Determination of Antibiotic Effect of *Jatropha gossypiifolia* Leaf Extract on Selected Pathogenic Microbes

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Abstract
Medicinal plants remain the feasible source of new compounds for drug development process. The natural products provide clues for the synthesis of new structural types of antimicrobial and antifungal chemicals that are cost effective and relatively safe for man. The aim of this study was to determine the antibacterial activity of the methanol extract of *Jatropha gossypiifolia* leaf on *Escherichia coli* and *Staphylococcus aureus. Jatropha gossypiifolia* leaves were dried and extraction of phytoconstituents was done following standard procedures. Antimicrobial susceptibility test was carried out on the selected clinical isolates using agar well diffusion technique. The minimum inhibitory concentration and the minimum bactericidal concentration were determined following standard techniques. Results of this study revealed the presence of phenols, tannins and flavonoids. The methanol extract of *Jatropha gossypiifolia* leaves was active against *Escherichia coli* and *Staphylococcus aureus.* The minimum inhibitory concentration (MIC) of the methanol extract *Jatropha gossypiifolia* leaves against *Escherichia coli* was 200 mg/mL and the minimum inhibitory concentration (MIC) of the methanol extract *Jatropha gossypiifolia* leaves against *Staphylococcus aureus* was 50 mg/mL. This study confirmed the folk medicine reports of the activity of *Jatropha gossypiifolia* leaves on some pathogens. It is therefore recommended that other parts of *Jatropha gossypiifolia* plant should be tested for their antimicrobial activity.

Key word: Medicinal plants, *Jatropha gossypiifolia*, *Escherichia coli*, *Staphylococcus aureus*, methanol extract, minimum inhibitory concentration

Introduction

Medicinal plants remain one of the most important sources of new compounds for drug development processes [1]. Herbal medicines are in great demand in both developed and developing countries as sources of primary health care due to their wide biological and medicinal activities, relative safety and cost effectiveness. Herbal molecules have been reported to have the ability to overcome the resistance problem developed by various pathogens [2]. Balick and Cox (1996) noted that a number of important modern drugs are derived from plants used by indigenous people [3].

Human beings have used plants for the treatment of diverse ailments for thousands of years [4]. The World Health Organization has noted that most populations are still relying on traditional medicines for mitigating problems related to their psychological and physical health [5]. This is because some of them cannot afford the products of Western pharmaceutical products [6]; [7]. Rural areas in many developing countries are still relying on traditional medicine for their primary health care needs. These medicines are relatively safer and cheaper than synthetic or modern medicine [8]; [9]; [10]. People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health [11]; [12].

Researchers have identified a number of compounds that are used in mainstream medicine which were derived from "ethnomedical" plant sources [13]. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [14]; [15].

Jatropha gossypiifolia is a small shrub with purplish-red coloured leaves. The leaves are 16 to 19 cm long and 10 to 13 cm wide. The small red flowers of the plant have yellow centres and are unisexual. *Jatropha gossypiifolia* has been considered as a source of many bioactive substances. These substances have been extracted from *Jatropha gossypiifolia* leaves, stem, bark, roots, latex and seeds. Some of the chemical constituents in the extracts of the plant include but not limited to lignoids, terpenoids, and steroids [16].

Staphylococcus aureus is a Gram-positive coccus, which has been implicated in a number of human infections. It is catalase positive and reduces nitrate. It is among the common causes of skin infections like abscesses, respiratory infections like sinusitis and food poisoning. *Staphylococcus aureus* has also been implicated in conditions ranging from minor skin infections such as pimples, impetigo, boils, cellulitis, scalded skin syndrome, ear infection to life threatening diseases like pneumonia, meningitis, osteomyelitis, toxic shock syndrome and bacterial sepsis. It is still one of the most common causes of hospital acquired infections like postsurgical wound diseases and a host of others. Although *Staphylococcus aureus* is susceptible to most beta-lactam antibiotics like penicillin, there are various reports on the increase in resistance to antibiotic. Staphylococcal strains have evolved mechanisms of inhibiting most antibiotics. These mechanisms include acquisition of the VanA gene, ribosomal mutations, modification of enzymes and active efflux of the drugs out of bacteria cells [17].

Escherichia coli is a Gram negative, facultative anaerobe which appears rod shape under the microscope. It is commonly found in the lower intestine of warm-blooded animals as a member of the normal microflora. Most strains are harmless, but some serotypes can initiate food poisoning in the host. Faecal-oral transmission is the major route through which pathogenic strains of the bacterium enters the host system. The pathogenic strains can cause gastroenteritis, urinary tract infections and neonatal meningitis. Although it is susceptible to many antibiotics, *Escherichia coli* has shown strong resistance to streptomycin and other antibiotics which it was earlier susceptible to [18].

The problem of antimicrobial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. This study aimed to determine the antibacterial activity of the methanol extract of *Jatropha gossypiifolia* leaf on *Escherichia coli* and *Staphylococcus aureus.*

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh *Jatropha gossypifolia* leaves were obtained from Islamic village, Ilorin, kwara State, Nigeria. The plants were taken to Herbarium Unit of Plant Biology Department, University of Ilorin where they were identified as *Jatropha gossypiifolia*.

The leaves were separated from the mid rib and gently washed under running water 2-3 times to remove dust and dirt and were then rinsed with distilled water. The leaves were drain and air-dried for about 2weeks at room temperature. The dried leaves were reduced into small size and milled into fine powder using blender. The leaf powder was weighed, packed and stored in sterile air-tight containers.

Extraction of Phytoconstituents

Extraction was done by transferring 25g of *Jatropha gossypiifolia* powdered leaves into a conical flask containing 250 mL methanol in the ratio of 1:10 (w/v). The conical flask was corked and placed on shaker for 48 hours. The percolates were then filtered and the resulting volume on filtration were then completely evaporated at 45±10ºC under reduced pressure using the rotary evaporator (Model RE Zhengzhou, Henan China). The concentrated extract was stored at 4°C for further use. The extracts were then collected, weighed, packed in sterile air-tight containers and labeled. The extracts were then kept at 4ºC until needed for analysis [19].

Sterility Test

Serial dilution of 1 g of the extract was made up to 10^{-1}. Two millilitre (2 mL) of each extract was inoculated into 10 mL of nutrient broth, incubated at 37ºC for 24 hours. The absence of turbidity or clearness of the broth indicated sterility of the extract [20].

Phytochemical Screening

Preliminary phytochemical screening of the extracts was done following standard procedures as described below:

Test for Saponins

A quantity (0.5g) of the extract was weighed into a sterile test tube and 5mL of distilled water was added. A few drops of olive oil were then added to the solution. The formation of emulsion indicated the presence of saponins [21].

Test for Flavonoids

Few drops of diluted NaOH solution was added to 1mL of the extract. Appearance of intense yellow colour indicated the presence of flavonoids [22].

Test for Phenols
Methanol leaf extract weighing 0.2g was dissolved in 3-4 drops of ferric chloride solution. Formation of blue-black or brown colour indicates the presence of phenol [22].

Test for Tannins
Two millilitre (2 mL) of plant extract was added to 2 mL of distilled water. About 2-3 drops of 5% ferric acids were added to the solution. The formation of green precipitate indicated the presence of Tannins [22].

Test for Anthraquinones
A quantity (3 mL) of plant extract was mixed with 10 mL of Bezene the content was shaken properly and filtered. Five millilitres (5 mL) of 10% ammonia was added to the filtrate and was allowed to mix by shaking. The appearance of pink, red or violet colouration in the ammonia layer indicated the presence of anthraquinones [23].

Test for Alkaloids
Two milliliter (2 mL) of the extract was stirred with few drops of hager's reagent. Appearance of yellow precipitates indicated the presence of alkaloids [22].

Test for Steroids
Two millilitres (2 mL) of the plant extract was mixed with 2 mL of chloroform and concentrated sulphuric acid. A reddish violet ring at the junction in the lower chloroform indicated the presence of steroids [24].

Test for Glycosides
Two millilitres (2 mL) of plant extract was mixed with 2 mL of chloroform and acetic acid. The content was shaken properly. Appearance of violet blue to green colouration indicated the absence of glycosides [22].

Test Organisms
The isolates used in this study *Escherichia coli* and *Staphylococcus aureus* were obtained from the Microbiology Laboratory of University of Ilorin Teaching Hospital. They were maintained on Nutrient agar slant. The cultures of the isolates were purified following standard techniques [25].

Standardization of Inoculum
Colonies from microbial culture (3-5 colonies) was aseptically suspended into 5 mL of peptone broth using a sterile loop and incubated at 37°C for 2 hours. This was adjusted to 0.5 McFarland turbidity ($1.5x10^8$ CFU/mL). The inoculum was used within 20 minutes of standardization [26].

Antibacterial Sensitivity Assay
Agar well diffusion techniques was used to determine the antimicrobial activity of the extract. The standardized inoculum was spread on sterile solidified Nutrient Agar using sterile glass spreader. A sterile cork borer of size 6 mm in diameter was used to make wells on the plates. About 0.5 ml of the leaf extract was then put into each appropriately labelled well in the concentration of 200 mg/mL, 150 mg/mL, 100 mg/mL and 50 mg/mL with the aid of a sterile micropipettes. Control experiments were also set-up where the holes were filled with sterile distilled water [27].

The inoculated plates were left on the table for 1 hour for the extracts to diffuse into the agar. The plates were then incubated at 37°C for 24 hours. After the incubation period, a zone of clearance around each well signified inhibition and the zones were measured with a transparent ruler. The diameter of the zone of inhibition around each well was measured to the nearest millimetre.

Determination of Minimum Inhibitory Concentration (MIC) of the Extract
The minimum inhibitory concentration of the plant extract was determined using broth dilution method. Varying concentrations of the extracts were weighed and mixed with 3ml of Mueller-Hinton broth in test tubes. The contents were thoroughly mixed and the tubes were inoculated with 0.2mL of standardized isolates. The tubes were then incubated at 37°C 24 hours [28]. The least concentration of the extract that did not permit any visible growth of the test isolates in the broth medium was regarded as the minimum inhibitory concentration (MIC) in each case [27].

Determination of the Minimum Bactericidal Concentration (MBC)
Sample from the tubes used in the MIC assays which did not show signs of turbidity after

incubation were streaked out on solidified Nutrient Agar plates using a sterile glass spreader and incubated at 37°C afor 24 hours. The lowest concentration of the extract which showed no growth on plates after 24 hours of incubation indicated the MBC [27].

Antibiotic Susceptibility Test Using Conventional Antibiotic Discs

Antibiotic Susceptibility Test was carried out using antibiotic discs. The standard disc diffusion procedure was adapted for the analysis. The following antibiotic discs were used: augmentin (AUG; 30 µg), ceftriazone (CRO; 30 µg), nitrofuranoton (NIT; 300 µg), gentamycin (GEN; 10 µg), otrimozazole (COT; 23.2 µg), ofloxacin (OFL; 5 µg), amoxycillin (AMX; 25 µg), ciprofloxacin (CPX; 5 µg), tetracycline (TET; 30 µg), pefloxacin (PFX; 5 µg). Standardized inoculum was transferred onto the Nutrient agar plates. Antibiotic discs were carefully placed on the plates. Plates were incubated at 37°C for 18 to 24 hours. The zones of inhibition were measured, recorded and interpreted according to the Clinical Laboratory Standard Institute (CLSI) provided.

Statistical Analysis

The experiments were done in triplicate and data were expressed as mean ± standard error of mean. Statistical comparison was performed between the groups by one-way Analysis of Variance (ANOVA) and Duncan Multiple Range Test using IBM SPSS 20.

Results

Phytoconstituent analysis revealed the presence of flavonoid, phenol, tannin, anthraquinones, alkaloid and steroid. The zones of inhibition produced by the methanol extract of *Jatropha gossypiifolia* leaves against *Escherichia coli* and *Staphylococcus aureus* were 20.33±0.88 and 13.67±0.67 respectively. The Minimum Inhibitory Concentrations (MICs) of methanol extract of *Jatropha gossypiifolia* leaves against *Escherichia coli* and *Staphylococcus aureus* were 200mg/mL and 50mg/mL respectively. The Minimum Bactericidal Concentration (MBC) of the plant extract against *Staphylococcus aureus* was 200mg/mL. The extract had no bactericidal effect on *Escherichia coli*.

The antibiotics used included Pefloxacin (10 µg), Gentamycine (10 µg), Ampiclox (30 µg), Zinnacef (20 µg), Amoxacillin (30 µg), Rocephin (25 µg), and Ciprofloxacin (10 µg). The zones of inhibition observed from these conventional antibiotics ranged between 1.47mm and 2.40mm for the two test organisms.

Table 1: Qualitative Phytochemical Screening of Methanol Extract of *Jatropha gossypiifolia* Leaves

Test	Interpretation
Saponin	Absent
Flavonoid	Present
Phenol	Present
Tannin	Present
Anthraquinones	Present
Alkaloid	Present
Steroid	Present
Glycoside	Absent

Table 2: Antimicrobial Susceptibility Test of *Jatropha gossypiifolia* Leaf Extract Against Test Isolates

S/N	Concentration of Plant Extract (mg/mL)	Zone of Inhibition (mm)	Test Organism
1	150	20.33±0.88	*Escherichia coli*
2	150	13.67±0.67	*Staphylococcus aureus*

Values are means of three replicates ± standard error of mean

Table 3: Minimum Inhibitory Concentration (MIC) of the Methanol Extract of *Jatropha gossypiifolia* Leaves on *Escherichia coli*

S/N	Concentration of Plant Extract (mg/mL)	Microbial Growth
1	50	1
2	100	1
3	150	1
4	200	0
5	250	0

Key: 1 = Growth observed, 0 = No growth observed

Table 4: Minimum Inhibitory Concentration (MIC) of the Methanol Extract of *Jatropha gossypiifolia* Leaves on *Staphylococcus aureus*

S/N	Concentration of Plant Extract (mg/mL)	Growth
1	50	0
2	100	0
3	150	0
4	200	0
5	250	0

Key: 1 = Growth observed, 0 = No growth observed

Table 5: Minimum Bactericidal Concentration (MBC) of the Methanol Extract of *Jatropha gossypiifolia* Leaves on *Escherichia coli*

S/N	Concentration of Plant Extract (mg/mL)	Growth
1	50	1
2	100	1
3	150	1
4	200	1
5	250	1

Key: 1 = Growth observed, 0 = No growth observed

Table 6: Minimum Bactericidal Concentration (MBC) of the Methanol Extract of *Jatropha gossypiifolia* Leaves on *Staphylococcus aureus*

S/N	Concentration of Plant Extract (mg/mL)	Growth
1	50	1
2	100	1
3	150	1
4	200	0
5	250	0

Key: 1 = Growth observed, 0 = No growth observed

Table 7: Comparative Analysis of Antibacterial Activity of the Conventional Antibiotics and the Methanol Extract of *Jatropha gossypiifolia* Leaves on *Escherichia coli*

S/N	Types of Antibiotics	Zone of Inhibition (mm)
1	Tarivid	2.50 ± 0.12^{a}
2	Gentamicin	1.63 ± 0.09^{a}
3	Ciprofloxacin	1.63 ± 0.15^{a}
4	Streptomycin	1.87 ± 0.06^{a}
5	Amoxacilin	2.10 ± 0.12^{a}
6	Septrin	1.80 ± 0.12^{a}
7	Augmentin	2.33 ± 0.09^{a}
8	Sparilioxacin	1.90 ± 0.12^{a}
9	Chloramphenicol	1.77 ± 0.09^{a}
10	Plant Extract	20.33 ± 0.88^{b}

Values are mean ± standard error of mean. Values with the same superscript are not significantly different across the column ($p\leq0.05$) based on Duncan's multiple range test

Table 8: Antibiotics Sensitivity Test Result for *Staphylococcus aureus*

S/N	Types of Antibiotics	Zone of inhibition (mm)
1	Pefloxacin	1.77 ± 0.09^{ab}
2	Gentamicin	1.53 ± 0.09^{a}
3	Ciprofloxacin	2.17 ± 0.12^{ab}
4	Streptomycin	1.77 ± 0.09^{ab}
5	Amoxacilin	1.47 ± 0.12^{a}
6	Septrin	1.53 ± 0.07^{a}
7	Zinnacef	1.57 ± 0.09^{a}
8	Ampiclox	1.93 ± 0.06^{ab}
9	Erythromycin	2.40 ± 0.10^{b}
10	Plant Extract	13.67 ± 0.67^{c}

Values are mean ± standard error of mean. Values with the same superscript are not significantly different across the column ($p\leq0.05$) based on Duncan's multiple range test

Discussion

The results obtained from the phytoconstituents analysis is suggestive of the antibacterial activity of the plant extract. Arekemase *et al.* (2011) posited that the presence of alkaloid, tannin and flavonoid enhance the antimicrobial activity of plant extracts [29].

The presence of these compounds has been reported in the leaf and root parts of the plant. These phytochemical compounds are known to play important roles in bioactivity of medicinal plants. Flavonoids which are part of the phytochemical constituents of leaves of *Jatropha gossypifolia*, exhibit a wide range of biological activities, one of which is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals. Flavonoids also exhibit anti-inflammatory, anti-allergic effects, analgesic and antioxidant properties. These observations support the usefulness of *Jatropha gossypifolia* in folklore remedies for the treatment of various infections. Plant originated antimicrobial drugs are of great importance in view of the undesirable side effects of orthodox antibiotics and multi-drug resistance.

The result of this study revealed the antimicrobial activities of the crude methanol extract of *Jatropha gossypiifolia* leaves on

Escherichia coli and *Staphylococcus aureus.* The results in this study also showed the minimum inhibitory concentration (MIC) of *Jatropha gossypiifolia* leaf extract on the test organisms. The results further showed the minimum bactericidal concentration (MBC) of *Jatropha gossypiifolia* leaf extract on *Staphylococcus aureus*. The values obtained in these analyses are within the range of results reported by Aiyelaagbe *et al.* 2007 [30].

Zones of inhibition reported by Ashraf *et al.* (2018) against *Escherichia coli* and *Staphylococcus aureus* when ethanolic extract of *Syzygium aromaticum* was used were 11.9±0.34 and 15.8±0.41 respectively [31]. These values were close to the values reported in this work against the two isolates. Using selected standard antibiotics as reference drugs, the test organisms cannot be considered sensitive to the antibiotics according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS).

Conclusion

The methanol extract of the leaves of *Jatropha gossypiifolia* has a number of phytochemicals which are responsible for its various pharmacological activities. Based on the results obtained from this study *Jatropha gossypiifolia* leaves can be considered an alternative therapeutic agent that may be used to treat infections caused by *Escherichia coli* and *Staphylococcus aureus*. It is therefore recommended that future work on this study should include further purification of the extract fractions in order to isolate the most active compound(s) responsible for antibacterial activity. The plant material should also be tested on fungi to establish its antifungal activities.

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