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Original Research Article

Biocidal Evaluation of Ethanol Leaf Extract of *Jatropha tanjorensis* by Inhibition of Dehydrogenase Activity of *Staphylococcus aureus* and *Candida albicans*

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ABSTRACT

Assay of enzyme activity generates accurate biochemical data for evaluating microbial activities. Assay of dehydrogenase activity of test organisms eliminates underestimation of viable cells and lack of homogeneity in distribution which characterize cultural methods. In this study the antibacterial and antifungal activities of ethanol leaf extract of *Jatropha tanjorensis* (ELJT) was investigated against *Staphylococcus aureus and Candida albicans* using the inhibition of total dehydrogenase activity (DHA) method. Inhibition of DHA of the test organisms by ELJT was determined and compared to standard antibiotic (Ciprofloxacin). Total DHA was assayed using 2,3,5-triphenyltetrazolium chloride as artificial electron acceptor which was reduced to red-coloured triphenylformazan (TPF). Pure cultures of *Staphylococcus* and *Candida species* were exposed to varied concentrations of ELJT (0 – 2000 µg/ml). ELJT exhibited concentration dependent response against tested organisms. Total DHA was progressively inhibited mostly in a logistic dose-response fashion in the test organism by the extracts and standard drug. The extract and standard drug achieved 80% inhibition within the tested doses (0-2000 µg/ml). Threshold inhibitory concentrations (IC_{50}) and IC_{80} for ELJT against *S. aureus* were 102.350 ± 6.14 µg/ml, and 440.930 ±26.46 µg/ml respectively, while ciprofloxacin showed 100% inhibition at 447.911 ± 26.87 µg/ml. The IC_{50} and IC_{80} for ELJT against *C. albicans* were 26.821 ± 1.34 µg/ml, and 58.895 ± 4.12 µg/ml respectively, while ciprofloxacin showed 80% inhibition at 5.742 ± 0.40 µg/ml. These results indicate *J. tanjorensis* extract as a viable antimicrobial source, useful in complimentary management and treatment of infections caused by *S. aureus* and *C. albicans.*

Keywords: Biocidal, *Candida albicans*, Dehydrogenase, Enzyme inhibition, *Jatropha tanjorensis*, *Staphylococcus aureus*.

Introduction

Plants as a source of medicinal compounds play important role in the maintenance and management of various human health challenges. Numerous medicinal plants and the purified bioactive constituents express therapeutic activities.[1] Use of plant and plant products is the initial medical care used by Nigerians and most developing and developed countries.[2,3] The knowledge and use of plants as medicine is important for community health care and drug development.[4-7]

Antibiotics have shown to be indispensable in the treatment of microbial infections and have improved life expectancy,[8,9] reduced morbidity and mortality rate induced by bacterial infections in humans.[10] Antibiotics abuse and misuse increases antibiotic resistance by microorganisms, a major global health threat. To counteract increasing antibiotic resistance, the search for alternative sources of antimicrobial agents, such as medicinal plants is currently explored.[11]

Extracts of many plants exhibit antimicrobial activities and there are new and sophisticated techniques used in the search for additional resources of raw materials for pharmaceutical industry.[12-14] Medicinal plants exhibit antibacterial, antifungal and antiviral activities due to the content of secondary metabolites,[7,15,15.17] which either inhibit or kill pathogens with little or no adverse effect to host cells. Plant secondary metabolites express clinical value in treating resistant microbial strains[7,17,18,19] and traditionally used medicinal plant produce a variety of compounds of known therapeutic properties. Bioactive compounds of plant origin that destroy or inhibit growth of pathogens, and have little or no toxicity to host cells are candidates for developing new antimicrobial drugs. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogen.

Jatropha tanjorensis (Figure 1), is a plant from the Euphorbiaceae family, commonly called '*Ugu Oyibo*' by Igbos and '*EfoIyana-Ipaja*' by Yorubas of Southeastern and Southwestern Nigeria respectively. *J. tanjorensis* plant is known also as 'hospital too far' because of its medicinal values.[20] It is a rich source of antioxidant nutrients including phosphorus, selenium, zinc, vitamin C and vitamin E. It exhibits antioxidant, anti-inflammatory, analgesic, antidiarrheal, and antibacterial properties. *J. tanjorensis* leaf extract has been shown to possess a wide spectrum of bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycosides, antraquinones and saponins.[21,22] Plant alkaloids and their synthetic derivatives are important basic medicinal agent due to its antimicrobial properties.[23,24]

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Figure 1: *Jatropha tanjorensis* plant, picture taken by C.O. Ujowundu

Phyto-constituents, such as phenols, flavonoids, alkaloids, tannins, and terpenoids have been explored and reported to be useful antimicrobials.[25,26,27,28]

This study determined the antibacterial activity of ethanol extract of *Jatropha tanjorensis* against *Staphylococcus aureus* and *Candida albicans* by assay of inhibition of total dehydrogenase activity (DHA) with a view to provide and/or discover newer, safer, alternative, inexpensive, simple and effective method of preventing, controlling and treating bacteria and fungi infections.

Materials and Methods

Chemicals/Reagents

The electron acceptor 2,3,5-Triphenyl Tetrazolium Chloride was obtained from BDH-England, nutrient agar, nutrient broth (TM media, India), butanol (JHD, China); while Ciprofloxacin tablets (Ciprogem) (Gemini Pharmaceuticals Nigeria Ltd.) were purchased at Right-Care Pharmacy, Owerri, Imo State, Nigeria. All reagents and media used were of analytical grade.

Microorganism

Microorganisms used for the study were obtained from the National Institute for Pharmaceutical Research and Development Abuja, Nigeria. The microorganisms and their identification numbers were: *Staphylococcus aureus* with ATCC number 22018 and *Candida albicans* with ATCC number 25932.

Plant sample collection

Fresh aerial leaves of *Jatropha tanjorensis* were collected in April 2018, from Ihiagwa village in Owerri West Local Government Area of Imo State, Nigeria. The plants were authenticated by a plant taxonomist at the School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology Owerri. Voucher specimen (FUTO/BCH/0046) was retained at the authors' laboratory.

Preparation of extract

Ethanol extraction was carried out by the modified method of Ujowundu.[29] Fresh leaves of *J. tanjorensis* were sorted, washed and air-dried to constant weight at room temperature (28±2°C) for 21 days. This was homogenized to fine powder using a mechanical grinder and stored in airtight containers at room temperature. Some portions (200 g) were macerated in 1.0 litre of 80% ethanol; this was intermittently shaken for active component extraction for 72 hours. The extract was filtered using muslin clothe and further passed through Whatman No.1 filter paper to obtain ethanol extract. The extract was concentrated by evaporation in a water bath at 49°C to obtain an ethanol-free extract slurry; this was stored in a refrigerator at <4.0°C and used for antimicrobial studies.

Preparation of test organisms

Pathogenic bacteria, *Staphylococcus* sp and *Candida sp* were isolated from high vaginal swab of patients on admission at the Federal Medical Centre Owerri, Imo State, Nigeria. Isolates were purified on nutrient agar (Fluka) plates and characterizations and identification followed the method of Holt et al.[30] The isolated *S. aureus* and *C. albicans* were subjected to modified processes.[29,31] Briefly; the microbes were purified on nutrient agar plates; and grown to mid exponential phase in nutrient broth (Lab M) on a rotatory incubator (Marrienfeld, Germany) at room temperature (28±2°C) and 150 rpm. Furthermore, the setup was centrifuged for 10 min at 400 rpm to harvest the cells, which were washed three times in deionised distilled water. Finally, the washed cells were re-suspended in distilled water and turbidity adjusted to an optical density of 0.1 at 540 nm. A portion (0.1 ml) of the cell suspension was used as inoculum in the dehydrogenase assay.

Total dehydrogenase activity (DHA) assay

Total dehydrogenase assay method as described by Alisi *et al.*[31] was employed to determine the antimicrobial activity of ethanol leave extract of *J. tanjorensis*. Briefly, total dehydrogenase activity was assayed using 2,3,5-triphenyltetrazolium chloride (TTC) (BDH England) as the artificial electron acceptor, which was reduced to the red-coloured triphenyl-formazan (TPF). The dehydrogenase activity of the microbial community was carried out in a 4 ml volumes of nutrient broth-glucose-TTC medium supplemented with varying concentrations (0, 10, 20, 50, 80, 100, 200, 500, 1000 and 2000 µg/ml) of extract in separate 20 ml screw-capped test tubes. Portions (0.1 ml) of *S. aureus* and *C. albicans* suspensions were inoculated into triplicate glass tubes containing 2.5 ml phosphate-buffered (pH 6.8) nutrient broth-glucose medium amended with *J. tanjorensis* and Ciprofloxacin and pre-incubated on a rotary incubator (150 rpm) at room temperature (28 ± 2°C) for 30 min. Thereafter, 0.1 ml of 0.1 % (w/v) TTC in deionised distilled water was added to each tube to obtain final extract concentrations of 0, 10, 20, 50, 80, 100, 200, 500, 1000 and 2000 µg/ml in different test tubes. The final concentrations of nutrient broth, glucose and TTC in the medium were 2, 2 and 0.25 mg/ml, respectively. The controls consisted of isolates and media without *J. tanjorensis* extract and Ciprofloxacin. The reaction mixtures were further incubated statically at room temperature (28 ± 2°C) for 8 h. The TPF produced were extracted in 4 ml of butanol and using spectrophotometer at 500 nm.

Data Analysis

Percentage inhibitions of total dehydrogenase activity of the isolates were calculated relative to control using equation 1 as described by Nweke *et al.*[32] The inhibition responses were generated as mean and their standard deviations from triplicate determinations.

$$\text{Inhibition of DHA (\%)} = 100 - \left(\frac{\text{Absorbance of test}}{\text{Absorbance of control}}\right) \times 100 \quad (1)$$

The dose response data of *J. tanjorensis* extract and ciprofloxacin were plotted and fitted into 4-parameter logistic dose response model (equation 2) and sigmoidal model (equation 3).

$$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^d} \quad (2)$$

Where x represents the concentration of the fraction, a and b are the minimum and maximum response of negative control respectively, c represents the IC_{50}, d is a constant that determines the slope at IC_{50}.

$$y = y_0 + \frac{a}{\left(1 + exp - \left(\frac{x - x_0}{b}\right)\right)} \quad (3)$$

Where x is the concentration of the extract, b is the IC_{50}, the concentration of extract that caused a 50 % inhibition, yo is the minimum [Y], a is the maximum response of untreated control, x_0 is the parameter determining the relative slope at IC_{50}.

Results and Discussion

The present study assayed the inhibition of total dehydrogenase activity of *S. aureus* and *C. albicans* by ethanol extract of *J. tanjorensis*. Assessment of inhibition of total dehydrogenase activity is applied as marker of antimicrobial potential and ecotoxicological

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effects of environmental substrates.[17,29,32,33,34] Furthermore, inhibition of total dehydrogenase activity is a significant bio-indicator for evaluation of the magnitude of microbial metabolism.[35,36] Since the viability of microorganisms can be represented by the total dehydrogenase activity, therefore, observed reduction in total dehydrogenase activity indicates bactericidal effect of plant extract on microbial activities.

Inhibition curve of J. tanjorensis extract and ciprofloxacin to total dehydrogenase activity of S. aureus

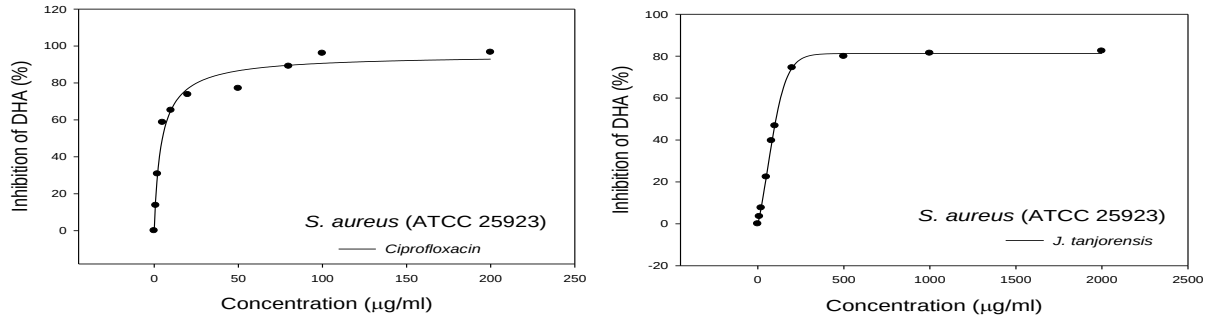
The result presented in Figure 2 shows the inhibition response curve of *J. tanjorensis* extract and ciprofloxacin to total dehydrogenase activity of *S. aureus*. Ciprofloxacin rapidly inhibited the isolate at low concentration and achieved complete inhibition of the isolate above 100 µg/ml but, *J. tanjorensis* extract achieved complete inhibition above 500 µg/ml. The inhibition of total dehydrogenase activity of *S. aureus* by *J. tanjorensis* extract closely fitted into a sigmoidal model (Sigmoid a,b,c,d); and was significantly (*P<0.05*) dose dependent. Whereas, the inhibition of *S. aureus* by ciprofloxacin strongly fitted into a logistic dose response model (LDR a,b,c,d). The threshold inhibitory concentration (IC_{50}) and (IC_{80}) value of *J. tanjorensis* extract on *S. aureus* were 102.350 ± 6.14 µg/ml and 440.930 ± 26.46 µg/ml respectively. Whereas, the IC_{50} and IC_{100} value of ciprofloxacin on *S. aureus* were 2.14 ± 0.13 µg/ml and 447.91 ± 26.87 µg/ml respectively. Comparatively, ciprofloxacin exhibited a higher inhibitory effect on *S. aureus* than the extract of *J. tanjorensis.*

The evaluation of low dose effect showed 5% inhibition of total dehydrogenase activity of *S. aureus* at 20.52 ± 1.03 *µ*g/ml. Also, the IC_{50} values showed that 50% of *S. aureus* total dehydrogenase activities were inhibited by 102.35 ± 6.14 *µ*g/ml of ethanol extract of *J. tanjorensis*. This trend of inhibition was consistent at higher doses of the extract, with 80% inhibition of *S. aureus* isolates achieved at 440.93 ± 26.46 *µ*g/ml of ethanol extract of *J. tanjorensis*. The inhibition of total dehydrogenase activity by *J. tanjorensis* extract was mathematically sigmoidal (Sigmoid (a,b,c,d)) for *S. aureus*.[1,17,34] However, the inhibitory trend of ciprofloxacin for *S. aureus* isolates were logistic (LDR (a,b,c,d).[34] This implies that inhibition of *S. aureus* total dehydrogenase activity at low doses were transitory, and presenting a slow change in percentage inhibition with increasing extract concentration; beyond the transition point, further increase in concentration yielded correspondingly increased inhibition. The correlation coefficient of the fitted models ranged from 0.98-0.99, showing a strong fit of the experimental data to the model.

Inhibition curve of J. tanjorensis extract and ciprofloxacin to total dehydrogenase activity of C. albicans

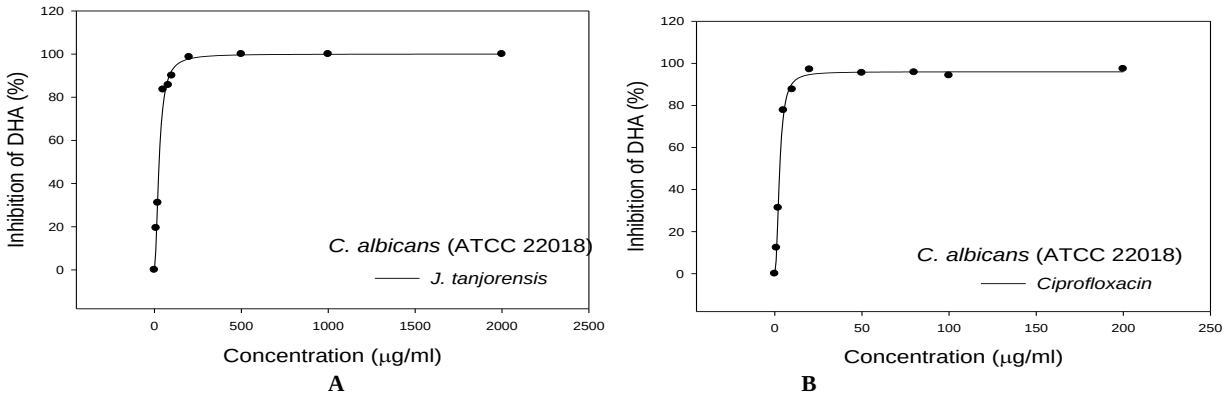
Figure 3 presents the inhibition curve of *J. tanjorensis* extract and ciprofloxacin to total dehydrogenase activity of *C. albicans*. The inhibition of total dehydrogenase activity of *C. albicans* by the extract and ciprofloxacin closely fitted into a logistic dose response model (LDR a,b,c,d), and were significantly (*P<0.05*) dose dependent. The threshold inhibitory concentration (IC_{50}) and (IC_{80}) values of the extract on *C. albicans* were 26.82 ± 1.34 µg/ml and 58.90 ± 4.12 µg/ml respectively. Whereas, ciprofloxacin presented IC_{50} and IC_{80} values on *C. albicans* of 2.81 ± 0.20 µg/ml and 5.74 ± 0.40 µg/ml respectively. Further increment in concentration of *J. tanjorensis* extract did not result in corresponding inhibition of total dehydrogenase activity. Comparatively, ciprofloxacin exhibited a higher inhibitory effect on *C. albicans* (10 times more effective) than the extract of *J. tanjorensis.*

Furthermore, the low dose effect indicated 5% inhibition of total dehydrogenase activity of *C. albicans* at 5.09 ± 0.20 *µ*g/ml. Similarly, the IC_{50} value shows that 50% of *C. albicans* total dehydrogenase activities were inhibited by 26.82 ± 1.34 *µ*g/ml of ethanol extract of *J. tanjorensis*. This observed inhibition trend was consistent when higher doses of the extract were applied. Furthermore, 80% inhibition of *C. albicans* activities were achieved at 58.90 ± 4.12 *µ*g/ml of ethanol extract of *J. tanjorensis*. The inhibition of total dehydrogenase activity by *J. tanjorensis* extract for *C. albicans*, fitted into a logistic dose response model LDR (a,b,c,d).[17,34] Whereas, the inhibitory trend of ciprofloxacin for *C. albicans* was logistic (LDR (a,b,c,d).[34]



A B

Figure 2: Inhibition curve of ciprofloxacin (A) and *J. tanjorensis* extract (B) to total dehydrogenase activity of *S. aureus*



A **B**

Figure 3: Inhibition curve of *J. tanjorensis* extract (A) and ciprofloxacin (B) to total dehydrogenase activity of *C. albicans*

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Table 1: Threshold inhibitory concentration (IC) and toxicity response model of *S. aureus* and *C. albicans* to *J. tanjorensis* extract and ciprofloxacin

Organism	Extract/	Threshold Inhibitory Concentration (µg/ml)									R^2	Inhibition response
	Standard	5	10	20	40	50	60	70	80	100		model
S. aureus	*J. tanjorensis*	20.52 ± 1.03	30.19 ± 1.51	46.26 ± 2.31	79.88 ±4.79	102.35 ± 6.14	134.90 ±8.09	195.02 ± 11.70	440.93 ± 26.46	ND	0.99	Sigmoid (a,b,c,d)
	Ciprofloxacin	2.00 ± 0.12	2.00 ± 0.10	2.00 ± 0.16	2.02 ±0.06	2.14 ±0.13	2.78 ± 0.17	5.88 ± 0.35	20.08 ±1.20	447.91 ± 26.87	0.98	LDR (a,b,c,d)
C. albicans	*J. tanjorensis*	5.09 ± 0.20	7.76 ± 0.31	12.26 ± 0.49	21.33 ±1.07	26.82 ±1.34	33.74 ± 1.69	43.34 ± 3.03	58.90 ± 4.12	ND	0.99	LDR (a,b,c,d)
	Ciprofloxacin	0.69 ± 0.02	0.99 ± 0.03	1.44 ± 0.12	2.31 ±0.18	2.81 ±0.20	3.43 ± 0.17	4.30 ± 0.34	5.74 ± 0.40	ND	0.99	LDR (a,b,c,d)

Also, results from this study indicate that the extracts of *J. tanjorensis* did not achieve 100 % inhibition of total dehydrogenase activity on both isolates using the Sigmoidal model (Sigmoid a, b, c, d) and logistic dose response model (LDR a, b, c, d). The inability of the model to describe a complete inhibition of total dehydrogenase activity may be attributed to a saturation effect of the extract on the isolates, resulting in no net change in dehydrogenase activity beyond a threshold concentration. Comparatively, the extract of *J. tanjorensis* was more effective against *C. albicans* than *S. aureus* at low and higher doses of the extract. This implies that, ethanol leaf extract of *J. tanjorensis* when used may be highly potent in the treatment of infections caused by *C. albicans* than *S. aureus* even at low concentrations.

Threshold inhibitory concentration (IC) and toxicity response model

Table 1 presents the threshold inhibitory concentration (IC) and inhibition response model of *S. aureus* and *C. albicans* to *J. tanjorensis* extract and ciprofloxacin. The $IC_{5,}$ $IC_{10,}$ $IC_{20,}$ $IC_{40,}$ $IC_{50,}$ $IC_{60,}$ $IC_{70,}$ IC_{80} and IC_{100} values are shown on Table 1. However, the IC_{100} value was non-determinable for the extract against the two isolates and for ciprofloxacin against *C. albicans*. The inhibition of total dehydrogenase activity of the isolates were largely logistic and followed the mathematical model LDR (a,b,c,d), with the exception of *J. tanjorensis* inhibition of *S. aureus* which was sigmoidal (Sigmoid (a,b,c,d)). The extract, and ciprofloxacin significantly ($P<0.05$) inhibited the total dehydrogenase activity of *S. aureus* and *C. albicans*. The correlation coefficient of the fitted models ranged from 0.98-0.99.

Although, the extract showed promising antimicrobial effect, the antimicrobial properties of ciprofloxacin was mostly 10 times greater than the ethanol leaf extract of *J. tanjorensis* at each threshold inhibition level. These findings were consistent with previous reports on antimicrobial property of *J. tanjorensis* against some pathogenic gram-positive, gram-negative bacteria and fungi using agar-well diffusion and disk diffusion methods.[37,38,39,40] However, the inhibition of total dehydrogenase activity method was preferred over culture method for enumeration of microorganisms which can underestimate the number of viable cells due to lack of homogeneity in distribution or difficulty in being readily desorbed from the substrate matrix.[41] The medicinal properties of all ethno-medicinal plants have been associated with the phytochemicals constituents.[1,21]The leaf extracts of *J. tanjorensis* having been shown to possess a wide spectrum of bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycosides, antraquinones and saponins,[21,22] indicate that the phytochemicals of ethanol leaf extract of *J. tanjorensis* was potentially responsible for the observed antimicrobial activity in the present study.

Conclusion

This study showed that the ethanol leaf extracts of *J. tanjorensis* significantly inhibited total dehydrogenase activity of *S. aureus* and *C. albicans*, and the inhibition were concentration dependent. These indicate that, ethanol leaf extract of *Jatropha tanjorensis* possesses bioactive compounds with promising antimicrobial effect against *C. albicans* and *S. aureus* isolates. It has also provided evidence for the traditional use of the plant in the management of infections and has shown promise in complementing conventionally used antibiotics.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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