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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ASSAY OF *JATROPHA CURCAS* STEM BARK EXTRACTS

Everlyne Moraa Isoe* and Okoye George Kubende

[1]Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Pwani University address: P.O Box 195-80108 Kilifi, Kenya.

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***Corresponding Author**
Dr. Everlyne Moraa Isoe
Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Pwani University address: P.O Box 195-80108 Kilifi, Kenya.

ABSTRACT

The study aimed to evaluate the phytochemical analysis and antimicrobial activity of *Jatropha curcas* ethanolic, methanolic, hexane, and ethyl acetate extracts obtained from stem bark. The methods used for phytochemical analysis and antimicrobial activity were standard qualitative tests and disc diffusion method respectively. Alkaloids, terpenoids, glycosides, steroids, and flavonoids were present in all extracts. Saponins and phenols were absent in ethyl acetate and hexane extracts but they were present in ethanolic and methanolic extracts the anthraquinones and tannins were absent in all the extracts. All the extracts managed to inhibit the growth of bacteria and fungi used. The *Escherichia coli* was observed to be most sensitive to hexane extract with a mean inhibition zone of 18.5mm. The *Staphylococcus aureus* was observed to be most sensitive to methanol extract with a mean inhibition zone of 15.5mm. The *Pseudomonas aeruginosa* was observed to be most sensitive to ethanol extract with the mean zone of inhibition of 17mm. *Bacillus subtilis* were observed to be most sensitive to methanolic extract with the mean zone of inhibition of 12mm. Finally, the *Fusarium oxysporium* was observed to be the most sensitive to ethanol extract with a mean inhibition zone of 14mm. Therefore, the outcome study shows that *Jatropha curcas* stem bark could be exploited for new potent antibacterial and antifungal agents.

KEYWORDS: Phytochemicals, *Jatropha curcas*, Antibacterial, Antifungal, extracts.

1. INTRODUCTION

Antimicrobial agents are substances that either inhibits the growth or eliminate microorganisms. Generally, antimicrobial drugs kill the pathogen by either blocking cell wall synthesis, preventing the synthesis of nucleic acid and protein, blocking metabolic pathways by inhibiting the key enzymes, or disrupting microbial cell membrane structure and function.[1] Novel antimicrobial drugs have been developed by exploring plants, animals, minerals, and microbiological sources.[2] Antimicrobial resistance is a great problem for public health in the fight against bacterial infections in Kenya and the entire world.[3] In addition, antimicrobial drugs which are being used currently are having more adverse effects such as hypersensitivity, immune suppression, and allergic reaction.[4] Also, many people are dying in the entire world due to the increased rate of emerging infectious diseases caused by microorganisms.[5]

Plants are considered the greatest source to obtain new antibiotics.[2] Plants are used as a source of new drugs because attention has been given to the medicinal value of herbal products for safety, efficacy, and economy because plants contain bioactive constituents which act as an antioxidant and antimicrobial agents.[6] Patil & Deshmukh,[7] stated that medicinal plants apart from therapeutic agents are also a great source of information for many bioactive constituents which could give a clue for newer modern drug design.

A medicinal plant is any plant that contains phytochemicals that can be used for healing purposes.[8] Phytochemicals are secondary metabolites such as alkaloids, phenolic compounds, terpenes, glycosides, anthraquinones, saponins, and flavonoids which have different benefits for both humans and animals. Secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as a source of pharmaceutical agents.[9]

Kenya has different medicinal plants which may be studied and evaluated for potential antimicrobial activities. The outcome of research may contribute to the development of novel antimicrobial agents through phytochemical screening, analysis, and antimicrobial assay with plant species. Therefore, it is vital to identify the phytochemical components of local medicinal plants which are used by herbalists in the treatment of diseases because the investigation into the antimicrobials activities of local medicinal plants will reveal potential phytochemical sources of therapeutic agents.[10]

Jatropha curcas is a stress-resistant perennial plant growing on marginal soils.[11] It is mainly found in the Northern part of South America, Asia, and Africa.[12] It belongs to the kingdom of Plantae, the family of Euphorbiaceae, Genus of *Jatropha*, species of *curcas*, and order of malpighiales. The English name of *Jatropha curcas* is known as a physic nut.

Medicinal plants like *Jatropha curcas* have been used in treating fungal and bacterial infections and many other diseases.[13] In Kakamega County, Kenya *Jatropha curcas* is a popular medicinal herb that has been part of traditional remedies for treating fresh cuts, wounds, tetanus, snake bite, constipation, kidney disorders, and chest pain. *Jatropha curcas* has been used in treating fungal and bacterial infections and many other diseases. Prasad[14] reported that *Jatropha curcas* leaves are used for wound healing, anti-parasitic, malaria treatment, and fungal infections. Stem barks, branches, and twigs are used as strong antimicrobial agents. Since they plant that grows in Western Kenya has not been evaluated in the laboratory to ascertain its use as a medicinal plant, there is need for this current study to evaluate its phytochemical components as well as antimicrobial potency.

Therefore, the aim of study is to evaluate the phytochemical analysis and antimicrobial activity of *Jatropha curcas* ethanolic, methanolic, hexane, and ethyl acetate extracts obtained from stem bark. The objective of our present study is to carry out phytochemical analysis and antimicrobial assay of *Jatropha curcas* stem bark extracts against selected microbes such as *Staphylococcus aureus, Escherichia coli, Bacillus subtills, Pseudomonas aureginosa and Fusarium oxysporum* known to caused disease.

2. MATERIALS AND METHODS

2.1 Plant material

The plant material used was *Jatropha curcas* stem bark. It belongs Euphorbiaceae family, kingdom Plantae, the genus of *Jatropha*, species of *J. curcas*, and order of malpighiales.

Figure 1: Showing a picture of *Jatropha curcas* plant.

Figure 1: illustrates *a picture of Jatropha curcas plant t*aken in Kakamega county, Matungu sub county, Koyonzo ward, Ikulumwoyo village, Kenya. It belongs Euphorbiaceae family, kingdom Plantae, the genus of *Jatropha*, species of *J. curcas*, and order of malpighiales. It is a popular medicinal herb that has been part of traditional remedies for treating fresh cuts, wounds, tetanus, snake bite, constipation, kidney disorders, and chest pain. *Jatropha curcas* has also been used in treating fungal and bacterial infections and many other diseases. *Jatropha curcas* seeds are used for constraining biodisel and finally, *Jatropha curcas* plant contains cyanide which is poisonous.

2.2 Test Organisms

The test organisms that were used included *Staphylococcus aureus, Escherichia coli, Bacillus subtills, Pseudomonas aureginosa, and Fusarium oxysporium*. They are common infectious microorganisms isolated in the Microbiology Laboratory Pwani University. The positive control used was an antibiotic drug (Ampicillin) and an antifungal drug (clotrimazole) and the negative control used was sterile distilled water.

2.3 Equipment, Machines, and Apparatus

Equipment, machines, and apparatus that were used included blender, conical flasks, test tubes, boiling tubes, corks, shaker, filter papers, McCartney bottles, refrigerator, incubator, clean bench, falcon tubes, water bath, cotton swabs, sterile paper discs, Petri plates, HAHNVAPOR Rotary evaporator HS-2005S, and a metric ruler.

2.4 Reagents

The Reagents that were used included distilled water, ethanol, methanol, hexane, ethyl acetate, nutrient agar media, Potato Dextrose Agar media (PDA), Muller Hinton agar - HIMEDIA, ferric chloride solution, conc. Sulphuric vi acid (H_2S0_4), conc. hydrochloric acid (HCL), Mayer's reagent, chloroform, glacial acetic acid, ferric solution, alcohol, sodium hydroxide solution (NaOH) and ammonium solution. All reagents were of analytical grade.

2.5 Collection of Plant Material

The stem bark of *Jatropha curcas* used was collected in a clean polythene bag at Ikulumwoyo village, Koyonzo ward, Matungu sub-county, Kakamega county, Kenya. Then the stem bark was transported to Pwani university's biological laboratory and then was washed with distilled water, cut into pieces, and shade-dried.

2.6 Preparation of Extracts

The plant stem bark was ground with a blender to form a coarse powder. The powder was stored aseptically to be used in extraction. Ethanolic, hexane, methanolic, and ethyl acetate extracts of *Jatropha curcas* stem bark were prepared using the cold maceration method described by De Silva et al,[15] with slight modification.

2.6.1 Ethanolic Extract
48 grams of ground stem bark was measured into a conical flask and 240ml of cold 95% ethanol was added, covered with a cork, mixed, and left on the shaker at 100 r.p.m for 24 hours after which the extract was placed in the freezer at -20℃ for 48 hours. Then the mixture was filtered using the filter papers. The filtrate obtained was subjected to a rotary evaporator machine for the ethanol to evaporate at its boiling point of 78.37℃. The extracts were reduced to 8ml and then stored in sterile McCartney bottles and kept in the refrigerator at 4℃. Later on, the crude extract was used for phytochemical analysis and antimicrobial assay.

2.6.2 Hexane Extract
48 grams of ground stem bark was measured into a conical flask and 240ml of hexane was added, covered with a cork, mixed, and left on the shaker at 100 r.p.m for 24 hours after which the extract was placed in the freezer at -20℃ for 48 hours. Then the mixture was filtered using the filter papers. The filtrate obtained was subjected to a rotary evaporator machine for the ethanol to evaporate at its boiling point of 69℃. The extracts were reduced to 8ml and then stored in sterile McCartney bottles and kept in the refrigerator at 4℃. Later on, the crude extract was used for phytochemical analysis and antimicrobial assay.

2.6.3 Methanolic Extract
48 grams of ground stem bark was measured into a conical flask and 240ml of methanol was added, covered with a cork, mixed, and left on the shaker at 100 r.p.m for 24 hours after which the extract was placed in the freezer at -20℃ for 48 hours. Then the mixture was filtered using the filter papers. The filtrate obtained was subjected to a rotary evaporator machine for the ethanol to evaporate at its boiling point of 64.7℃. The extracts were reduced to 8ml and then stored in sterile McCartney bottles and kept in the refrigerator at 4℃. Later on, the crude extract was used for phytochemical analysis and antimicrobial assay.

2.6.4 Ethyl Acetate Extract
48 grams of ground stem bark was measured into a conical flask and 240ml of ethyl acetate was added, covered with a cork, mixed, and left on the shaker at 100 r.p.m for 24 hours after which the extract was placed in the freezer at -20℃ for 48 hours with occasional shaking. Then the mixture was filtered using the filter papers. The filtrate obtained was subjected to a rotary evaporator machine for the ethanol to evaporate at its boiling point of 77.1℃. The extracts were reduced to 8ml and then stored in sterile McCartney bottles and kept in the refrigerator at 4℃. Later on, the crude extract was used for phytochemical analysis and antimicrobial assay.

2.7 Sterility Test of Plant Extracts
Each of the above extracts (ethanolic, hexane, methanolic, and ethyl acetate) was tested for contaminants. This was carried out by inoculating 0.1ml of each extract on nutrient agar and incubating at 37℃ for 24 hours to test for bacterial contamination and also by inoculating 0.1ml of each extract on potato Dextrose Agar at 30℃ for 72 hours to test for fungal contamination. Then plates were observed for growth. The extract was then assessed for phytochemical analysis and antimicrobial activity as described by Arekemase et al., (2011).[16]

2.8 Collection and Maintenance of Test Organisms
The organisms were selected based on their availability and they were collected from Pwani university Biological Sciences Laboratory. The organisms used were *Staphylococcus aureus, Escherichia coli, Fusarium oxysporum, Bacillus subtilis and Pseudomonas aeruginosa.* All bacteria were maintained on Muller Hinton agar slant and stored in the refrigerator at a temperature of 4℃. *Fusarium oxysporuim* was maintained on Potatoes Dextrose Agar (PDA) and stored in the refrigerator at a temperature of 4℃. Both bacteria and *fusarium oxysporuim* were sub-cultured onto fresh media before they were used for the antimicrobial assay as described by Arekemase et al., (2011).[16]

2.9 Phytochemical Screening of Stem Bark Extracts
Standard biochemical methods were followed for phytochemical analysis of the *Jatropha curcas* stem bark extracts as described by De Silva et al., (2017) **[15]** with slight modification.

2.9.1. Testing for Saponins
0.2ml of each extract was mixed with 3ml of distilled water and shaken, then it was heated to a boil. Frothing or foaming indicated the presence of saponinsas described by De Silva et al., (2017).[15]

2.9.2. Test for Tannins
To 0.5ml of each extract solution, 1ml distilled water and 1-2 drops of ferric chloride solution were added and blue coloration indicated the presence of tannins.as described by De Silva et al., (2017).[15]

2.9.3. Test for Alkaloids
2 drops of Mayer's reagent were added along the side of the test tube into a few amounts of each extract solution. The presence of alkaloids was indicated by a white creamy precipitate.as described by De Silva et al., (2017)[15]

2.9.4. Test for Steroids and Terpenoids
0.5ml of each extract solution was mixed with 2ml of chloroform and then 2ml of concentrated Sulphuric acid was added to form a layer. A reddish-brown coloration of the interface was formed to indicate the presence of terpenoids while the red color at the lower surface indicated the presence of steroids as described by De Silva et al., (2017).[15]

2.9.5. Test for Glycosides
0.5ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric solution, this was under-layered with 1ml of concentrated Sulphuric acid. A brown ring at the interface indicated the presence of cardiac glycoside (A violet ring might appear below the ring while in the acetic acid layer a greenish ring might be formed).as described by De Silva et al., (2017)[15]

2.9.6. Test for Phenols
To 0.5ml of each extract solution, 2ml of alcohol and a few drops of ferric chloride solution were added and coloration was observed (Blue-black or blue-green coloration or white precipitate indicated the presence of phenol)as described by De Silva et al., (2017).[15]

2.9.7. Test for Flavonoids
0.2ml of each extract will be dissolved in a 10% sodium hydroxide solution. Yellow coloration indicated the presence of flavonoids as described by De Silva et al., (2017).[15]

2.9.8. Test for Anthraquinones
To 0.2ml of each extract, 2ml of chloroform and 1ml ammonia solution were added. The formation of bright pink coloration indicated the presence of anthraquinonesas described by De Silva et al., (2017).[15]

2.10. Antimicrobial Test
The antimicrobial properties of the *Jatropha curcas* stem bark extracts were determined using the Agar diffusion method described by Fateh et al., (2017).[4] with slight modification.

2.10.1. Antibacterial Test
Broth cultures containing test organisms were swabbed onto different sterile Petri plates containing solidified Muller Hinton Agar. Then the 3 sterile filter paper discs were used per petri plate, whereby the first one, the second, and the third were dipped inside the stem bark extract, positive control (ampicillin), negative control(distilled water) respectively using the 3 different sterilized forceps. Then the filter paper discs were removed after being impregnated with the stem bark extract, ampicillin, and distilled water and then were placed on the Petri plate that was containing the solidified Muller Hinton Agar and a specific bacterium. The experiments were done in duplicates for *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtills*, and *Pseudomonas aureginosa*. Each *Jatropha curcas* stem bark extract which included the methanol, ethanol, hexane, and ethyl acetate extracts was tested for antibacterial activity against the bacteria named above. Then inoculated plates were left for an hour at room temperature for the extracts to diffuse before being incubated at 37℃ for 24 hours after which the results were read by measuring the diameter of the zone of inhibition with the aid of a metric ruler and recorded. Since the antibacterial assay was done in duplicates and diameters of zone of inhibition (mm) were expressed as means. A larger zone of inhibition showed that the substance is active against bacteria, a small zone of inhibition showed that the substance is slightly active against bacteria and no zone of inhibition showed no activity against bacteria as described by Fateh et al., (2017).[4]

2.11. Antifungal Test
Broth culture containing *Fusarium oxysporuim* was swabbed onto the sterile Petri plate that was containing the solidified Potatoes Dextrose Agar medium. Then 3 sterile filter paper discs were used, whereby the first one, the second, and the third were dipped inside the stem bark extract, positive control (clotrimazole) and negative control (distilled water) respectively using the 3 sterilized forceps. Then the filter paper discs were removed after being impregnated with the stem bark extract, clotrimazole, and the respective solvents and then were placed on the Petri plate that was containing the solidified Potato Dextrose. The experiment was done in duplicates. Each *Jatropha curcas* stem bark extracts which included the methanol, ethanol, hexane, and ethyl acetate were tested for antifungal activity against the fungus. Then inoculated plates were left for an hour at room temperature for the extracts to diffuse before the plates were incubated at 30℃ for 72 hours after which the results were read by measuring the diameter of the zone of inhibition with the aid of a metric ruler and recorded. The antifungal assay was done in duplicates and diameter of zones of inhibition (mm) were expressed as means. A larger zone of inhibition showed that the substance against active against fungi, a small zone of inhibition showed that the substance is slightly active against fungi and no zone of inhibition showed no activity against fungi as described by Fateh et al., (2017).[4]

3. DATA ANALYSIS

Data analysis of means of the diameter of zone of growth inhibition produced by the effect of *Jatropha curcas* stems bark extracts such as methanol, ethanol, ethyl acetate, and hexane on selected microorganisms known to cause disease was analyzed by the use of Analysis of Variance (ANOVA). From the analysis, the *p*-value of the zone of inhibition of each microbe was less than the F critical value an indication of statistical significance in the difference observed in the means of the diameter of the zone of inhibition of each microbe.

4. RESULTS AND DISCUSSION

4.1. Sterility Test of Plant Extracts
No growth in the extracts after incubation indicated that the extracts were sterile.

4.2. Phytochemicals Screening of *Jatropha curcas* Stem Bark Extracts
Alkaloids, terpenoids, glycosides, steroids, and flavonoids were present in all *Jatropha curcas* stem bark

extracts. Saponins and phenols were absent in only ethyl acetate and hexane extracts and finally, the anthraquinones were absent in the all *Jatropha curcas* stem bark extracts. The outcome of phytochemicals screening of stem bark is shown in tables (1 and 2) below.

Table 1: Showing the outcome of phytochemical screening of *Jatropha curcas* methanolic and ethanolic stem bark extracts.

Methanolic Extract	**Result**	**Ethanolic Extract**	**Result**
Phytochemical	***Presence*** (+)/(-)	***Phytochemical***	***Presence*** (+)/(-)
Flavonoids	+ +	Flavonoids	+ +
Steroids	+	Steroids	+
Phenolics	+	Phenolics	+
Glycosides	+ +	Glycosides	+
Terpenoids	+	Terpenoids	+
Tannins	_	Tannins	_
Alkaloids	+	Alkaloids	+ +
Saponins	+	Saponins	+
Anthraquinones	_	Anthraquinones	_

Key: (+) = Slightly Present (++) =Present (-) = Absent

Table 1 illustrates the outcome of phytochemical screening of *Jatropha curcas* methanolic and ethanolic stem bark extracts. Table 1 above shows that all the phytochemicals tested in the methanol and ethanolic extracts were present except anthraquinones and tannins. In the methanolic extract flavonoids and glycosides were more than all the other phytochemicals tested. In the ethanolic extract, flavonoids and alkaloids were more than the other present phytochemicals. Some stem bark extracts were richer in metabolites than others which may be due to their ability to extract different components based on solvents polarity.

Table 2: Showing the outcome of phytochemicals screening of *Jatropha curcas* Ethyl acetate and hexane stem bark extracts.

Ethyl acetate Extract	**Result**	***Hexane Extract***	**Result**
Phytochemicals	***Presence (+)/(-)***	Phytochemicals	***Presence (+)/(-)***
Flavonoids	+	Flavonoids	+ +
Steroids	+	Steroids	+
Phenolics	_	Phenolics	_
Glycosides	+ +	Glycosides	+
Terpenoids	+ +	Terpenoids	+
Tannins	_	Tannins	_
Alkaloids	+ +	Alkaloids	+ +
Saponins	_	Saponins	_
Anthraquinone	_	Anthraquinones	_

Key: (+) = Slightly Present (++) =Present (-) = Absent

Table 2 illustrates the outcome of phytochemicals screening of *Jatropha curcas* ethyl acetate and hexane stem bark extracts. The ethyl acetate extract showed more terpenoids, glycosides and alkaloids as compared to steroids and flavonoids. Saponins, tannins, anthraquinones and phenolics were absent in the ethanol extract and in hexane extracts. Flavonoids and alkaloids were more prevalent as compared to steroids, glycosides and terpenoids

Jatropha curcas stem bark, leaves, and sap extracts contained glycosides, terpenoids, flavonoids, steroids, alkaloids, phenol, tannins, and saponins in a research done by Doughari & Abraham[17] their results are comparable to our findings although in our case tannins were absent.

Idrees et al[18] reported that *Jatropha curcas* extracts contains glycosides, terpenoids, flavonoids, steroids, alkaloids, phenol, tannins, coumarin, and saponins which correlates with our study with an exception of the coumarins that were not tested. Arekemase et al., (2011)[16] reported that all the roots extracts of *Jatropha curcas* except the hexane extract were rich in steroids but in our studies the stem bark hexane extract had steroids. Qualitative screening of phytochemicals indicated that *Jatropha curcas* contained flavonoids, cardiac glycosides, steroids, and tannins, but it lacked anthraquinones, phlorotannin, alkaloids, and terpenoids. Saponins were exclusively absent in ethanolic extractin a research carried out by Dada et al,[19] in the current study alkaloids, terpenoids, saponins were present in ethanolic extract and anthraquinones were absent.

The leaf, latex, bark, and fruit of the *Jatropha curcas* plant contain phytochemicals such as flavonoids, sapogenins phytosterols, glycosides, tannins, and flavonoids as reported in a research by Debnath & Bisen,[11] in our study, the stem bark lacked tannins. Tomar et al[20] said that leaves extract of *Jatropha curcas* contained phenols, phytic acid, and tannins. Furthermore, Oyama et al[21] reported that *Jatropha curcas* contained secondary metabolites such as cyanogenic glycosides, saponins, alkaloids, tannins, flavonoids, and oxalates. In this study, the *Jatropha curcas* stem bark lacked tannins and anthraquinones, but reports other phytochemicals which is in line with these studies.

In our case, some stem bark extracts were richer in metabolites than others which may be due to their ability to extract different components based on solvents polarity. The saponins and phenols were absent in ethyl acetate extract and hexane extract while the anthraquinone was absent in all the stem bark extracts. In addition, ethyl acetate extract had more content of glycosides and terpenoids, hexane extract had more content of flavonoids and alkaloids, methanol extract had more flavonoids and glycosides and ethanol extract had more flavonoids and alkaloids.

4.3. Antimicrobial and antimicrobial Test

Both the antibacterial and antifungal assays were done. For the antibacterial assay, all the *Jatropha curcas* stem bark extracts which included ethanolic, methanolic, hexane, and ethyl acetate extracts, and positive control (ampicillin) were able to inhibit the growth of bacteria such as the *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* leading to zones of inhibition. The negative control used showed no zone of growth inhibition of the all bacteria listed above. For the antifungal assay, all the *Jatropha curcas* stem bark extracts and positive control (clotrimazole) were able to inhibit the growth of *Fusarium oxysporuim* leading to zones of inhibition of which the diameter was measured. The negative control led to the lack of growth zone of inhibition of the *Fusarium oxysporuim.* Both the antibacterial and antifungal assays were done in duplicate for each microorganism. Therefore, the zones of growth inhibition that were obtained for both the antibacterial and antifungal activity of *Jatropha curcas* stem bark extracts are shown in table (3, 4, 5, 6 and 7) and figure 2, 3 ,4, 5, 6, 7, 8, 9, 10, and 11 below.

Figure 2: A plate showing the zone of growth inhibition of ethyl acetate of *Jatropha curcas* stem bark on Staphylococcus aureus.

Figure 2 illustrates the zone of growth inhibition of ethyl acetate of *Jatropha curcas* stem bark on *Staphylococcus aureus.* Ethyl acetate extract of *Jatropha curcas* stem bark extract had a zone of growth inhibition diameter of 20mm. Therefore, the ethyl acetate can be used as a suitable organic solvent extract on stem barks. The postive control used was ampicillin and had a zone of growth inhibition diameter of 30mm because it is a conventional antibiotic drug already on the market. The negative control used was distilled water and had no zone of growth inhibition because it lack bioactive constituents that kills the microbes.

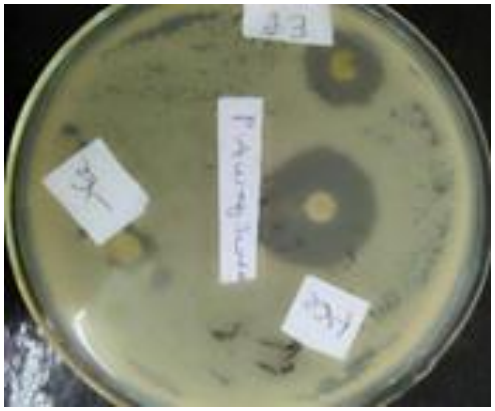

Figure 3: A plate showing the zone of growth inhibition of ethanolic extract of *Jatropha curcas* stem bark on *Pseudomonas aureginosa.*

Figure 3 illustrates the zone of growth inhibition of ethanolic extract of *Jatropha curcas* stem bark on *Pseudomonas aureginosa.* Ethanolic extract of *Jatropha curcas* stem bark had a zone of growth inhibition diameter of 18mm. Therefore, the ethanolic extract can be used as a suitable organic solvent extract on stem barks. The postive control used was ampicillin and had a zone of growth inhibition diameter of 26mm because it is a conventional antibiotic drug already on the market. The negative control used was distilled water and had no zone of growth inhibition because it lack bioactive constituents that kills the microbes.

Figure 4: A plate showing the zone of growth inhibition of ethanolic extract of *Jatropha curcas* stem.

Figure 4 illustrates the zone of growth inhibition of ethanolic extract of *Jatropha curcas* stem bark on *Fusarium oxyporum.* Ethanolic extract of *Jatropha curcas* stem bark had a zone of growth inhibition diameter of 16mm. Therefore, the ethanolic extract can be used as a suitable organic solvent extract on stem barks. The postive control used was clotrimazole and had a zone of growth inhibition diameter of 12mm because it is a conventional antifungal drug already on the market. The negative control used was distilled water and had no zone of growth inhibition because it lack bioactive constituents that kills the microbes bark on *Fusarium oxysporum.*

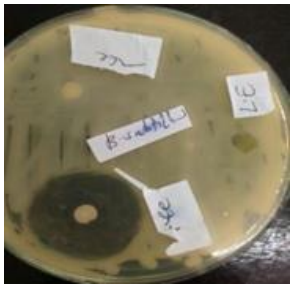

Figure 5: A plate showing the zone of growth inhibition of ethyl acetate extract of *Jatropha curcas* stem bark on *Bacillus subtills.*

Figure 5 illustrates the zone of growth inhibition of ethanolic extract of *Jatropha curcas* stem bark on *Bacillus subtills.* Ethyl acetate extract of *Jatropha curcas* stem bark had a zone of growth inhibition diameter of 6mm. Therefore, the ethanolic extract can be used as a suitable organic solvent extract on stem barks. The postive control used was ampicillin and had a zone of growth inhibition diameter of 30mm because it is a conventional antibiotic drug already on the market. The negative control used was distilled water and had no zone of growth inhibition because it lack bioactive constituents that kills the microbes.

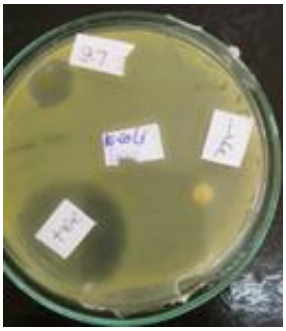

Figure 6: A plate showing the zone of growth inhibition of ethyl acetate extract of *Jatropha curcas stem* bark on *Escherichia coli.*

Figure 6 illustrates the zone of growth inhibition of ethanolic extract of *Jatropha curcas* stem bark on *Escherichia coli.* Ethyl acetate extract of *Jatropha curcas* stem bark had a zone of growth inhibition diameter of 15mm.Therefore, the ethanolic extract can be used as a suitable organic solvent extract on stem barks. The postive control used was ampicillin and had a zone of growth inhibition diameter of 30mm because it is a conventional antibiotic drug already on the market. The negative control used was distilled water and had no zone of growth inhibition because it lack bioactive constituents that kills the microbes.

Table 3: Showing the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Escherichia coli.*

	M.E	P.C	N.C	E.A.E	P.C	N.C	H.E	P.C	N.C	E.E	P.C	N.C
1st diameter (mm)	8	30	0	15	30	0	14	24	0	6	13	0
2nd diameter (mm	18	30	0	4	30	0	23	30	0	26	40	0
Mean	13	30	0	9.5	30	0	18.5	27	0	16	26.5	0

Key

M.E – Methanolic extract E.E – Ethanolic extract P.C – Positive control

N.C – Negative control E.A.E – Ethyl acetate extract H.E – hexane extract

Table 3 illustrates the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Escherichia coli.* The hexane extract of *Jatropha curcas* stem bark extract had a greater mean diameter of zone growth inhibition of 18.5mm on *Escherichia coli*. Because hexane extract of *Jatropha curcas* stem bark had bioactive constituents which prevented the growth of microbes. The ampicillin (antibiotic drug) used as postive control had a greater mean diameter of growth inhibition of 30mm. The distilled water used as negative control had a mean diameter of zone of growth inhibition of 0.0mm because it lacks bioactive constituents which kills the microbes.

Table 4: shows the mean diameter of the zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Staphylococcus aureus.*

	M.E	P.C	N.C	E.A.E	P.C	N.C	H.E	P.C	N.C	E.E	P.C	N.C
1st diameter (mm)	25	30	0	14	40	0	9	32	0	6	40	0
2nd diameter (mm	6	35	0	20	30	0	20	30	0	6	26	0
Mean	15.5	32.5	0	17	35	0	14.5	31	0	6	33	0

Key
M.E – Methanolic extract E.A.E – Ethyl acetate extract H.E – hexane extract
E.E – Ethanolic extract P.C – Positive control N.C – Negative control

Table 4 illustrates the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Stapylococcus aureus.* The ethyl acetate extract of *Jatropha curcas* stem bark had a greater mean diameter of zone growth inhibition of 17mm on *Staphylococcus aureus*. Because ethyl acetate extract of *Jatropha curcas* stem bark had bioactive constituents which prevented the growth of microbes. The ampicillin (antibiotic drug) used as postive control had a greater mean diameter of growth inhibition of 35mm. The distilled water used as negative control had a mean diameter of zone of growth inhibition of 0.0mm because it lacks bioactive constituents which kills the microbes.

Table 5: shows the mean diameter of the zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Pseudomonas aureginosa.*

	M.E	**P.C**	**N.C**	**E.A.E**	**P.C**	**N.C**	**H.E**	**P.C**	**N.C**	**E.E**	**P.C**	**N.C**
1st diameter (mm)	6	10	0	10	10	0	6	8	0	18	26	0
2nd diameter (mm)	14	10	0	0	16	0	6	8	0	16	22	0
Mean	10	10	0	10	13	0	6	8	0	17	24	0

Key
M.E – Methanolic extract E.A.E – Ethyl acetate extract H.E – hexane extract
E.E – Ethanolic extract P.C – Positive control N.C – Negative control

Table 5 illustrates the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Pseudomonas aureginosa.* The ethanolic extract of *Jatropha curcas* stem bark had a greater mean diameter of zone growth inhibition of 17mm on *Pseudomonas aureginosa*. Because ethanolic extract of *Jatropha curcas* stem bark had bioactive constituents which prevented the growth of microbes. The ampicillin (antibiotic drug) used as postive control had a greater mean diameter of growth inhibition of 24mm. The distilled water used as negative control had a mean diameter of zone of growth inhibition of 0.0mm because it lacks bioactive constituents which kills the microbes.

Table 6: shows the mean diameter of the zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Bacillus subtills.*

	M.E	**P.C**	**N.C**	**E.A.E**	**P.C**	**N.C**	**H.E**	**P.C**	**N.C**	**E.E**	**P.C**	**N.C**
1st diameter (mm)	12	26	0	6	30	0	6	26	0	6	30	0
2nd diameter (mm)	12	30	0	6	30	0	16	3	0	6	30	0
Mean	12	28	0	6	30	0	11	28	0	6	30	0

Key
M.E – Methanolic extract E.A.E – Ethyl acetate extract H.E – hexane extract
E.E – Ethanolic extract P.C – Positive control N.C – Negative control

Table 6 illustrates the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Bacillus subtills.* The methanolic extract of *Jatropha curcas* stem bark had a greater mean diameter of zone growth inhibition of 12mm on Bacillus subtills. Because methanolic extract of *Jatropha curcas* stem bark had bioactive constituents which prevented the growth of microbes. The ampicillin (antibiotic drug) used as postive control had a greater mean diameter of growth inhibition of 30mm. The distilled water used as negative control had a mean diameter of zone of growth inhibition of 0.0mm because it lacks bioactive constituents which kills the microbes.

Table 7: showing the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Fusarium oxysporuim.*

	M.E	**P.C**	**N.C**	**E.A.E**	**P.C**	**N.C**	**H.E**	**P.C**	**N.C**	**E.E**	**P.C**	**N.C**
1st diameter (mm)	16	6	0	8	8	0	6	6	0	16	12	0
2nd diameter (mm	6	10	0	10	10	0	6	12	0	14	16	0
Mean	11	8	0	9	9	0	6	9	0	15	14	0

Key
M.E – Methanolic extract E.A.E – Ethyl acetate extract H.E – hexane extract
E.E – Ethanolic extract P.C – Positive control N.C – Negative control

Table 7 illustrates the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Fusarium oxysporum*. The ethanolic extract of *Jatropha curcas* stem bark had a greater mean diameter of zone growth inhibition of 15mm on *Fusarium oxysporum*. Because ethanolic extract of *Jatropha curcas* stem bark had bioactive constituents which prevented the growth of microbes. The clotrimazole (antifungal drug) used as postive control had a greater mean diameter of growth inhibition of 14mm. The distilled water used as negative control had a mean diameter of zone of growth inhibition of 0.0mm because it lacks bioactive constituents which kills the microbes.

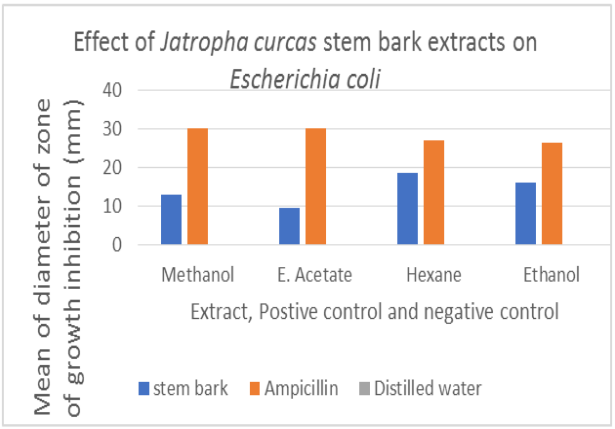

Figure 7: Illustrates the effect of Jatropha extracts on *Escherichia coli.*

Figure 7 illustrates the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Escherichia coli*. The hexane extract of *Jatropha curcas* stem bark extract had a greater mean diameter of zone growth inhibition of 18.5mm on *Escherichia coli*. Because hexane extract of *Jatropha curcas* stem bark had bioactive constituents which prevented the growth of microbes. The ampicillin (antibiotic drug) used as postive control had a greater mean diameter of growth inhibition of 30mm. The distilled water used as negative control had a mean diameter of zone of growth inhibition of 0.0mm because it lacks bioactive constituents which kills the microbes.

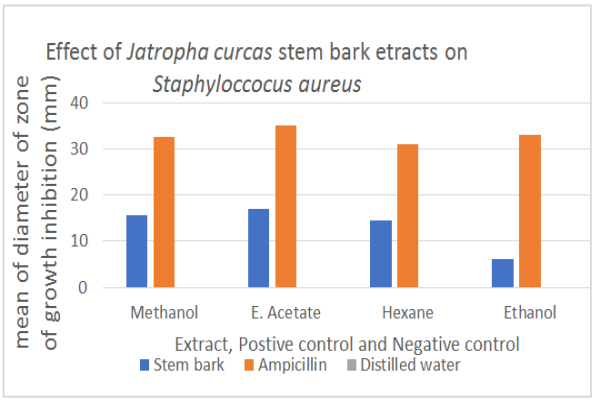

Figure 8: showing the effect of *Jatropha curcas* stem bark extracts on *Staphylococcus aureus.*

Figure 8 illustrates the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Stapylococcus aureus*. The ethyl acetate extract of *Jatropha curcas* stem bark had a greater mean diameter of zone growth inhibition of 17mm on *Staphylococcus aureus*. Because ethyl acetate extract of *Jatropha curcas* stem bark had bioactive constituents which prevented the growth of microbes. The ampicillin (antibiotic drug) used as postive control had a greater mean diameter of growth inhibition of 35mm. The distilled water used as negative control had a mean diameter of zone of growth inhibition of 0.0mm because it lacks bioactive constituents which kills the microbes.

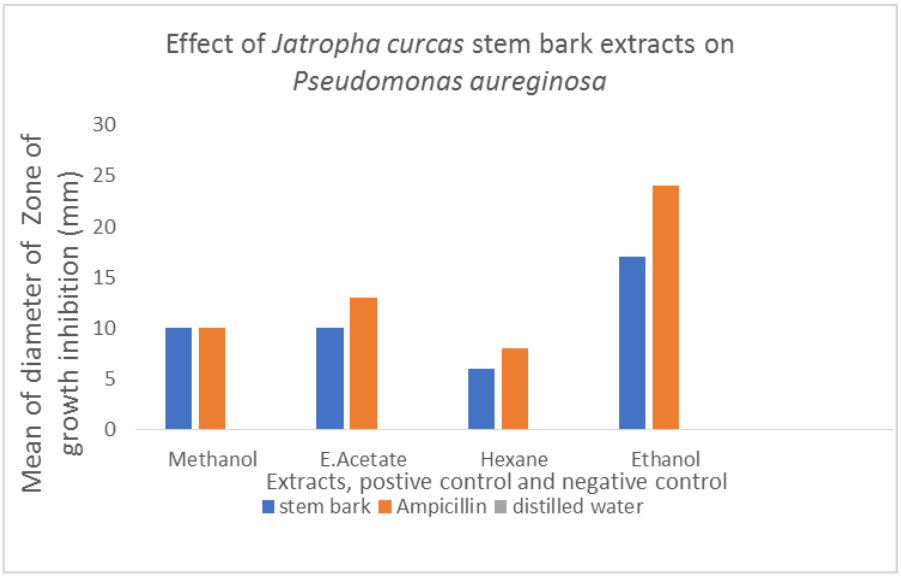

Figure 9: Showing the effect of *Jatropha curcas stem bark extracts* on *Pseudomonas aureginosa*.

Figure 9 illustrates the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Pseudomonas aureginosa.* The ethanolic extract of *Jatropha curcas* stem bark had a greater mean diameter of zone growth inhibition of 17mm on *Pseudomonas aureginosa*. Because ethanolic extract of *Jatropha curcas* stem bark had bioactive constituents which prevented the growth of microbes. The ampicillin (antibiotic drug) used as postive control had a greater mean diameter of growth inhibition of 24mm. The distilled water used as negative control had a mean diameter of zone of growth inhibition of 0.0mm because it lacks bioactive constituents which kills the microbes.

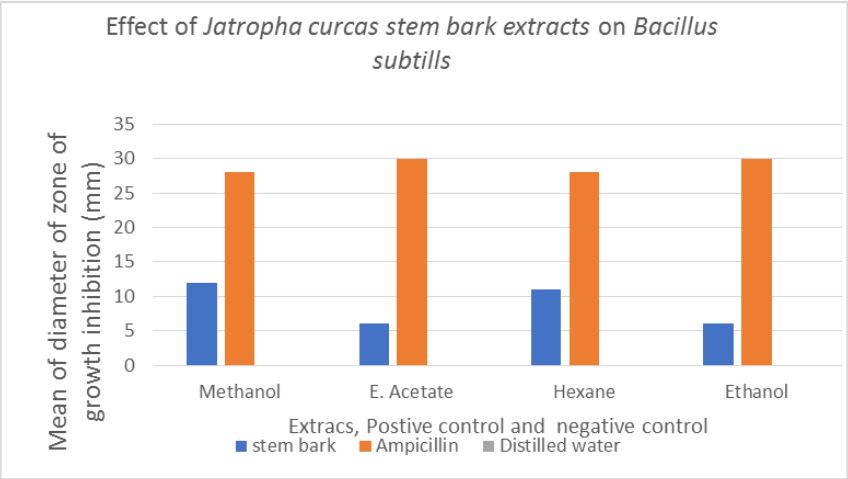

Figure 10: showing the effect of *Jatropha curcas* stem bark extracts on *Bacillus subtills.*

Figure 10 illustrates the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Bacillus subtills.* The methanolic extract of *Jatropha curcas* stem bark had a greater mean diameter of zone growth inhibition of 12mm on Bacillus subtills. Because methanolic extract of *Jatropha curcas* stem bark had bioactive constituents which prevented the growth of microbes. The ampicillin (antibiotic drug) used as postive control had a greater mean diameter of growth inhibition of 30mm. The distilled water used as negative control had a mean diameter of zone of growth inhibition of 0.0mm because it lacks bioactive constituents which kills the microbes.

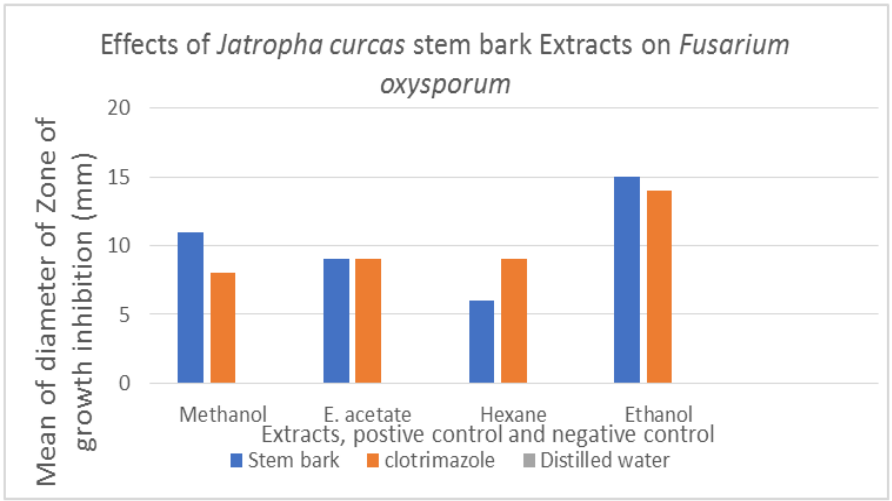

Figure 11: Illustrates the effect of *Jatropha curcas* stem bark extracts on *Fusarium oxysporum*.

Figure 11 illustrates the mean diameter of zone of growth inhibition caused by *Jatropha curcas* stem bark extracts on *Fusarium oxysporum*. The ethanolic extract of *Jatropha curcas* stem bark had a greater mean diameter of zone growth inhibition of 15mm on *Fusarium oxysporum*. Because ethanolic extract of *Jatropha curcas* stem bark had bioactive constituents which prevented the growth of microbes. The clotrimazole (antifungal drug) used as postive control had a greater mean diameter of growth inhibition of 14mm. The distilled water used as negative control had a mean diameter of zone of growth inhibition of 0.0mm because it lacks bioactive constituents which kills the microbes.

The current study also looked at the antibacterial and antifungal assay of *Jatropha curcas* stem bark extracts against *Escherichia coli, Staphylococcus aureus, Pseudomonas aureginosa,* and *Bacillus subtilis* and the fungi *Fusarium oxysporum*.

All the *Jatropha curcas* stem bark extracts managed to inhibit the growth of *Escherichia coli, Staphylococcus aureus, Pseudomonas aureginosa, Bacillus subtilis, and* the fungus *Fusarium oxysporum* (table (3, 4, 5, 6 and 7) and (figure 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11) because of the phytochemicals such as alkaloids, steroids, glycosides, terpenoids, flavonoids, saponins and phenols that were present in the stem bark extracts. Therefore, these secondary metabolites were able to inhibit the growth of both bacteria and fungi because of their antifungal and antibacterial activity.

Escherichia coli was observed to be most sensitive to hexane extract with a mean inhibition zone of 18.5mm and was also observed to be least sensitive to ethyl acetate extract with a mean inhibition zone of 9.5mm (figure 7). The *Staphylococcus aureus* was observed to be most sensitive to methanol extract with a mean inhibition zone of 15.5mm and also was observed to be least sensitive to ethyl acetate extract with a mean inhibition zone of 6mm (figure 8). The *Pseudomonas aureginosa* was observed to be most sensitive to ethanol extract with a mean zone of inhibition of 17mm and was observed to be the least sensitive to hexane extract with a mean inhibition zone of 6mm (figure 9). *Bacillus subtilis* were observed to be most sensitive to methanol extract with a mean zone of inhibition of 12mm and also was observed to be the least sensitive to ethanol and ethyl acetate extracts with a mean zone of inhibition of 6mm (figure 10). Finally, the *Fusarium oxysporum* was observed to be the most sensitive to ethanol extract with a mean inhibition zone of 15mm and also was observed to be the least sensitive to hexane extract with a mean inhibition zone of 6mm (figure 11).

Shinde[22] reported that *Jatropha curcas L*. leaf extract managed to inhibit the growth of *Klebsiella pneumoniae*, *Proteus sp*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aureginosa.* In our study, *Jatropha curcas* stem bark extracts also managed to suppress the growth of *Staphylococcus aureus*, *Escherichia coli*, and *pseudomonas aeruginosa.*

Furthermore, Prastiyanto and his colleagues[23] reported that the latex extract of *Jatropha curcas*, *Jatropha gossypilofia*, and *Jatropha mulfida* managed to inhibit the growth of methicillin-resistant *Staphylococcus aureus* and carbapenemase resistant *Pseudomonas aeruginosa* with the zone of inhibition of (20.4–23.7mm and 12–15mm) respectively. In our study, *Jatropha curcas* ethyl acetate and ethanolic stem bark extracts managed to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with a zone of inhibition diameter of 17mm each. The results are comparable.

Arekemase et al[16] reported that *Staphylococcus aureus* was observed to be the most sensitive to root extracts of *Jatropha curcas* with an inhibition diameter of 7mm at 0.1ml. *Escherichia coli* and *Pseudomonas aeruginosa* were not sensitive to hexane extract of *Jatropha curcas* root with no zone of inhibition. But for our study, hexane extract of *Jatropha curcas* stem bark was able to inhibit the growth of *Escherichia coli* and *Pseudomonas aeruginosa* with a mean inhibition zone of 18.5mm and 6mm respectively (figures 7 and 9). This could be attributed to the difference in the parts of extraction; they used the roots while we used the stem bark. Arekemase et al[16] also reported the latex and root extract of the *Jatropha curcas* indicated a potential antimicrobial activity against *Pseudomonas aeruginosa, Escherichia coli, Aspergillus flavus Staphylococcus aureus, Neisseria gonorrhea, and Candida albican*. Compared to our study, *Jatropha curcas* stem bark also managed to inhibit the growth of *Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus. Jatropha mollissima* indicated antibacterial activity and curbed the activity of the antibiotic Norfloxacin, encouraging more studies on medicinal plant extracts.[24]

Extracts from leaves and seeds of *Jatropha curcas* were used as antifungal agents to mitigate *Colletrotricum gleosporioides* which leads to anthracnose disease in papaya *in vitro*,[25] which correlates to our case where the stem bark extract managed to inhibit the growth of *Fusarium oxyporium*. On the other hand, Doughari & Abraham,[17] reported that *Jatropha curcas* stem bark, leaves, and sap extracts managed to inhibit the growth of *Candida albican* and *Candida tropicals* with a zone of inhibition of 13.00mm, 11.00mm, and 20.00mm respectively, in our study, the ethanolic extract of stem bark managed to inhibit the growth of fungi known as *Fusarium oxysporium* with the zone of inhibition of 15.00mm.

Jatropha curcas leaf extracts contain antifungal properties since they repressed the growth of *P. personata*. Furthermore, the fungicidal effect increased with an increase in concentration.[26] Ethanol stem extract of *Jatropha curcas* showed a growth zone of inhibition of 40mm on *Klebsiella pneumonia* and aqueous root extract had a growth zone of inhibition of 35.25mm on *Escherichia coli*. Compared to our study, the growth of *Escherichia coli* was also inhibited by hexane stem bark extract with an 18.5mm diameter. In addition, *Jatropha curcas* methanol extract of root and ethanol extract of flower had an antifungal activity of 27.25mm and 38.5mm against *Penicillium notatum* and *Aspergillus niger* respectively according to Idrees et al.[18] The results clearly show that the plant can be used against a variety if fungal species.

Aqueous extracts of *Jatropha curcas* related most favorably with the gentamycin on entire coliform bacteria exclusively on *Escherichia coli* and *Klebsiella pneumoniae*.[19] Tripathi et al[27] reported that methanolic

extract of the *Jatropha curcas* seed cake indicated antibacterial activity on both gram-negative and gram-positive bacteria. The ethyl acetate stem bark extract inhibited the growth of both the gram-positive and gram-negative bacteria. Furthermore, ethyl acetate extract also repressed the growth of fungi such as *Candida oryzae, Candida albican, Aspergillus niger*, and *Saccharomyces cerevisiae*.[28] In our study, *Jatropha curcas* stem bark extracts also managed to inhibit the growth of both the gram-negative and gram-positive bacteria and the fungi known as *Fusarium oxysporium.*

The positive control (Ampicillin) for the antibacterial assay was observed to have the greatest mean zone of inhibition of the 35mm on the *Staphylococcus aureus* (figure 8) while it had the lowest mean zone of inhibition of 8mm on *Pseudomonas aeruginosa* (figure 9). The positive control (clotrimazole) for the antifungal assay was observed to have the mean zone of inhibition of 14mm on *Fusarium oxysporum* (figure 11). The negative control (distilled water) was used for both antifungal and antibacterial assay and it had no zone of inhibition because it had no bioactive compound that can inhibit the growth of microbes.

The *Jatropha curcas* stem bark extracts had different diameters zones of growth of inhibition because different extracts were unable to extract the sufficient content of specific phytochemicals like alkaloids which have antimicrobial activity. Also, maybe the phytochemicals known to have antimicrobial activity were impregnated on the paper discs and the phytochemicals were unevenly distributed in the Eppendorf tubes where the paper discs were dipped before placing on the Petri plate.

The results presented above indicated that stem bark extracts from *Jatropha curcas* exhibited antimicrobial properties thus justifying scientifically its traditional use as a medicinal plant. In this study, the antimicrobial test of stem bark extracts showed that the *Jatropha curcas* plant exhibited a broad spectrum of activities by inhibiting the growth of five bacteria and one fungus used. It is worth noting that this plant showed activity against microbes causing diseases and since these test organisms are also implicated in a wide variety of infections, it, therefore, means that the constituents of the stem bark of the *Jatropha curcas* plant are potential agents useful in phytomedicine.

5. CONCLUSION

The study revealed the presence of many secondary metabolites in the *Jatropha curcas* stem bark. It further confirmed that the plant extracts were able to inhibit the growth of both bacteria and fungi. These results show that *Jatropha curcas* could be exploited for new potent antibiotics and antifungal agents.

6. ACKNOWLEDGEMENT

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7. AUTHORS CONTRIBUTION STATEMENT

Everlyne Isoe designed the study and did the article writing while George Kubende did the laboratory assays and data analysis.

8. CONFLICT OF INTEREST

There is no conflict of interest among the authors

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