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# **In-vitro Anti-bacterial Effects of Jatropha curcas on Salmonella Typhi and Salmonella Typhimurium Isolated from Presumptive Typhoid Fever Patients in Akure Metropolis, Nigeria**

**O. E. Ajayi1\*, S. I. Awala<sup>1</sup> , F. N. Okogbue<sup>1</sup> , A. G. Ogunleye<sup>1</sup> and T. O. Adeyeye<sup>1</sup>**

<sup>1</sup>Department of Microbiology, Federal University of Technology, Akure, Nigeria.

# **Authors' contributions**

This work was carried out in collaboration between all authors. Authors OEA and SIA designed the study. Author AGO performed the statistical analysis. Authors OEA and SIA wrote the protocol and first draft of the manuscript. Authors FNO and TOA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

## **Article Information**

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# **ABSTRACT**

**Aim:** The present study determined the in-vitro anti-bacterial effects of Jatropha curcas on Salmonella Typhi and Salmonella Typhimurium isolated from presumptive typhoid fever patients in Akure metropolis, Nigeria.

**Study Design:** The study evaluated the prospective use of J. curcas as an alternative to conventional drugs in the treatment of typhoid fever and gastroenteritis.

**Place and Duration of Study:** Five selected hospitals within Akure metropolis in Ondo State, Nigeria were used for the study. The study was conducted between June and September, 2015. **Methodology:** Salmonella Typhi and Salmonella Typhimurium were isolated from two hundred (200) blood samples collected from presumptive typhoid fever patients attending Federal University of Technology, Akure (FUTA) Health Centre, First Mercy, Don-Bosco, Sijuade and Skye hospitals

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\*Corresponding author: E-mail: ajayigbengaemmmanuel87@gmail.com;

in Akure, Ondo State, Nigeria using selective media. Their identities of the isolates were verified using conventional microbiological techniques. The antibacterial effect of methanolic, acetone and N-hexane extracts of the plant leaves on Salmonella Typhi and S. Typhimurium isolates were thereafter evaluated. Quantitative and qualitative phytochemical screening were performed on the leaf extracts. Antibiotics susceptibility profile of the isolates was also assessed using commercial antibiotics.

**Results:** Highest zone of inhibition (20.00±0.58 mm) was observed with the hexane extract at a concentration of 100 mg/ml, while the least  $(2.00\pm0.58$  mm) was observed with the methanolic extract at concentrations of 12.5 mg/ml and 25 mg/ml respectively for the S. Typhi isolate. Highest zone of inhibition (25.00±0.58 mm) was however observed with the hexane extract at a concentration of 100 mg/ml and the least (1.00±0.58 mm) at a concentration of 6.25 mg/ml for same isolate. S. Typhimurium was inhibited at all concentrations by the methanolic and hexane extract of J. curcas but resistant to the acetone extract. Phytochemical analysis of the extracts revealed the presence of secondary metabolites such as steroids, alkaloids and saponins.

**Conclusion:** These findings showed that J. curcas is a promising source of reliable phytotherapy in combating salmonellosis.

Keywords: In-vitro; anti-bacterial; phytochemical; inhibition; Jathropha curcas; salmonellosis.

# **1. INTRODUCTION**

In recent years, there has been a rapid upsurge in the emergence of multi-drug resistance among pathogenic bacteria due to extensive use of antibiotics [1]. To this end, the pharmaceutical industry world around is in a continuous struggle to keep up with the production of new synthetic antibiotics to combat the emerging multi-drug resistant bacterial strains. The consumption of these synthetic antimicrobials have been shown to have deleterious effects on humans, one of which is the disruption of the natural human microbiota known to play key roles in nutrition, development, metabolism, pathogen resistance and regulation of the human immune responses [2].

Therefore, there is a need for the development of new naturally sourced antimicrobial drugs to reduce the risk associated with the synthetic ones. Medicinal plants thus remain a feasible source of new compound for the drug development process. Jatropha curcas Linn. is fast becoming a very useful economic resource in phytomedicine development and development of new lead compounds [3,4].

The plant belongs to the family Euphorbiaceae, and is closely related to other important cultivated plants like rubber and castor plants. It originated from South America and Africa but has spread to other parts of the world [5]. The plant has been demonstrated to be useful in the treatment of infectious and non-infectious ailments such as gonorrhoea, dropsy, gout,

paralysis, scabies, eczema, dermatitis and rheumatoid arthritis [6].

Many parts of this plant such as leaves, stem bark and latex have been reported to exhibit antibacterial activity [7]. Over 35,000 species of the plant exist with various phytochemicals in them being used for medicinal purposes around the world. J. curcas has a long history of use in Africa [8]. In Nigeria specifically, the leaf and stem decoction is used singly or in combination with other recipes for the treatment of skin diseases, and stomach disorders. Typhoid fever is a systemic disease caused by the bacterium Salmonella enterica subspecies enterica serovar Typhi. Typhoid fever is endemic in many parts of the world, notably Asia and Africa, where it is an important cause of febrile illness in crowded, lowincome settings. People with this disease may experience mild or severe symptoms. Persons with typhoid fever usually have a sustained fever as high as  $103^\circ$  to  $104^\circ$  (39° to  $40^\circ$ C). The symptoms of typhoid fever may also include weakness, headache, stomach pains, or loss of appetite. Constipation or diarrhoea may also occur. In some cases, persons may develop a rash of flat, rose-colored spots on the trunk of the body [9]. Typhoid fever is spread by eating or drinking food or water contaminated with the feaces of an infected person [9]. Risk factors include poor sanitation and poor hygiene [10].

Based on the urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases, this study was designed to evaluate the

phytochemical properties and in vitro antibacterial activity of the extracts of J. curcas leaves on Salmonella Typhi and S. Typhimurium isolated from blood samples of presumptive typhoid fever patients attending selected hospitals in Akure metropolis, Nigeria.

# **2. MATERIALS AND METHODS**

# **2.1 Ethical Clearance**

Informed consent was sought in clinically suspected cases and approval for the study was obtained from the Ethics Committee of the Ondo State Ministry of Health. Confidentiality was maintained in accordance with standards of medical practice.

# **2.2 Collection of Blood Samples**

A total of two hundred (200) blood samples were collected from clinically suspected typhoid fever patients attending FUTA Health Centre, First Mercy, Don-Bosco, Sijuade and Skye hospitals in Akure, Ondo State, Nigeria. The blood samples were kept in an EDTA (Ethylene diamine tetra acetic acid) bottle and thereafter transferred to the laboratory for analysis.

## **2.2.1 Isolation of bacteria from blood samples**

Bacteria (Salmonella Typhi and S. Typhimurium) were isolated from the blood samples using the Brain-Heart Infusion broth. Collected blood samples (2 ml) were introduced into 20 ml of sterile brain-heart infusion broth and the mixture was gently shaken to enhance homogeneity. The samples were thereafter incubated at 37°C for 48-72 hrs. This served as inoculum for subsequent analysis.

## **2.2.2 Sub-culturing of bacteria isolates**

This was done using the Salmonella-Shigella agar. About 6.3 g of the agar was dissolved in 100ml of distilled water. It was heated briefly using a hot plate and it was allowed to cool. It was then poured into Petri dishes and allowed to gel. A loopful from the cultured plate was taken and streaked on the solidified agar. It was thereafter incubated for 24 hrs at 37°C and viewed for growth.

## **2.2.3 Identification of the bacterial isolates**

The bacterial isolates were identified using their colony morphological characteristics. The appearance of each colony on the agar media and characteristics such as shape, edge, colour, elevation and texture were observed as described by Olutiola et al. [11]. The isolates were thereafter subjected to relevant biochemical tests and identified using the taxonomic scheme<br>of Bergey's Manual of Determinative of Bergey's Manual of Determinative Bacteriology.

# **2.3 Collection of Plant Materials**

The fresh leaves of Jatropha curcas was collected from Elesare estate, along Ijare road, Akure, Ondo State, Nigeria. The plant's identity was authenticated at Crop Science and Pest Department, Federal University of Technology, Akure, using standard manuals.

## **2.3.1 Preparation of plant extracts**

Extracts were prepared as described by Harbone [12] with slight modifications. The leaves were air dried for three weeks and pulverized using an electric blender (Marlex Electrolyne IS: 250). The solvents used for the extraction were methanol, acetone and hexane. About 200 g of the powdered leaf was soaked in each solvent. Each solution was allowed to stand for 72 hours, after which it was sieved with a muslin cloth and filtered using No 1 Whatman filter paper. The filtrate was collected in a beaker and concentrated in a vacuo using rotary evaporator (Resona, Germany). The extracts were reconstituted in tween 20 (10% v/v) prior to use and sterilized with the aid of membrane filter  $(0.22 \mu m)$ .

## **2.3.2 Antibacterial assay of the plant**

The antibacterial activity of the plant leaf extract was determined using the agar well diffusion method as described by Irobi et al. [13]. Muller Hinton agar was prepared according to the manufacturer's instruction and allowed to cool to about 40-50°C. The freshly prepared and cooled media was poured into Petri dishes and allowed to solidify at room temperature. About 0.2 ml of the standardized test inoculum was evenly spread on the surface of the solidified media using a sterile swab stick. Five equidistant wells of 5 mm in diameter were then made on the seeded agar plate using a sterile cork borer and the plant extracts with concentrations ranging from 6.25-100 mg/ml were introduced into the bored holes. The plates were then incubated at 37°C for 24 hrs. The antibacterial activity was

determined by measuring the zones of inhibition around the isolates in millimetres.

## **2.3.3 Determination of minimum inhibitory concentration**

Agar well diffusion method as described by Irobi, et al. [13] was used to monitor the antibacterial effect of different concentrations of the extracts. The concentration of the extracts which ranged from 6.25-100 mg/ml was tested on the bacterial isolates. The minimum inhibitory concentration was obtained by taking the lowest concentration that did not permit any visible growth of the tested organisms.

# **2.3.4 Phytochemical screening of the leaf extract of Jatropha curcas**

The methods described by Trease and Evans [14] were used to test for the presence of saponins, tannins, phenolics and alkaloids, Lieberman Burchad reaction was used to test for steroids, while the Salkowski test was used to test for the presence of glycosides.

## 2.3.4.1 Testing for saponins

Each extract (0.5 g) was mixed with water in test tube. Foaming which persisted on warming was taken as an evidence for the presence of saponins.

## 2.3.4.2 Testing for tannins and phenolics

Each extract (0.5 g) was separately stirred with 10 mL of distilled water and then filtered. Few drops of  $5\%$  FeCl<sub>3</sub> reagent was added to the filtrate. Blue-black or blue-green colouration or precipitation was taken as an indication of the presence of phenolics and tannins.

## 2.3.4.3 Testing for alkaloids

Each extract (0.5 g) was stirred with 5 mL of 1% HCl on a steam bath. The solution obtained was filtered and one 1 mL of the filtrate was treated with a few drops of Mayer's reagent. The turbidity of the extract filtrate on addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the extracts.

## 2.3.4.4 Testing for steroids

A 0.5 g of each extract was separately added with 5 drops of acetic anhydride and then a drop of concentrated  $H_2SO_4$ . The mixture was steamed for 1 hour and neutralized with sodium hydroxide (NaOH), followed by the addition of chloroform. The appearance of a blue-green colour indicated the presence of steroid.

# 2.3.4.5 Testing for glycosides

A 0.5 g of each extract was dissolved in 2mL of chloroform. Tetraoxosulphate VI acid  $(H_2SO_4)$ was carefully added to form a lower layer. A reddish brown colour at the interface indicated the presence of a steroidal ring, that is, a glycone portion of the cardiac glycosides.

# **2.4 Determination of the Antibiotics Sensitivity Profile of the Bacterial Isolates**

The antibiotics sensitivity test was carried out in order to compare the sensitivity of the organisms to the different commercially available antibiotics. The commercial antibiotics (abtek biologicals) which included Ciprofloxacin (10 µg), Amoxycillin (25 µg), Ofloxacin (5 µg), Cotrimozazole (25 µg), Gentamycin (10 µg), Nitrofuranton (200 µg), Ceftriazone (30  $\mu$ g), Pefloxacin (5  $\mu$ g), Tetracycline (30 µg), and Augmentin (30 µg) were used. The disc diffusion method decribed by Willey et al. [15] was used to determine the effect of standard antibiotics on the bacterial isolates. Test organisms were standardized as described by [16] and sterile Petri-dishes were seeded aseptically with 1 ml each of standardized broth culture of the test organisms while about 20 ml of sterilized Muller-Hinton agar was poured aseptically on the plates. The plates were swirled carefully for even distribution of the agar and later allowed to gel. With the aid of sterile forceps, the antibiotics disc were firmly placed on solidified plates and incubated for 24 hrs at 37°C. After incubation, clear zones around the disc (which represent the zones of inhibition) were measured. Seeded agar plates without antibiotics served as the control experiment. The zones of inhibition were measured in millimetres and the experiment was carried out in triplicates.

# **2.5 Statistical Analysis**

All experiments were carried out in triplicate. Numerical data obtained were analysed using one way Analysis of Variance (ANOVA) and treatment means were compared using New Duncan's Multiple Range Test. Differences were considered significant at P<0.05.

# **3. RESULTS**

#### **3.1 Antibacterial Assay of the Plant**

The antimicrobial effects of the methanolic, acetone and hexane extracts of J. curcas on Salmonella Typhi is depicted by Table 1. At concentration 6.25 mg/ml, no zone of inhibition was observed for the methanol and acetone extracts but an inhibition zone of 12.00±0.58 mm was observed for the hexane extract. At a concentration 12.50 mg/ml however, there was no zone of inhibition for the acetone extract but methanol and hexane recorded 2.00±0.58 mm and 13.00±0.58 mm respectively. At concentration 25 mg/ml, the zones of inhibition remained the same while at 100 mg/ml, methanol extract had a 3.00±0.58 mm zone while hexane recorded 20.00±0.58 mm. For all the extracts, zone of inhibition increased as concentration increased.

Table 2 represents the activity of methanolic, acetone and N-hexane extracts respectively on Salmonella Typhimurium. At all concentrations, no zone of inhibition was observed for the acetone extract. At 6.25 mg/ml concentration, methanol recorded a 2.00±0.58 mm zone and hexane 21.00±0.58 mm. At concentration 12.50 mg/ml however, the zone of inhibition increased to 2.00±0.58 mm and 23.00±0.58 mm for the methanolic and hexane extracts respectively. Increments were Ajayi et al.; EJMP, 12(2): 1-10, 2016; Article no.EJMP.23308

also observed in the zones of inhibition around the S. Typhimurium isolate when the concentrations were increased to 50 mg/ml and 100 mg/ml for the methanolic and hexane extracts.

Table 3 shows the qualitative phytochemical screening profile of the extracts. The three extracts were found to be rich in saponins, tannins, terpenoids, and alkaloids while steroid and phlobatanin were absent in them. Table 4 represents the quantitative analysis of the plant extracts and reveals the various quantities of the secondary metabolites present in the plant. Nhexane extract had the highest quantity of the cardiac glycosides and flavonoids while methanolic extract had the highest quantity for terpenoid, alkaloid, tannin, and saponin. Cardiac glycosides were highest (14.71±0.11 mg/g) in hexane extract and least (0.53±0.58 mg/g) in methanol while terpenoid was highest in methanol extract (12.13±0.03 mg/g) mg/g and least (4.64±0.04 mg/g) in hexane extract. As for tannins, highest tannin content (3.65±0.01 mg/g) was obtained in the methanol extract and the least  $(7.74\pm0.03 \text{ mg/g})$  in acetone. The trend was the same for the alkaloid content, with the highest (35.15±0.07) mg/g being recorded with methanol extract and the least (20.31±0.06 mg/g) with hexane extract. For the flavonoids however, hexane extract recorded the highest (8.63±0.05 mg/g) and methanol, the least  $(1.78 \pm 0.04 \text{ mg/g})$ . These are shown in Table 4.



## **Table 1. Antibacterial effect of J. curcas on S. Typhi**

Values with the same alphabet along the column are not significantly  $(P<0.05)$  different





Values with the same alphabet along the column are not significantly (P<0.05) different

# **3.2 Antibiotics Sensitivity of the test Organisms**

Fig. 1 depicts the antibiogram of the bacteria isolates when tested with commercially available gram negative antibiotics sensitivity disc. Salmonella Typhi and Salmonella Typhimurium recorded the highest zones of inhibition (14 mm and 24 mm) respectively with Ciprofloxacin (10 µg) while the least zone of inhibition (2 mm) was obtained with ofloxacin (5 µg) and tetracycline (30 µg) respectively for the two isolates.

Table 3 showed that all the extracts were not rich in steroids, phlobatannin, and anthraquinone but they all possess saponin, tannin, flavonoid, terpenoid, alkaloid, and cardiac glycosides.

# **4. DISCUSSION**

Medicinal plants like Jatropha curcas play a major role in the treatment of various diseases including bacterial infections. In this study, the antibacterial efficacy of the plant's leaf extract on S. Typhi and S. Typhimurium, phytochemical screening and antibiogram of the S. Typhi and S. Typhimurium isolates were assessed. The results of the antibacterial assay showed that the leaf extract was active against Salmonella Typhi and S. Typhimurium isolates. The susceptibility of Salmonella Typhimurium to methanolic and hexane extracts of J. curcas strengthens the

suggestion that it could be useful in the treatment of gastroenteritis and food poisoning. The crude extracts of the plant tested showed varying degree of antibacterial activities against the test bacteria. The inhibitory activity of plant extract is largely dependent on the concentration, parts of the plant used and the microbes tested [17]. The methanol extract had the highest activity against the bacterial isolates; this may be attributed to the presence of soluble phenolic and polyphenolic compounds. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol [18]. Methanol has a high polarity index [19] and thus is able to extract more phenolic and flavonoid compounds**.** These phytocompounds have been reported to be effective antimicrobial agents [19]. The qualitative estimation of the phytocompounds of J. curcas extracts revealed the presence of alkaloids, saponins, flavonoids, tannins and steroids. Using qualitative analysis, Igbinosa et al. [20] and Akinpelu et al. [21] observed the presence of the same compounds in J. curcas stem bark and leaves extracts respectively. These phytocompounds are known to support bioactive activities in medicinal plant and therefore aid the antimicrobial activity of J. curcas [22]. These compounds have been associated with medicinal uses for centuries and have been reported as the most efficient, therapeutically significant plant substance [23,24].

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N-hexane	+										
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**Table 3. Qualitative phytochemical components of the plant extracts** 

**Table 4. Quantitative phytochemical components of the plant extracts** 

<b>SN</b>	<b>Phytochemicals</b>	Methanol (mg/g)	Hexane (mg/g)	Acetone (mg/g)
	Glycoside	$0.53^{\circ}$ ±0.08	$14.71^{\circ}$ ±0.11	$7.06^{\circ}$ ±0.02
2	Terpenoid	$12.13^d \pm 0.03$	$4.64^b \pm 0.04$	$7.74^{\circ}$ ±0.03
3	Tannin	$3.65^{\circ}$ ±0.01	$2.90^a \pm 0.02$	$1.65^a \pm 0.02$
4	Saponnin	$35.00^{\circ}$ ±0.27	$19.03^{\circ}$ ±0.24	$16.02^{\circ}$ ±0,20
	Flavonoid	$1.78^{\circ}$ ±0.04	$8.63^{\circ}$ ±0.05	$4.93^{\circ}$ ±0.01
6	Alkaloid	$35.15^{\circ}$ ±0.07	$20.31^{\frac{t}{2}} + 0.06$	$27.97^{\dagger}$ ±0.04

Values with the same alphabet along the column are not significantly (P<0.05) different



#### **Fig. 1. Antibiotics sensitivity profile of Salmonella Typhi and Salmonella Typhimurium to commercial antibiotics**

Key: CPX= Ciprofloxacin 10 µg, AMX= Amoxycillin 25 µg OFL= Ofloxacin 5 µg, COT= Cotrimozazole 25 µg, GEN= Gentamycin 10 µg, NIT= Nitrofuranton 200 µg, CRO= Ceftriazone 30 µg, PFX= Pefloxacin 5 µg, TET= Tetracycline 30 µg, AUG= Augmentin-30 µg

The absence of some of the phytochemicals in the plant extracts could be due to the fact that a particular type of solvent may not be selective for a single compound as a result of multicomponent nature of plant materials with complex interactions [25]. The presence of various phytochemicals in the plant extract supports the therapeutic potentials of the plant in folkloric medicine for the treatment of infectious diseases. Saponin for instance has been reported to have anti-inflammatory and immune stimulating activity. Aside this, saponins demonstrate antimicrobial properties particularly against bacteria [26].

Tannin compounds have also been reported to have some antibacterial potential [27]. The large amount of tannin reported in this work suggests that Jatropha curcas can also be useful in the production of drugs for treatment of bacterial infections. Terpenoids which were also present have also been reported to have antimicrobial effect [28]. Phytochemicals confer pharmacological activities on natural products derived from plants. Many drugs in common use in modern medicine today were isolated and purified from plants. Olajire and Azeez, [29], Upadhyay, [30] have both reported that natural products such as plants have been found to possess a range of antimicrobial activities. Rabe, [31] opined that secondary metabolites in plants are generally known to confer plants with

therapeutic activities. The results of the present study also confirm the presence of alkaloids in acetone, methanol and hexane extracts of J. curcas. The presence of toxic alkaloids like curcin and phorbal ester supports their observed antimicrobial activities [32]. In particular, alkaloids have been reported as the most efficient, therapeutically significant plant substance [24] hence, the moderate abundance of alkaloids, glycosides, and terpenoids detected in the tested extract of  $J$  curcas leaves, might explain the basis for its reported efficacy in ethnomedicinal therapy. Flavonoids, another constituent of J. curcas leaves extract are known for their wide range of biological activities like antimicrobial, anti-inflammatory, and antioxidant properties [33]. Flavonoids have biological activities that are of benefit in the prevention and management of many ailments [34]. Earlier, Erah et al. [35] had associated antimicrobial activity with the presence of flavonoids. Flavonoids are secondary metabolites, which are the most common group of polyphenolic compounds found ubiquitously in plants [30]. These compounds have been associated with medicinal uses for centuries and were reported as the most efficient, therapeutically significant plant substance [23,24]. The active principles of many drugs found in plant are secondary metabolites. Fortunately, these compounds exert antibacterial activity through different mechanisms [31,36]. In this study, result of antibiotics sensitivity test

revealed varying antibiogram of the bacterial isolates to the commercially available antibiotics. The resistance of human pathogens to antibiotics may be due to indiscriminate use of antibiotics by patients and improper use of antibiotics as prescribed by physician [37,38]. Commercial antibiotics were found to exert higher inhibitory effect on the isolate compared to the leaf extracts. This may be due to its excellent tissue penetration and high level of purification of the antibiotics as reported by Crafton, [39]. He stated that antibiotics in a refined state may record higher antimicrobial activity than plant in crude state. Furthermore, antibiotics in its small portion can easily penetrate through the cell wall into the cytoplasm of the microorganism [40]. The comparatively better antibacterial effects of the commercial antibiotics is in line with several findings that have reported the higher potency of antibiotics to their high degree of purity as compared to the crude state of the extract [41,42]. As product of large scale industrial fermentation, antibiotics are usually pure, owing to good manufacturing practice and quality control which ensures that standard is met [43]. The molecular size of antibiotics which aid their solubility in diluent may be another reason for their better performance. This could enhance their penetration through the cell wall into the cytoplasm of the organism where they act [40]. The active principles of many drugs found in plant are secondary metabolites and these compounds exert antibacterial activity through different mechanisms [31,36]. It is worth noting that the plant extracts showed activity against both Salmonella Typhi and Salmonella Typhimurium except acetone extract, which doesn't have effect on Salmonella Typhimurium at the given concentrations. Therefore, the leaf extract of this plant could be very useful in chemotherapy.

# **5. CONCLUSION**

The investigations carried out in vitro on the crude extracts of J. curcas leaves showed that the plant leaves extracts has antibacterial activity against both Salmonella Typhi and Salmonella Typhimurium. The ability of the crude extracts to inhibit the growth of tested bacterial isolates has confirmed the usefulness of J. curcas for the treatment of typhoid fever and gastroenteritis. The phytochemical screening of leaves extracts of J. curcas revealed the presence of saponins, alkaloids, steroids; tannins and flavonoids. The quantity of the phytocompounds observed varied according to the extraction method and the

solvent used. The result of this study gives credence to the scientific validation for the use of Jatropha curcas in ethnomedicine. It also serves as a guide for selection of the plant for further<br>phytochemical work on isolation and phytochemical work on isolation and identification of the active compounds.

The inhibitory effect of J. curcas against Salmonella Typhi and Salmonella Typhimurium can introduce this plant as a potential candidate in bio-prospecting for antibiotic drugs.

## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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