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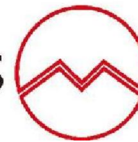

Phyto-chemical Screening of Seed Oil, Yield and Antibacterial Effect Of *Jatropha Curcas* And *Ricinus Communis* Oils Fractions Against Selected Bacteria

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Abstract: The antibacterial effect of *Jatropha curcas* and *Ricinus communis* oils fractions against *Staphylococcus aureus* (ATCC 6538)*, Bacillus subtilis* (ATCC 6633)*, Escherichia coli* (LMG 21766) and *Pseudomonas aeruginosa* (ATCC 9027) was carried out. The extracts were obtained by mechanical exhaustive extraction using the hydraulic press to obtain crude oil extracts from the seeds. The evaluation of phytochemicals, the crude of extract were fractionated by thin layer and column chromatography carried out qualitatively following standard methods. Phytochemical screening showed the presence of steroids, alkaloids, saponins, tannins and glycosides in both oil extracts. Five (5) fractions each were obtained for both *J. curcas* and *R. communis.* Fraction 2 had significantly higher (P<0.05) effect against the test organisms for both oil extracts. Fractions1, 3, 4 and 5 also showed significant effects (8.0±0.50-26.5±0.50 mm) against all the test organisms for both oil extracts. The fractions and their combinations were more effective for *J. curcas* than *R. communis* against the test organisms. The combination of fractions 1 & 2 for *J. curcas* had significantly higher (P<0.05) effect against *Staphylococcus aureus* (32.0±0.20 mm), *Bacillus subtilis* (28.0±0.00 mm) and *Escherichia coli* (26.0±1.50 mm) than for *Pseudomonas aeruginosa* (21.0±0.50 mm) at 100 mg/ml.
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Keywords: *Jatropha curcas, Ricinus communis* oils, Phytochemical, fractions.

1. Introduction

The trend of developing drugs from natural sources scientifically is not new. Most drugs are either herbal based or were developed as synthetic products from the natural sources. The use of natural/crude herbs or drugs to cure ailments is the oldest medicinal practice the world over. *Jatropha curcas* and castor plants have been reported to have a lot of health benefits because of their wide range of medicinal uses (Edeoga *et al*., 2005). Plants that have medicinal and anti-microbial values have either alkaloid; steroids, tannins, glycosides and various oils and they tend to be sites for the active medicinal ingredient of such plants (Odungbemi, 2006). Edeoga *et al.* (2005) found that the extract of *J. curcas* seeds has natural phytochemicals that could serve as agents against bacterial and fungal phytopathogens which could be used for agricultural applications at a low cost and safe practice.

The name *Jatropha curcas* meaning Doctor's nutrient, was related to its numerous medicinal uses. The medicinal uses of this species range from external, internal and even teeth (Agbogidi and Ekeke, 2011). Different parts of the plant including the leaves, fruits, latex and bark have been reported to contain glycosides, tannins, phytosterol, flavonoids and steroidal sapogenins that exhibits wide range of medicinal properties (Agbogidi and Eruotor, 2012). Flavonoids are phenolic compounds that are involved in plant-plant interaction (allelopathy, inhibition of germination and growth) while glycosides are synthesized for amino acids. The oil from *J. curcas* seeds is helpful with the management of rashes and parasitic skin diseases (Edeoga *et al*., 2005). From the report of Prminik (2002), when the oil is mixed with benzyl benzoate, it becomes effective against microbial infections such as scabies and dermatitis. The oil from the seed can also be applied to soothe rheumatic pain. *Jatropha* kernel oil together with about 36% linoleic acid is a possible interest for skin care industry(Prminik, 2002).

Ricinus communis seed oil and its compounds are utilized as a part of the making of

cleansers, oils, brake solvents, paints, inks, and color pigments, coatings, anti-low temperature safe plastics, polishes, nylon fiber, and in medicines (Bhagat and Kulkarni, 2010). According to Odungbemi (2006), ricinoleic acid is uncommon around the list of fatty acids in a sense that it holds an -OH group on the carbon at 12th number. Due to the presence of -OH group on ricinoleic acid *R. communis* seed oil shows more polarity as compared to other fats. The chemical reactivity of *R. communis* seed oil due to alcoholic -OH allows the chemical binding to other functional groups present which is not conceivable with many seed oils (Odungbemi, 2006). Therefore, there is need to evaluate fractions of *Jatropha curcas* and *Ricinus communis* oils for their antibacterial effects against *Staphylococcus aureus* (ATCC 6538)*, Bacillus subtilis* (ATCC 6633)*, Escherichia coli* (LMG 21766) and *Pseudomonas aeruginosa* (ATCC 9027).

2. Materials and Method

2.1 Study Area

This study was carried out at the Microbiology laboratory of University of Abuja, Gwagwalada Federal Capital Territory, Abuja.

2.2 Sample collection and identification

Healthy and mature *Jatropha curcas* and *Ricinus communis* seeds were collected from Gwagwalada FCT-Abuja and identified at, the University of Abuja Herbarium. The *Jatropha curcas* and *Ricinus communis* seeds collected were sorted, de-hulled, cleaned and dried (sun drying) to constant weights and the oils in the kernels were extracted mechanically.

2.3 Extraction and sterilization of *Jatropha curcas* and *Ricinus communis* oils

Extraction of oils from the kernels of *Jatropha curcas* and *Ricinus communis* was done according to the method used by Muzenda *et al.* (2012) that involved hot pressing using a hydraulic press. The clean dry kernels were crushed and then placed in the hydraulic press and pressed until they became cake to extract the oils. The resultant solid and colloidal matters were removed by sedimentation and filtered using a filter press. Finally, the oils were measured and sterilized using membrane filtration according to Muzenda *et al.* (2012) and stored in sterile bijoux bottles at 4^oC.

2.4 Qualitative phytochemical screening

Using the oil extracts, some phytochemical screening tests were carried out as described by Prabha and Vasantha (2012) for the presence of saponins, tannins and alkaloids, Lieberman Burchad reaction as described by Prabha and Vasantha (2012) was used to test for steroids, flavonoids and phenols, while the Salkowski test described by Prabha and Vasantha (2012) was used to test for the presence of glycosides.

2.4.1 Alkaloids

About 1ml each of *Jatropha curcas* and *Ricinus communis* oil was added to 2 ml of Mayer's reagent in each of the test tubes. The turbidity of the oil extract on addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the oil extract.

2.4.2 Saponins

About 0.2 ml each of *Jatropha curcas* and *Ricinus communis* oil was shaken with 5 ml of distilled water in test tubes and heated to boil. Frothing (appearance of creamy-like bubbles) showed the presence of saponins.

2.4.3 Tannins

About 1ml each of *Jatropha curcas* and *Ricinus communis* oil was poured in test tubes and boiled with 5 ml of distilled water for five minutes in a water bath. When cool, a drop (2-3) of 10% ferric chloride was added and observed for any colour change. A brownish–green precipitate indicated the presence of tannins.

2.4.4 Steroids

About 0.5 ml each of *Jatropha curcas* and *Ricinus communis* oil was added to 5 drops of acetic anhydride in test tubes followed by a drop of concentrated H_2SO4 into each of the tubes. The mixture was steamed for 1 hour and neutralized with drops of sodium hydroxide (NaOH), followed by the addition of two drops of chloroform. The appearance of a blue-green colour showed the presence of steroid.

2.4.5 Glycosides

About 0.5 ml each of *Jatropha curcas* and *Ricinus communis* oil was dissolved in 2ml of chloroform. Drops of concentrated H_2SO4 were 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.

2.4.6 Flavonoids

About 1 ml each of *Jatropha curcas* and *Ricinus communis* oil was added to 1 ml of 10% ferric chloride. A green precipitate indicated the presence of flavonoids.

2.4.7 Phenols

About 1 ml each of *Jatropha curcas* and *Ricinus communis* oil was added to 1 ml of 10% ferric chloride. The presence of blue precipitate confirmed the presence of phenols.

2.5 Preparation of *Jatropha curcas* and *Ricinus communis* oil extract concentrations

The stock solutions of the oil samples were prepared in screw capped bijoux bottles. Six different concentrations of each oil sample were prepared at 100 %, 50 %, 25 %, 12.5 %, 6.25 % and 3.1 % according to Olutiola *et al*. (2000). Doubling dilutions of the stock were made in screw capped bijoux bottles using dimethyl sulfoxide (DMSO) as diluent. Some 1.0 ml of stock sample was aseptically pipetted into a sterile bijoux bottle containing 1.0 ml of dimethyl sulfoxide (DMSO). The contents were mixed thoroughly. Then 1.0 ml of the dilution sample was aseptically pipetted into the next sterile bijoux bottle containing 1.0 ml of dimethyl sulfoxide (DMSO). The contents were mixed thoroughly. Other dilutions of solutions were similarly made up to 3.1%.

2.6 Test organisms

The test organisms (*Escherichia coli* (LMG 21766), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538) were obtained from the Diagnostic Division, NVRI, Vom, Jos. They were resuscitated by streak inoculation on Nutrient agar, incubated at 37 ^{0}C for 24 hrs and later on their various selective media and tested for purity by microscopy following Gram staining and subjected to conventional tests and preserved on fresh nutrient agar slants in a refrigerator at 4°C.

2.7 Identification of test organisms

The test organisms were identified on the basis of microscopy following Gram Staining and the characteristics used included growth patterns, colonial characteristics, color, shape, arrangement and entire surface of pure isolates which were observed by visual examinations.

Isolates from Nutrient Agar and Eeosin Methylene Blue agar (EMB) with green metallic sheen were subjected to IMViC series of tests. This provided additional evidence for the identification of *Escerichia coli*. It consists of Indole Production, Methyl red test, Voges Proskauer test and the citrate utilization test while *Staphylococcus aureus* was isolated on manitol salt agar (MSA). *Bacillus subtilis* and *Staphylococcus aureus* were subjected to catalase and coagulase tests followed by spore staining to further confirm *Bacillus subtilis*.

2.8 Standardization of the test organisms

The test organisms were standardized using standard curves. An inoculum of the slant culture of each test organism, *Escherichia coli* (LMG 21766), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538) was subculture unto freshly prepared nutrient agar plates and incubated for 18hrs at 37 ^{0}C.Ten - fold serial dilutions of each suspension were made from a discrete colony of each. A loop full of each test organism was separately incorporated in 10 ml of sterile distilled water as the stock culture. Ten - fold serial dilutions of the stock culture were made using sterile water as diluent. Then 1.0 ml of the dilution sample was aseptically pipetted into a sterile test tube containing 9.0 ml of sterile water. The contents were mixed thoroughly. Other ten-fold dilutions of solution were similarly made up to 10^{-6}. One (1) milliliter was taken and discarded from the last tube. Spectrophotometer was standardized using distilled sterile water. From the dilution tubes, samples were taken from every dilution into cuvettes to measure their optical densities and each dilution was plated by the spread plate technique for viable count. Finally, a graph of colony forming unit per ml (cfu/ml) against optical density was plotted to obtain standard curve of the test organisms.

2.9 Fractionation of oil extracts

The oils extracts were fractionated using thin Layer and column chromatography and the fractions were tested for antibacterial activity.

2.9.1 Thin Layer chromatography of *Ricinus communis* Oil and *Jatropha curcas* oil extracts

The thin layer chromatography (TLC) was carried out based on the method described by Philip (2003). The TLC profile of the oil extracts was obtained on pre-coated TLC plate using hexane/ethyl acetate and petroleum ether mixture in the ratio of 6:3:1 for *Jatropha curcas* oil and methanol and petroleum ether in the ratio of 4:1 for *Ricinus communis* oil in a beaker and covered to keep the concentration. The TLC plates of sizes 5.0 cm in width and 10cm in length were used. A line was drawn across the width of the plates at 1.0cm mark from the bottom of the plate as a starting mark using a ruler and a pencil. A capillary tube was then used to take the oil extracts on the centre of the line drawn on the TLC plate. The plates were taken into a chromatography chamber and observed till the solvent rose to the 6.1cm mark on the TLC plate which was the solvent front. The TLC plates were carefully removed from the chromatography chamber and observed under a UV lamp at 254/366nm to see

the separated components and each of the components were mark with a pencil. Also, the plates were sprayed with a mixture of 90% ethanol and 10% sulphuric acid followed by heating on a hot plate to get the colour and permanent spot on the TLC plate. The resolution front (RF) value was calculated for each of the fractions using the formula:

Rf = Distance travelled by solute.

Distance travelled by solvent. (Philip, 2003).

2.9.2 Column chromatography of *Ricinus communis* oil and *Jatropha curcas*oil extracts

The flash column was washed, dried and clamped vertically unto a retort stand. A piece of cotton wool was introduced into the clean dry column followed by 30g absorbent silica gel. The column was tapped gently to give a uniform packing. Some 15g of oil extract was weighed and poured into the column followed by the addition of solvent. Solvent elution was started with 100% hexane followed by hexane/ethyl acetate (80:20 %, 60:40 %, 40:60 %, 20:80 % v/v, 100% ethyl acetate and 100% methanol respectively) for both *J. curcas* and *R. communis* oils. The fractions were collected in 50ml beakers and evaporated. The fractions were further purified using thin layer chromatography (TLC) using 100% chloroform for *J. curcas oil* and Hexane-ethyl acetate-methanol (4:4:1) for *R. communis* oil as the solvent system and 10% sulphuric acid (H_2SO_4) as spray reagent for both oil extracts. This was done according to Philip (2003). The fractions were tested for antibacterial activity individually and in combinations.

2.10 Antibacterial assay

Antibacterial activity of the oils against the test organisms was determined using agar well diffusion method described by Irshad *et al*. (2012). With the aid of a sterile pipette, 1 ml of an 18 hour broth culture of each test organism was aseptically seeded on the sterile solidified surfaces of Mueller Hinton Agar plates by flooding and the excess was aseptically drained. The plates were left undisturbed for about 15 minutes and with the aid of a sterile 5mm diameter cork borer, three wells were born on every seeded agar plate and were sufficiently separated and kept away from the edge of the plate and 25 mm from well to well to prevent overlapping of zones. The base of each well was sealed using 2 drops of molten Mueller Hinton agar. Into each of the wells was added 2 drops (0.4 ml) of a known concentration of each oil sample on well 1, chloramphenicol into well 2 and the diluent (DMSO) into well 3, of the plates seeded with *Escherichia coli* and *Pseudomonas aeruginosa* while ampicillin was added into well 2 for the plates seeded with *Staphylococcus aureus* and *Bacillus subtilis* using sterile Pasteur pipettes. Chloramphenicol and Ampicillin were used as positive controls while the diluent (DMSO) was used as Negative Control. The plates were allowed to stand undisturbed for about 30 minutes at room temperature for the oils to diffuse and were incubated at 37°C for 24 hours. After 24 hrs, the diameters of the zones of inhibition around the wells were measured with the aid of a transparent metric ruler and recorded. The antibacterial study was done in triplicates and mean zone diameters of inhibition (mm) were determined. The standard of the antibacterial susceptibility testing according to Irshad *et al*. (2012), which is 10 mm was used for result interpretations.

2.10 Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined using the method described by Olutiola *et al*. (2000). In this assay, the broth dilution technique was utilized where the MIC was tested against six different concentrations (100%, 50%, 25 %, 12.5 %, 6.25 % and 3.1 %), of each oil sample obtained through doubling dilution using DMSO as the diluent. For every test organism, 18hrs broth culture of the test organism was diluted and the number of organisms determined from the standard curve. This assay was done by mixing 10 ml of nutrient broth with 100µL of oil samples of the different concentrations. After that, 10µL of standardized bacterial culture was added to all the tubes, initial optical densities were taken and were incubated for 24 hours at 37°C. After 24 hours, final optical densities were taken again and all the tubes were compared to Control tubes for turbidity. Chloramphenicol and Ampicillin were used as positive controls while DMSO was used as Negative Control. The least concentration were there was no increase in number from the standard curve(No increase in optical density)and visually was taken as the minimum inhibitory concentration (MIC).

2.11 Determination of Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) of the oils was determined as described by Doughari *et al.* (2007). This was determined from the broth dilution resulting from the minimum inhibitory concentration (MIC) tubes. Samples were taken from tubes with no increases in optical density and visually in the minimum inhibitory concentration (MIC) assay and inoculated using a sterile wire loop on freshly prepared nutrient agar plates and incubated at 37°C for 48 hours. The lowest concentration of the oil

samples which showed no bacterial growth was taken as the minimum bactericidal concentration (MBC).

2.12 Statistical analysis

Data obtained in this study were analyzed statistically using the Statistical package for social sciences (SPSS) for windows version involving parametric test such as ANOVA at P<0.05.

3. Results

The results are presented as follows:

3.1 Phytochemical components of *Jatropha curcas* and *Ricinus communis* oil extracts

Table 1 shows the results of phytochemical screening of *Jatropha curcas* and *Ricinus communis* oil extracts. From the data, both *Jatropha curcas* and *Ricinus communis* oil extracts tested positive for the phytochemical tested which showed the presence of steroids, alkaloids, saponins, tannins and glycosides.

Table 1: Qualitative phytochemical constituents of *Jatropha curcas* and *Ricinus communis* oil extracts

Phytochemical Components	Extracts	
	***Jatropha curcas* oil**	***Ricinus communis* oil**
Alkaloids	+	+
Phenols	-	+
Tannins	+	+
Saponins	+	+
Steroids	+	+
Flavonoids	+	-
Glycosides	+	+

Key: += Present, -=Negative

4.2 Antibacterial effects of fractions of *Jatropha curcas* and *Ricinus communis* oil extract

Tables 2 and 3 show the effects of column fractions of *Jatropha curcas* and *Ricinus communis* oil extracts respectively against the test organisms. It can be seen that fraction 2 of *J. curcas* had significantly higher (P< 0.05) effect against *Staphylococcus aureus* (26.5±0.50 mm), *Bacillus subtilis* (24.0±0.00 mm), *Escherichia coli* (21.0±0.00 mm) and *Pseudomonas aeruginosa* (20.0±1.00 mm) at 100 % (Table 8). From the same Table 6, fractions1, 3, 4 and 5 show significant effect against all test organisms. The antibacterial effects of *J. atropha curcas* and *Ricinus communis* oil fractions increased with concentrations, whereas the antibacterial effects of the controls were significantly higher (P< 0.05) against all the fractions.

Likewise, it can be seen that fraction 2 of *R. communis* oil extracts also had significantly higher (P< 0.05) effect against *Staphylococcus aureus* (23.5±0.50 mm), *Bacillus subtilis* (21.0±0.00 mm), *Escherichia coli* (19.0±1.00 mm) and *Pseudomonas aeruginosa* (17.5±2.50 mm) at 100 % (Table 7). From the same Table 7, fractions1, 3, 4 and 5 show significant effect against all test organisms. The antibacterial effect of *Ricinus communis* oil fractions increased with concentrations. The antibacterial effect of *Jatropha curcas* and *Ricinus communis* oil extracts against the test organisms were significantly different (P< 0.05).

Table 2: Antibacterial effects of fractions of *Jatropha curcas* oil extract showing mean zones of inhibition in millimeter

Fractions	Test Organisms	Concentrations in %					
		100	50	25	12.50	6.25	3.13
Fraction 1	Ps	18.0±0.50	17.5±0.50	16.0±3.00	15.5±0.50	14.0±0.50	10.0±1.00
	Bs	23.0±2.00	21.0±1.00	19.5±0.50	18.0±1.50	16.0±1.00	13.0±2.00
	Sa	25.0±1.00	23.0±2.50	21.0±2.00	19.5±1.50	18.0±2.00	16.5±1.50
	Ec	20.0±1.50	19.5±0.00	17.5±0.50	16.0±1.50	15.0±1.50	11.0±0.00
Fraction 2	Ps	20.0±1.00	18.5±1.50	17.0±1.00	16.5±0.50	15.0±3.00	11.0±1.50
	Bs	24.0±0.00	22.0±2.00	21.0±1.00	19.0±1.00	17.0±2.00	14.5±0.50
	Sa	26.5±0.50	25.0±1.00	23.5±1.50	21.0±1.00	19.5±1.00	18.0±1.00
	Ec	21.0±0.00	20.0±0.50	18.5±0.00	16.5±0.00	15.5±0.50	12.5±2.00
Fraction 3	Ps	19.5±1.00	18.0±0.50	14.5±0.50	14.0±1.50	12.0±0.50	9.0±1.00
	Bs	21.0±0.00	19.0±0.00	18.0±2.00	15.0±1.00	13.0±1.00	11.0±0.00
	Sa	22.0±1.00	20.0±1.50	19.0±0.00	16.0±2.00	14.0±2.00	12.0±2.00
	Ec	20.0±2.00	19.0±1.00	16.0±1.00	15.0±0.50	13.0±3.00	10.5±0.50
Fraction 4	Ps	18.0±0.00	16.0±1.50	14.0±2.00	12.0±0.50	10.0±0.50	10.0±2.00
	Bs	22.0±1.00	20.0±1.00	17.0±1.00	15.0±1.00	14.0±1.00	11.5±1.50
	Sa	23.0±0.00	21.0±0.00	18.0±2.00	17.0±0.00	15.0±2.00	13.0±0.00
	Ec	21.0±1.50	19.0±2.00	15.0±1.50	13.0±1.00	12.0±1.00	11.0±1.00
Fraction 5	Ps	16.5±0.00	15.0±0.50	13.0±0.50	10.5±2.50	10.0±1.00	8.0±0.50
	Bs	18.0±0.50	16.0±0.00	15.0±1.00	12.0±0.50	11.0±0.50	10.0±2.00
	Sa	20.0±2.00	18.0±0.50	17.0±2.00	14.0±0.00	12.0±2.00	11.0±1.50
	Ec	17.0±1.50	15.0±1.50	14.0±0.00	11.0±1.00	10.5±1.50	10.0±0.00
Controls							
CH	Ps	26.0±1.00	24.0±2.00	22.0±1.00	18.5±2.00	16.0±1.00	14.0±2.00
AMP	Bs	30.0±2.00	28.0±1.00	26.0±1.00	21.0±0.00	17.0±1.00	16.0±0.00
AMP	Sa	34.0±0.00	30.0±2.00	28.0±2.00	23.0±1.00	19.0±2.00	18.0±0.00
CH	Ec	28.0±1.00	26.0±1.00	25.0±1.50	21.0±2.00	18.5±1.50	15.0±1.00

Each value represents Mean± standard deviation from three replicate values.

Keys: Fraction 1= 100 % Hexane, Fraction 2 = 80: 20 % Hexane/Ethyl acetate, Fraction 3 = 60: 40 % Hexane/Ethyl acetate, Fraction 4 = 20: 80 % Hexane/Ethyl acetate, Fraction 5= 100% Methanol, Ps= *Pseudomonas aeruginosa*, Bs= *Bacillus subtilis*, Sa= *Staphylococcus aureus*, Ec= *Escherichia coli*

Table 3: Antibacterial effects of fractions of *Ricinus communis* oil extracts showing mean zones of inhibition in millimeter

Fractions	Test Organisms	Concentrations in %					
		100	50	25	12.50	6.25	3.13
Fraction 1	Ps	16.0±0.50	14.0±1.50	13.0±0.40	12.5±0.50	11.0±0.50	10.0±0.00
	Bs	19.5±0.00	17.0±0.50	16.0±0.70	14.0±1.00	13.0±0.50	12.0±1.00
	Sa	22.0±0.00	19.0±0.00	18.0±1.00	16.0±0.00	15.0±0.00	13.0±0.00
	Ec	18.0±0.50	16.0±1.00	14.0±2.00	13.8±1.20	12.0±1.50	10.5±1.50
Fraction 2	Ps	17.5±2.50	16.0±1.00	13.5±2.00	13.0±1.00	12.0±0.00	9.50±1.00
	Bs	21.0±0.00	18.0±0.50	17.0±1.00	15.0±2.50	13.0±0.50	11.0±0.50
	Sa	23.5±0.50	19.0±0.00	18.5±0.00	16.0±0.00	14.0±1.50	12.0±1.00
	Ec	19.0±1.00	17.0±0.50	15.0±0.50	14.2±1.10	12.0±0.00	10.0±0.00
Fraction 3	Ps	14.0±1.00	16.0±0.50	14.0±0.50	12.0±2.50	11.0±1.00	10.0±2.00
	Bs	18.0±2.00	18.0±0.00	17.0±0.50	16.5±1.00	13.0±2.00	12.0±0.50
	Sa	20.0±0.50	19.0±0.50	19.0±0.00	18.0±2.00	15.0±1.00	12.5±1.00
	Ec	16.0±0.00	16.0±0.00	15.5±1.00	15.0±0.50	13.0±0.00	11.0±0.00
Fraction 4	Ps	15.5±0.00	15.0±0.50	13.0±0.50	12.8±1.40	10.0±0.50	10.0±0.00
	Bs	18.5±1.00	18.0±0.00	17.3±0.11	15.0±0.50	13.0±0.00	11.0±1.50
	Sa	23.0±0.00	20.0±0.00	18.0±0.50	15.0±0.00	14.0±0.00	12.0±0.00
	Ec	16.0±1.50	16.0±0.50	15.0±0.00	14.0±2.00	13.0±0.50	11.0±1.00
Fraction 5	Ps	15.0±0.00	14.0±0.50	13.0±2.00	11.0±0.50	10.0±0.50	9.0±0.00
	Bs	17.0±0.50	15.0±0.00	12.0±0.20	10.0±0.00	10.0±0.00	10.0±0.10
	Sa	19.5±0.70	17.0±1.00	15.0±1.00	13.0±0.00	12.0±0.50	11.0±0.50
	Ec	16.0±0.00	15.0±0.00	15.0±0.00	13.0±0.30	11.0±0.00	10.0±0.30
Control							
CH	Ps	26.0±1.00	24.0±2.00	22.0±1.00	18.5±2.00	16.0±1.00	14.0±2.00
AMP	Bs	30.1±2.00	28.0±1.00	26.0±1.00	21.0±0.00	17.0±1.00	16.0±0.00
AMP	Sa	34.0±0.00	30.0±2.00	28.0±2.00	23.0±1.00	19.0±2.00	18.0±0.00
CH	Ec	28.0±1.00	26.0±1.00	25.0±1.50	21.0±2.00	18.5±1.50	15.0±1.00

Each value represents Mean± standard deviation from three replicate values.

Keys: Fraction 1= 100 % Hexane, Fraction 2 = 80: 20 % Hexane/Ethyl acetate, Fraction 3 = 60: 40 % Hexane/Ethyl acetate, Fraction 4 = 20: 80 % Hexane/Ethyl acetate, Fraction 5= 100% Methanol, Ps= *Pseudomonas aeruginosa*, Bs= *Bacillus subtilis*, Sa= *Staphylococcus aureus*, Ec= *Escherichia coli*.

3.3 Minimum inhibitory concentration of fractions of *Jatropha curcas* oil extract.

Table 4 shows the minimum inhibitory concentrations of *Jatropha curcas* oil extract fractions respectively against the test organisms. The minimum inhibitory concentration of *Jatropha curcas* oil extract for fraction 1 and 2 against *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633) were 12.50% respectively whereas *Pseudomonas aeruginosa* and *Escherichia coli,* 25% were obtained. For fraction 3, *Staphylococcus aureus* (ATCC 6538) was 12.5 % and *Bacillus subtilis* (ATCC 6633) was 25% whereas *Pseudomonas aeruginosa* and *Escherichia coli were* 50% respectively. The minimum inhibitory concentrations of *Jatropha curcas* oil for fractions 4 and 5 against test organisms were higher than that of fractions 1, 2 and 3 (Table 5) whereas the minimum inhibitory concentrations of the control were significantly different ($P<0.05$).

Table 4: Minimum inhibitory concentrations of fractions of *Jatropha curcas* oil extracts against test organisms

Fractions	Test Organisms	Concentrations in %					
		100	50	25	12.50	6.25	3.13
Fraction 1	Ps	-	-	-*	+	+	+
	Bs	-	-	-	-*	+	+
	Sa	-	-	-	-*	+	+
	Ec	-	-	-*	+	+	+
Fraction 2	Ps	-	-	-*	+	+	+
	Bs	-	-	-	-*	+	+
	Sa	-	-	-	-*	+	+
	Ec	-	-	-*	+	+	+
Fraction 3	Ps	-	-*	+	+	+	+
	Bs	-	-	-*	+	+	+
	Sa	-	-	-	-*	+	+
	Ec	-	-*	+	+	+	+
Fraction 4	Ps	-*	+	+	+	+	+
	Bs	-	-	-*	+	+	+
	Sa	-	-	-	-*	+	+
	Ec	-	-*	+	+	+	+
Fraction 5	Ps	-*	+	+	+	+	+
	Bs	-	-*	+	+	+	+
	Sa	-	-	-	-*	+	+
	Ec	-*	+	+	+	+	+
Control							
CH	Ps	-	-	-	-	-	+
AMP	Bs	-	-	-	-	-	+
AMP	Sa	-	-	-	-	-	+
CH	Ec	-	-	-	-	-	+

Keys: + = Growth, - = No growth, Fraction 1= 100 % Hexane, Fraction 2 = 80: 20 % Hexane/Ethyl acetate, Fraction 3 = 60: 40 % Hexane/Ethyl acetate, Fraction 4 = 20: 80 % Hexane/Ethyl acetate, Fraction 5= 100% Methanol, Ps= *Pseudomonas aeruginosa*, Bs= *Bacillus subtilis*, Sa= *Staphylococcus aureus*, Ec= *Escherichia coli,* -* = MIC.

4.8 Minimum bactericidal concentration of fractions of *Jatropha curcas* oil extract.

Table 5 shows the minimum bactericidal concentrations of *Jatropha curcas* oil extract fractions respectively against the test organisms. The minimum bactericidal concentration of *Jatropha curcas* oil extract for fraction 1 and 2 against *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633) were 12.50 % whereas *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (LMG 21766) were 25% respectively. For fraction 3, 4 and 5 only *Staphylococcus aureus* had minimum bactericidal concentration of 12.50%.

Table 5: Minimum bactericidal concentrations of fractions of *Jatropha curcas* oil extract against test organisms

Fractions	Test Organisms	Concentrations in %					
		100	50	25	12.50	6.25	3.13
Fraction 1	Ps	-	-	-*	+	+	+
	Bs	-	-	-	-*	+	+
	Sa	-	-	-	-*	+	+
	Ec	-	-	-*	+	+	+
Fraction 2	Ps	-	-	-*	+	+	+
	Bs	-	-	-	-*	+	+
	Sa	-	-	-	-*	+	+
	Ec	-	-	-*	+	+	+
Fraction 3	Ps	-	-*	+	+	+	+
	Bs	-	-	-*	+	+	+
	Sa	-	-	-	-*	+	+
	Ec	-	-*	+	+	+	+
Fraction 4	Ps	-*	+	+	+	+	+
	Bs	-	-	-*	+	+	+
	Sa	-	-	-	-*	+	+
	Ec	-	-*	+	+	+	+
Fraction 5	Ps	-*	+	+	+	+	+
	Bs	-	-*	+	+	+	+
	Sa	-	-	-	-*	+	+
	Ec	-*	+	+	+	+	+
Control							
CH	Ps	-	-	-	-	-	+
AMP	Bs	-	-	-	-	-	+
AMP	Sa	-	-	-	-	-	+
CH	Ec	-	-	-	-	-	+

Keys: + = Growth, - = No growth, Fraction 1= 100 % Hexane, Fraction 2 = 80: 20 % Hexane/Ethyl acetate, Fraction 3 = 60: 40 % Hexane/Ethyl acetate, Fraction 4 = 20: 80 % Hexane/Ethyl acetate, Fraction 5= 100% Methanol, Ps= *Pseudomonas aeruginosa*, Bs= *Bacillus subtilis*, Sa= *Staphylococcus aureus*, Ec= *Escherichia coli,* -* = MBC.

3.4 Minimum inhibitory concentration of fractions of *Ricinus communis* oil extract.

Tables 6 shows the minimum inhibitory concentrations of *Ricinus communis* oil extract fractions against *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (LMG 21766) and *Pseudomonas aeruginosa* (ATCC 9027) respectively. It can be seen that the minimum inhibitory concentration of *Ricinus communis* oil fraction 2 against *Staphylococcus aureus* (ATCC 6538) was 12.50% whereas for *Bacillus subtilis*25 %, *Pseudomonas aeruginosa* and *Escherichia coli* were 50 %each. The minimum inhibitory concentrations of *Ricinus communis* oil for fractions 1, 3, 4 and 5 against test organisms were significantly higher than that of the control ($P< 0.05$).

Table 6: Minimum inhibitory concentrations of fractions of *Ricinus communis* oil extracts against test organisms

Fractions	Test Organisms	Concentrations in %					
		100	50	25	12.50	6.25	3.13
	Ps	-	-*	+	+	+	+
Fraction 1	Bs	-	-*	+	+	+	+
	Sa	-	-*	+	+	+	+
	Ec	-	-*	+	+	+	+
	Ps	-	-*	+	+	+	+
Fraction 2	Bs	-	-	-*	+	+	+
	Sa	-	-	-	-*	+	+
	Ec	-	-*	+	+	+	+
	Ps	-	-*	+	+	+	+
Fraction 3	Bs	-	-*	+	+	+	+
	Sa	-	-*	+	+	+	+
	Ec	-	-*	+	+	+	+
Fraction 4	Ps	-	-*	+	+	+	+
	Bs	-	-*	+	+	+	+
	Sa	-	-*	+	+	+	+
	Ec	-	-*	+	+	+	+
						+	
Fraction 5	Ps	-*	+	+	+	+	+
	Bs	-*	+	+	+	+	+
	Sa	-	-*	+	+	+	+
	Ec	-*	+	+	+	+	+
Controls							
CH	Ps	-	-	-	-	-	+
AMP	Bs	-	-	-	-	-	+
AMP	Sa	-	-	-	-	-	+
CH	Ec	-	-	-	-	-	+

Keys: + = Growth, - = No growth, Fraction 1= 100 % Hexane, Fraction 2 = 80: 20 % Hexane/Ethyl acetate, Fraction 3 = 60: 40 % Hexane/Ethyl acetate, Fraction 4 = 20: 80 % Hexane/Ethyl acetate, Fraction 5= 100% Methanol, Ps= *Pseudomonas aeruginosa*, Bs= *Bacillus subtilis*, Sa= *Staphylococcus aureus*, Ec= *Escherichia coli,* -* = MIC.

3.5 Minimum bactericidal concentration of *Ricinus communis* oil extracts fractions

Tables 7 shows the minimum bactericidal concentrations of *Ricinus communis* oil extract fractions against *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (LMG 21766)and *Pseudomonas aeruginosa* (ATCC 9027) respectively. The minimum bactericidal concentration of *Ricinus communis* oil for fraction 1, 3 and 4 against all test organisms were 50 % whereas for fraction 2, *Staphylococcus aureus* had MBC of 12.50%, *Bacillus subtilis* had 25%, *Pseudomonas aeruginosa* and *Escherichia coli* had 50 % respectively. Also for fraction 5, only *Staphylococcus aureus* had minimum bactericidal concentration of 50 %while *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* had 100% each which were significantly higher than that of the control ($P<0.05$).

Table 7: Minimum bactericidal concentrations of fractions of *Ricinus communis* oil extract against test organisms

Fractions	Test Organisms	Concentrations in %					
		100	50	25	12.50	6.25	3.13
Fraction 1	Ps	-	-*	+	+	+	+
	Bs	-	-*	+	+	+	+
	Sa	-	-*	+	+	+	+
	Ec	-	-*	+	+	+	+
Fraction 2	Ps	-	-*	+	+	+	+
	Bs	-	-	-*	+	+	+
	Sa	-	-	-	-*	+	+
	Ec	-	-*	+	+	+	+
Fraction 3	Ps	-	-*	+	+	+	+
	Bs	-	-*	+	+	+	+
	Sa	-	-*	+	+	+	+
	Ec	-	-*	+	+	+	+
Fraction 4	Ps	-	-*	+	+	+	+
	Bs	-	-*	+	+	+	+
	Sa	-	-*	+	+	+	+
	Ec	-	-*	+	+	+	+
						+	
Fraction 5	Ps	-*	+	+	+	+	+
	Bs	-*	+	+	+	+	+
	Sa	-	-*	+	+	+	+
	Ec	-*	+	+	+	+	+
Controls							
CH	Ps	-	-	-	-	-	+
AMP	Bs	-	-	-	-	-	+
AMP	Sa	-	-	-	-	-	+
CH	Ec	-	-	-	-	-	+

Keys: + = Growth, - = No growth, Fraction 1= 100 % Hexane, Fraction 2 = 80: 20 % Hexane/Ethyl acetate, Fraction 3 = 60: 40 % Hexane/Ethyl acetate, Fraction 4 = 20: 80 % Hexane/Ethyl acetate, Fraction 5= 100% Methanol, Ps= *Pseudomonas aeruginosa*, Bs= *Bacillus subtilis*, Sa= *Staphylococcus aureus*, Ec= *Escherichia coli,* -* = MBC.

4.0 Discussions

The broader spectrum of antibacterial activities observed in this study is interesting and could be due to the synergic effects of the various components in the *Jatropha curcas and Ricinus communis* oil extracts. In this study, the antibacterial effects of the fractions of *Jatropha curcas* were significantly different from that of the *Ricinus communis* oil (P< 0.0) against *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (LMG 21766) and *Pseudomonas aeruginosa* (ATCC 9027) whereas the effects of controls were significantly higher (P>0.05). Momoh *et al*. (2012) also reported the evaluation of the antimicrobial and phytochemical properties of oil from castor seeds (*Ricinus communis* Linn). In this study, Both *Jatropha curcas* and *Ricinus communis* combine fractions had antimicrobial effects on all the test microorganisms but *Jatropha curcas* had higher antimicrobial activity than the *Ricinus communis*. Antimicrobial properties of the different parts of castor plant have been reported by Ramos-L´opez *et al.* (2010). The antibacterial mechanism of the extracts is not known, but it can be attributed to the presence of the major phytochemicals that were detected in this studies. The presence of alkaloids, saponin, tannin, steroids and glycosides in *Jatropha curcas and Ricinus communis* has been reported by

Aiyelaagbe *et al*. (2007) which is in agreement with this study. Extract of *Jatropha curcas* has significantly higher (P<0.05) effects than *Ricinus communis* oil against *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (LMG 21766)and *Pseudomonas aeruginosa* (ATCC 9027).

The accumulation and concentration of secondary metabolites which are responsible for inhibitory activity varies accordingly with the plant parts. This may be a reason for the variation in the inhibitory activity of extracts of both *Jatropha curcas* and *Ricinus communis*. Results of this study support the folkloric usage of these test plants and suggest that their oil extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents for the management of infectious diseases caused by the pathogens which is in agreement with the report by Odungbemi (2006) that *Ricinus communis* is recognized as a rumored solution in gastropathy i.e. amadosa, constipation, irritations, ascitis, strangury, fever, bronchitis, chest infection, skin maladies, coxalgia, colic, and lumbago. The large zone of inhibition exhibited by the extracts on *S. aureus* and *E. coli* could justify their use by tradomedical practitioners in the treatment of sores, bores and control of diarrhea and dysentery. The low minimum inhibitory concentration exhibited by the oil extracts on *S. aureus* is of great significance in the health care delivery system since it could be exploited as an alternative to orthodox antibiotics in the treatment of infections due to the microorganisms especially as they frequently develop resistance to commonly used antibiotics. It was also observed from this work that the higher the concentration the more their activity and as the concentration decreases the lower the antimicrobial effect. Hence an acceptable and effective dosage can be prepared for the control and eradication of these pathogens.

4.1 Conclusion

This study has revealed the presence of metabolites in the seed oils of *Jatropha curcas* and *Ricinus communis.* It has further suggests that the extracts could be useful for the treatment of various infections caused by some bacterial pathogens.

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