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Phytochemical Screening and Antimicrobial Activity of Leaf Extract of *Jatropha curcas*

M. O. Oyama[1], O. I. Malachi[1*] and A. A. Oladejo[1]

[1]Afe Babalola University, Ado-Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author MOO designed the study and wrote the protocol. Author OIM performed the statistical analysis and wrote the first draft of the manuscript. Author AAO did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The study investigated the antibacterial activity of *Jatropha curcas* using aqueous, methanol and ethanolic extracts that were obtained from the leaf against growth inhibition of *Escherichia coli, Staphylococcus aureus, Proteus spp, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* Tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides were the phytochemicals present. All test microorganisms were significantly inhibited by the leaf extracts but the presence of oxalates and cyanogenic glycosides in the leaf could present potential toxicity and therefore reduces the application of this plant to topical but not systemic use. Clearly, additional pharmacology and safety studies are warranted to investigate clinical potential of *Jatropha curcas* as a topical antibiotic for use against susceptible pathogens.

Keywords: Jatropha curcas; phytochemical; antimicrobial.

**Corresponding author: E-mail: malachiseyi@gmail.com;*

1. INTRODUCTION

Microbial infections are major public health problems in the developed countries. Due to general use of commercial antibiotics, the relative incidence of multiple antibiotic resistances in human pathogens is not only large but also growing [1]. Intractable problems of resistance to antibiotics by microorganisms previously thought to have been brought under control have led to the resurface of interest in herbal products as source of potential compounds to suppress or possibly eradicate the ever increasing problems of emergence of newer diseases [2,3]. The World Health Organization estimated that about 80% of people still rely mainly on traditional remedies such as herbs for their medicines [4]. Most of the plants used by the rural residential areas have biologically active compounds that have been proven by generations to be potent against specific disorders [5-7]. The prevention and treatment of diseases by the use of available and accessible medicinal plants in a particular locality will continue to play important roles in medical health care implementation in the developing countries as plants make up the primary source of new pharmaceuticals and health care products [8]. Natural products are therefore gaining attention as an alternative for antimicrobial agents [9].

There have been major role played by *Jatropha curcas* in the treatment of different kinds of diseases, ranging from bacterial to fungal infections [10]. Glycosides, tannins, phytosterol, flavonoids and steroidal sapogenins are contained in different parts of the plants (leaves, fruits, latex and bark); a feature which enables it to demonstrate vast range of medicinal properties [11-15]. Also, the presence of ansovitexin, apigenin and vitexin in the leaf gives it the ability to serve as a cure for rheumatism and muscular pains [16-18]. Antibiotic activities of *Jatropha curcas* seed have been demonstrated against *Staphylococcus aureus* and *Escherichia coli* [10]. With the wealth of medicinal claims surrounding the use of *Jatropha curcas* leaves, [19] there is need for investigation of antibiotic potential of the leaves against some common pathogenic microorganisms. This research therefore investigates the growth inhibition of *E. coli, S. aureus, Proteus spp, Klebsiella pneumonia and Pseudomonas aeruginosa* by methanolic, ethanolic and aqueous leaf extracts of *Jatropha curcas.*

2. MATERIALS AND METHODS

2.1 Media Used

Nutrient media (Micro master) and MacConkey agar (Lab M).

2.2 Test Organisms

While carrying out this work, the following bacteria were used as the test organisms: *Escherichia coli, Staphylococcus aureus, Proteus spp, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* These organisms were obtained from the Microbiology Laboratory of Abia State University, Nigeria.

2.3 Leave Extracts

Fresh leaves of *Jatropha curcas* were collected from farm land in Agbani, Enugu State, Nigeria. The plant was identified and authenticated by the Department of Botany, University of Uyo, Nigeria. Voucher sample was prepared and deposited in the Herbarium for reference. The leaves of *Jatropha curcas* were allowed to air-dry at room temperature for fourteen (14) days until the leaves became brittle, and ground into fine powder in a clean mortar using a clean pestle. Twenty grams of the leaves powder were weighed and poured into three (3) conical flasks; 100ml of distilled water, 70% ethanol, and 70% methanol. Ethanol and methanol used were of analytical grade. The preparations were allowed to stand undisturbed, but with occasional agitation, for 24 hours and filtered using Whatman No. 1 filter paper. The residues were discarded and the filtrates (extracts) were divided into two portions. One portion was refrigerated at 4℃ until needed. The other was evaporated to dryness in a water bath at 100℃.

2.4 Phytochemical Analysis of the Plant Extracts

The extracts were subjected to qualitative phytochemical tests for tannins, saponins, flavonoids, alkaloids, oxalates and glycosides in accordance with Trease and Evans [20].

2.5 Antimicrobial Activity

2.5.1 Preparation of culture media

2.5.1.1 Nutrient agar preparation

The medium was prepared by suspending 2.8 g of the agar powder into a 100 ml of distilled

water, in a conical flask and shaken very well to dissolve. It was then autoclaved at 121℃ for 15mins and allowed to cool at 47℃ before pouring into plates aseptically in the required amount. The medium was allowed to solidify [21].

2.5.1.2 Macconkey agar preparation

This medium was prepared by weighing 4.8 g of agar powder into a conical flask; 100 ml of distilled water was poured into the flask. The flask was corked, shaken and allowed to soak for about 10 minutes. The medium was autoclaved at 121℃ for 15 minutes. After autoclaving, it was allowed to cool to 47℃ before pouring into the plates aseptically in the required amount and allowed to solidify [21].

2.5.2 Incubation and inoculation

The agar well diffusion method was used in the assessment of the antimicrobial activity of the plant. This method was achieved by using a sterile cork borer to make six wells on each Nutrient and MacConkey agar plates. Then 100 µl of the plant extracts at $1mgml^{-1}$ were dropped into wells and allowed to diffuse for 5 minutes. Tetracycline, at $1mgml^{-1}$, was used as control. Plates were then kept to stand in the incubator for 24hours at 37℃ [22]. Zones of inhibition were measured and recorded. The experiment was done in triplicate and the results are expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

The phytochemical screening of *Jatropha curcas* leaf, presented in Table 1, revealed the presence of tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides. These phytochemicals are biologically active and can be responsible for the antimicrobial activity of the plant. The mechanisms through which these secondary metabolites exert their antimicrobial activities differ. Tannins have been reported to form irreversible complexes with proline rich protein, [23] thereby inhibiting cell protein synthesis [24]. Intestinal disorders such as diarrhea and dysentery have been treated with herbs that have tannins as their main components [25].

Another secondary metabolite observed in the leave extract of *J. curcas* was alkaloid. Alkaloids have analgesic effects [26] and have been clinically used [27]. Alkaloids have been acclaimed for their antimicrobial activities, especially against gram negative bacteria [28]. Other secondary metabolites present in *J. curcas* whose antimicrobial activities have been documented are flavonoids and saponins [29].

Though traditionally used in the fermentation of cassava, *J. curcas* is inedible. This is due to the presence of oxalate and cyanogenic glycosides in it as presented in Table 1. Oxalate is an antinutrient and an acute toxin [30]. Cyanogenic glycosides are not just distasteful but also toxic, as they release hydrogen cyanide which generates free radicals and causes oxidative stress [31].

As presented in Table 2, all the extracts demonstrated significant inhibition against the test microorganisms. Ethanolic extract however demonstrated the greatest inhibition. This is consistent with the high level of phytochemicals in the ethanolic extract. The inhibition was most effective against *E. coli*. This is consistent with the inhibition by stem back extracts of *Jatropha curcas* [32]. The use of *J. curcas* as a medication for diarrhea may however not be feasible because of the presence of oxalate and cyanogenic glycosides in it. The extracts are also active against *Staphylococcus aureus* and *Klebsiella pneumonia*. This agrees with the report of Ekundayo and co workers [33] on the antimicrobial activities of the nut extracts of *J. curcas. Staphylococcus aureus* is the causative organism for skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning [34]. *Klebsiella pneumonia* infections are seen mostly in people with a weakened immune system. *Klebsiella* infections are often nosocomial. The most common condition caused by *Klebsiella* outside the hospital is pneumonia. [35] *Klebsiella* is also implicated in urinary tract, lower biliary tract, and surgical wound sites infections [36]. The inhibition of *S. aureus* and *K. pneumonia* by the leave extract substantiates the trado-medicinal use of *J. curcas* in wound healing, scrabies and other skin infections [10].

Other microorganisms inhibited include *Proteus spp* and *Pseudomonas aeruginosa.* Similar inhibitions by the nuts and stem bark of *J. curcas* have been reported [22,33]. *Pseudomonas aeruginosa* is a ubiquitous multi drug resistant pathogen that is associated with serious illnesses - especially nosocomial infections such as ventilator-associated pneumonia and various sepsis syndromes as well as hot-tub rash [37]. *Proteus spp* is implicated in urinary, septic and wound infections, often nosocomial [38].

Table 1. Phytochemical screening of methanolic, ethanolic and aqueous extract of *Jatropha curcas* leaves

Phytochemicals	Methanolic extract	Ethanolic extract	Aqueous extract
Saponin	-	-	+++
Tannins	-	++	+
Flavonoids	+++	+	-
Cyanogenic glycosides	+++	++	++
Alkaloids	-	+	+++
Oxalate	++	+++	-

+++Highly present, ++Moderately present, +Present, -Absent

Table 2. Antibacterial activity of *Jatropha curcas* leaves extracts using each solvent (Water, ethanol and methanol) at different concentrations

Organisms	Zone of Inhibition (mm)						
	W_1	W_2	E_1	E_2	M_1	M_2	Control
Escherichia coli	8.0±2.0	7.7±0.8	8.1±1.2	9.1±1.4	10.1±2.2	8.8±1.1	8.0±0.54.
Staphylococcus aureus	8.7±1.71	8.3±1.1	7.5±0.9	7.7±1.0	7.0±1.5	8.1±1.4	7.4±1.3
Proteus spp	6.3±0.76	8.7 ± 1.8	6.2±0.2	5.9±0.3	7.7±1.1	6.9±1.2	6.1±0.81
Klebsiella pneumonia	7.1±0.29	7.2±1.3	6.9±1.1	7.4±1.2	10.6±2.5	9.7±1.3	7.7±1.2
Pseudomonas aeruginosa	8.1±0.91	8.1±0.56	7.3±0.71	6.8±0.2	7.6±1.1	7.8±2.1	6.9±2.2

Means±Standard Deviation
W_1 =Water extract (refrigerated) 4℃
W_2 =Water extract (evaporated) 100^oC
E_1 = Ethanolic extract (refrigerated) 4℃
E_2 = Ethanolic extract (evaporated) 100℃
M_1 = Methanolic extract (refrigerated) 4℃
M_2 = Methanolic extract (evaporated) 100℃
Control = Tetracycline

However, it is opportunistic and only affects immuno-suppressed individuals [35]. The inhibition of *Proteus spp* by *Jatropha curcas,* as shown in Table 2, substantiates the reported wound healing ability of *Jatropha curcas* [39,40]. There were no significant differences between the inhibitions by the refrigerated and evaporated extracts. This demonstrates that the preservation methods have no significant effect on the antimicrobial properties of the extracts.

4. CONCLUSION

Phytochemical screening of *Jatropha curcas* leaf revealed the presence of several biological active compounds including tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides; which can be responsible for the observed antimicrobial action of the leave. The leaf demonstrated considerable inhibition zone against the test microorganisms but can only be used for topical treatment because of its inedibility.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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