageenan and histamine-induced paw edema method in a rat model.[16]

Carrageenan-induced paw edema in rats

Adult Wistar rats of both sexes weighing 130–200 g were randomly allocated into four groups of six. Each group was constituted 3 males and 3 females. The first and second groups received by oral route respectively 200 and 400 mg/kg body weight of crude leaves extracts of *J. multifida*; the third group was given isotonic saline (NaCl 9%) and the last group aspirin 1% mL/kg (control). Thirty minutes after the extracts administration, 0.1 mL of 1%W/V carrageenan freshly prepared in isotonic saline was injected into the sub plantar of left hind paw of rat. The paw volumes were then measured by the dislocation of water column in a plethsmometer at 0, 1, 2, 3, 4 and 5 h after carrageenan injection. The anti-inflammatory activity was calculated by using the formula below:

$$I(\%) = 100(\frac{Vt\ control - Vt\ extract}{Vt\ control}) \times 100$$

Vt control was the volume of the control, and Vt extracts were the volume of the extracts while I (%) correspondent to the inflammation inhibition percentage.

Histamine-induced paw edema in rats

Four groups of five Wistar adult rats were constituted. Carrageenan was replaced by 0.1% of freshly prepared histamine solution. Paw edema was produced by sub-plantar administration of 0.1% of freshly prepared solution of histamine into the left hind paw of rats. The animals were pretreated with vehicle (normal saline)/extracts/positive control. The paw volumes were measured after 0, 1, 2 and 3 h. The experiment was carried out in triplicate. All the drugs were administered orally. Aspirin constituted reference standard anti-inflammatory drug.

Statistical analysis

Results were expressed as the means ± standard deviation of three replicates. Data were subjected to different statistical analysis using SYSTAT 11 (software for Windows, 2004). Graphics were performed using Graphpad Prism version 5.00 (Graphpad Software, San Diego, USA).

RESULTS

Carrageenan and histamine-induced paw edema

The maximum paw anti-inflammatory effect occurred 3 and 5 h after administration of histamine and carrageenan, respectively [Tables 1 and 2]. *J. multifida* showed varying degrees of edema inhibition. The results indicated in carrageenan-induced inflammation that the hydroethanolic extracts showed significant anti-edema activity in dose-dependent manner from the 2nd to the 5th h for the both doses (200 mg/kg and 400 mg/kg). Their corresponding percentages inhibition were 31.57%; 52.05% and 40.35%; 53.42% at dose 200 mg/kg and 400 mg/kg, respectively. The significant inhibition began at the 2nd h for 200 mg/kg, and at the first for 400 mg/kg in histamine-induced paw edema. The values recorded were 16.66% and 30.61%. At 3 h, these values were 30.61% and 74.96%, respectively for the two doses.

Antibacterial activity of *Jatropha multifida*

MICs and MBCs of the tested bacteria were recorded in Figures 1 and 2. *J. multifida* exhibited antibacterial activity against the tested strains. Varying degrees of inhibition were observed. MICs range from 2.5 to 3.12 µg/mL for 15 strains and from 6.25 to 12.5 µg/mL for 22 strains of *S. aureus*. Relative to *P. aeruginosa* strains, the MICs were 6.25–12.5 µg/mL for 26 strains and 3.12 µg/mL for 8 strains. MBCs values were 3.12 for 9 strains and 12.5 µg/mL for 28 strains of *S. aureus*. Compared to *P. aeruginosa*, MBCs were 6.25; 6.50 and 50 µg/mL, respectively for 14; 8 and 12 strains. Extracts showed the bactericidal effect on 39 (86.67%) and bacteriostatic effect on 6 (13.33%) strains of *S. aureus*. Concerning *P. aeruginosa,* extracts exert a bactericidal effect on 14 (31.11%) and bacteriostatic effect on 20 (44.44%) strains [Table 3].

Phytochemical contents and antioxidant activity

Total phenolic content (TPC) value of the plant was 1.09 ± 0.26 mg GAE/g. *J. multifida* yielded a weak DPPH free radical scavenging activity. The fifty percent inhibition concentration of the plant extracts was IC50% =43.23 ± 8.079 µg/mL while the ferric reduction was 112 ± 5.54 µmoles Fe^{2+}/g.

DISCUSSION

The anti-inflammatory activity of the plant could be linked to its chemical composition. In fact, sterols, flavonoids, alkaloids, and saponins are usually responsible for the anti-inflammatory activity of

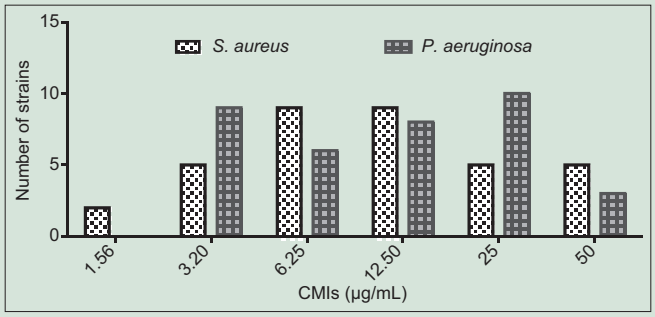

Figure 1: Antibacterial activity of *Jatropha multifida* expressed as minimal inhibitory concentrations (µg/mL). MIC: Minimum inhibitory concentration; *Jatropha multifida* extracts were tested on 45 strains of *Staphylococcus aureus* and 45 strains of *Pseudomonas aeruginosa*. MICs were determined for susceptible strains and antibacterial activity was evaluated. The highest antibacterial activity was observed on *Staphylococcus aureus* significantly when compared to *Pseudomonas aeruginosa* ($P < 0.001$)

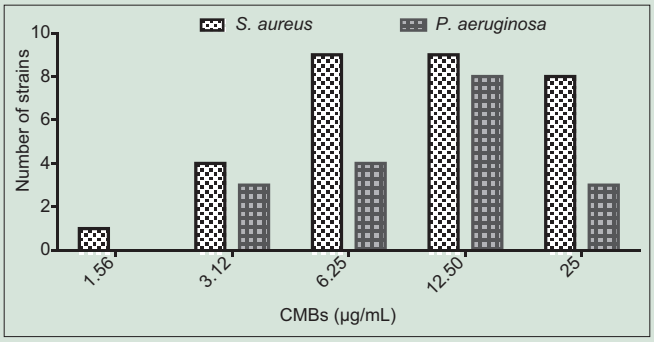

Figure 2: Minimal bactericidal concentration of *Jatropha multifida* on *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from wound (µg/mL). MBC: Minimal bactericidal concentration; *Jatropha multifida* extracts was tested on 45 strains of *Staphylococcus aureus* and 45 strains of *Pseudomonas aeruginosa*. MBC were determined for susceptible strains. The highest antibacterial activity was observed on *Staphylococcus aureus* significantly when compared to *Pseudomonas aeruginosa* ($P < 0.001$)

Table 1: Anti-inflammatory activity of *J. multifida* on carrageenan-induced paw edema

Treatment (mg/kg)	Edema volume (mL) (%)				
	1 h	2 h	3 h	4 h	5 h
Extracts 200	0.38±0.05 (9.5)	0.39±0.10 (31.57)*	0.36±0.09 (44.61)*	0.35±0.09 (50.70)*	0.35±0.11 (52.05)*
Extracts 400	0.36±0.07 (14.2)	0.34±0.08 (40.35)*	0.33±0.41 (49.23)*	0.32±0.07 (54.49)*	0.31±0.07 (53.42)*
Aspirin 100	0.31±0.04 (26.19)*	0.29±0.37 (49.12)*	0.30±0.08 (53.84)*	0.28±0.04 (60.56)*	0.26±0.27 (64.38)*
Saline	0.42±0.032	0.57±0.030	0.65±0.022	0.71±0.036	0.73±0.034

Values are mean±SD and the percentage inhibition of paw edema carrageenan-induced is given within parenthesis. From the 2nd to 5th hour, hydroethanolic extracts of *J. multifida* showed significant anti-edema activity at 200 and 400 mg/kg when compared to the control (*$P<0.05$). SD: Standard deviation; *J. multifida*: *Jatropha multifida*

Table 2: Anti-inflammatory activity of *J. multifida* on histamine-induced paw edema

Treatment (mg/kg)	Edema volume (mL) (%)		
	1 h	2 h	3 h
Extracts 200	0.36±0.16 (7.69)	0.35±0.06 (16.66)*	0.34±0.11 (30.61)*
Extracts 400	0.34±0.07 (12.82)*	0.33±0.07 (21.42)*	0.31±0.07 (74.96)*
Aspirin 100	0.29±0.061 (25.64)*	0.28±0.057 (33.33)*	0.25±0.033 (48.97)*
Saline	0.39±0.042	0.42±0.036	0.49±0.054

Values are mean±SD and the percentage inhibition of paw edema histamine-induced is given within parenthesis. From the 1st and 2nd hour, hydroethanolic extracts of *J. multifida* at doses 200 and 400 mg/kg showed, respectively, significant anti-edema activity when compared to the control (*P<0.05). SD: Standard deviation; *J. multifida*: *Jatropha multifida*

Table 3: Antibacterial effects of *J. multifida*

Strains	Susceptible strains	MICs (µg/mL)	MBC (µg/mL)	Antibacterial effects
S. aureus	8	12.5	12.5	Bactericidal
	14	6.25	12.5	Bactericidal
	9	3.12	3.12	Bactericidal
	6	2.5	12.5	Bacteriostatic
P. aeruginosa	12	12.5	50	Bacteriostatic
	14	6.25	6.25	Bactericidal
	8	3.12	6.50	Bacteriostatic

J. multifida extracts was tested on 45 strains of *S. aureus* and 45 strains of *P. aeruginosa*. MICs and MBCs were determined for susceptible strains, and antibacterial activity was evaluated. By comparing MICs, *J. multifida* demonstrated significantly the highest activity (P<0.001) on *S. aureus* than *P. aeruginosa*. Bactericidal effect if MBC/MIC ≤1 and bacteriostatic activity if MBC/MIC >2. MIC: Minimum inhibitory concentration, MBC: Minimal bactericidal concentration; *J. multifida*: *Jatropha multifida*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*

many plants.[16] Saponins are also involved in the early and final phase of the inflammatory process. They inhibit prostaglandins, preventing the triggering of the next phase.[17] Alkaloids as piperine have an inhibitory potency of the release of prostaglandin-mediated anti-inflammatory.[19] In the process of healing wounds, burns and inflammations, tannins contribute by forming a protective layer (tannin-protein/tannin-polysaccharide complex), over injured epithelial tissues permitting the healing process below to occur naturally.[19] Authors also demonstrated that tannins and flavonoids exhibit significant inhibitory activities on nitric oxide implicated in the physiological and pathological process as chronic inflammation.[20]

Aransiola *et al*.[22] using the cup diffusion on nutrient agar method, showed that the MICs of the *J. multifida* sap on *P. aeruginosa* and *S. aureus* were 66 mg/mL and 16 mg/mL, respectively.[21] Antibacterial property of this plant may be link to its phytochemicals content. *J. multifida* contains diterpenoid compounds as multifidanol and Jatrophone, which were reported to possess antibacterial activity.[8]

Tannins exhibit antibacterial effects on *S. aureus* and *P. aeruginosa*.[22] The antibacterial activity of tannins is characterized either by complexation with enzymes or bacterial substrates or by its action on the cell membrane of bacteria or either by complexation of metal ions.[23] The antibacterial activity of flavonoid results in lysis of the membrane, followed by the death of the cell.[24] The greater resistance of *P. aeruginosa* strains as those of *S. aureus* could be due to the difference between their cell wall. Gram-negative bacteria possess a highly hydrophilic outer surface of the membrane, while the lipophilic ends of lipoteichoic acids in the cell membrane of Gram-positive bacteria may facilitate penetration of the hydrophobic compounds.[25] Membrane disruption was suggested to start with insertion of the alkaloid moiety in the bilayer, followed by interactions between the sugar moieties, formation of a sterol/alkaloid matrix, chemical rearrangement, and lysis of the cell.[26]

Many plants have good antioxidant activity such as aqueous extracts of *Scophularia striata* (IC50 = 195 µg/mL) and *Toddalia asiatica* with (IC50 = 432.17 µg/mL).[27] However, the antioxidant activity of *J. multifida* is significantly higher than that of *Balanites aegyptiaca* (IC50 = 3 µg/mL).[28] Antioxidant activity was based on the phytochemical compounds. In fact, flavonoids are a phenolic compound with antioxidant activity due to their ability to reduce the free radical formation and also to scavenge them.[29]

CONCLUSION

The study revealed the presence of bioactive components in the leaves of *J. multifida*. The phyto constituents of this plant may be responsible for their usefulness in the management and treatment of wounds and the other diseases. These results warrant at least in part the properties attributed to the plant.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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