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Evaluation of the In-vitro effects of *Jatropha curcas* and *Ricinus communis O*ils Extract Against Selected Bacteria

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Abstract: The in-vitro activities of *Jatropha curcas* and *Ricinus communis* oils extract against selected bacteria was evaluated. The *Jatropha curcas* and *Ricinus communis* oils was extracted by mechanical exhaustive extraction using the hydraulic press to obtain crude oil extracts from the seeds. In-vitro activities of *Jatropha curcas* and *Ricinus communis* oils extracts were determined against the bacterial strains using agar well diffusion technique. The minimum inhibitory concentration (MIC) was investigated by broth dilution technique. Two commonly used antibiotics were used as controls against the test organisms. The in-vitro activity of both *J. curcas* and *R. communis* against all the test organisms increased as the concentration increased. Zone diameter of inhibition of *J. curcas* oil at 100% against *Bacillus subtilis* was 17.0±0.00 mm, *Staphylococcus aureus* 21.0±0.00 mm, *Escherichia coli* 15.0±3.00 mm, and *Pseudomonas aeruginosa*12.5±0.50 mm while for *R. communis* oil, *Bacillus subtilis* was 14.0±1.00mm, *Staphylococcus aureus* 16.5±0.00mm, *Escherichia coli* 12.0±2.00mm and *Pseudomonas aeruginosa* 10.5±0.50mm. zone diameter of inhibition of the controls (Ampicillin against *Bacillus subtilis* was 30.0±2.00 mm, *Staphylococcus aureus* 34.0±0.00 mm and Chloramphenicol against *Escherichia coli* was 28.0±1.00 mm, *Pseudomonas aeruginosa* 26.0±1.00 mm) were significantly ($P<0.05$) higher than that of the extracts of both *Jatropha curcas* and *Ricinus communis* oils at all concentrations of 3.13 %, 6.25 %, 25 %, 50 % and 100 %. The minimum inhibitory concentration of *R. communis* oil extract against *Staphylococcus aureus* (ATCC 6538) was 25%, *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (LMG 21766) were 50% while *Pseudomonas aeruginosa* (ATCC 9027) was 100 % (Table 4). The minimum inhibitory concentration of *J. curcas* and *R. communis* oil extracts against all the test organisms were significantly different ($P< 0.05$) from the controls. The minimum inhibitory concentration and the minimum bactericidal concentration of *J. curcas* and *R. communis* oil extracts against all the test organisms were significantly different ($P< 0.05$) from the controls.
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1.0 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* (Euphorbiaceae family) is a multipurpose plant that has a long history of cultivation in tropical America, Africa, and Asia with many attributes, mainly as potential source of bio-fuel because its seed kernels contain a high amount of oil (58-60%) (Esimone *et al*., 2015). Besides oils, *Jatropha* seeds also contain high protein, anti-nutritional factors including trypsin inhibitor, lectin, saponin, phytic acid and toxic compounds called phorbol esters (Martinez-Herrera *et al*., 2014). The natural compounds of *Jatropha* exhibit bioactive activities for fever, mouth infections, jaundice, guinea worm sores and joint rheumatism (Bhagat and Kulkarni, 2010). Furthermore, extracts from various parts of *Jatropha curcas*, such as seeds, seed oil, stem barks, roots and leaves have shown fungicidal (Saetae and Worapot, 2010) and bactericidal properties (Igbinosa *et al*., 2015). On the other hand, *Ricinus communis* (Castor plant) is a species of flowering plant in the spurge family, Euphorbiaceae. Its seed is the castor bean which, despite its name, is not a true bean. Castor plant is indigenous to the south eastern Mediterranean Basin, Eastern Africa, and India, but is widespread throughout tropical regions (Aiyelaagbe *et al*., 2015). *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). *R. communis* oil is well known because it is a major contributor of ricinoleic acid which is an 18 carbons containing fatty acid having unsaturation at single point. It is baffling how traditional healers, without the use of elaborate equipment or any formal schooling can be so adept at identifying plants, discern their uses and find effective remedies among the thousands of plants that surround them. They also manage to combine several species that may work together synergistically such that remedies could be administered at doses that cure, but kill when administered in other doses (Acharya and Shrivastava, 2015). According to Dias *et al.* (2012), today there are at least 20 distinct chemical substances derived from plants that are considered as important drugs currently in use in one or more countries in the world.Therefore, this study aim to determine the in-vitro effects of *Jatropha curcas* and *Ricinus communis* oils against *Escherechia coli*, *Pseudomonas aeruginosa Bacillus subtilis* and *Staphylococcus aureus*.

2.0 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^{o}C.

2.4 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%.

2.5 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^{o}C.

2.6 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*.

2.7 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.

2.8 In-vitro Assay

In-vitro 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 (0.4ml) 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^{o}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 in-vitro study was done in triplicates and mean zone diameters of inhibition (mm) were determined. The standard of the in-vitro susceptibility testing according to Irshad *et al*. (2012), which is 10 mm was used for result interpretations.

2.9 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).

2.10 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).

2.11 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.

3.0 Results

3.1 In-vitro Activity of *Jatropha curcas* and *Ricinus communis* Oil

Table 1 shows the in-vitro activities of *Jatropha curcas* and *Ricinus communis* oil extracts against *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (LMG 21766) and *Pseudomonas aeruginosa* (ATCC 9027) respectively. Both oils had in-vitro effects on all the test organisms but the effect of *J. curcas* oil is significantly higher (P< 0.05) than *R. communis* oil (See Appendix I and II). The in-vitro activity of both *J. curcas* and *R. communis* against all the test organisms increased as the concentration increased. Zone diameter of inhibition of *J. curcas* oil at 100% against *Bacillus subtilis* was 17.0±0.00 mm, *Staphylococcus aureus* 21.0±0.00 mm, *Escherichia coli* 15.0±3.00 mm, and *Pseudomonas aeruginosa* 12.5±0.50 mm while for *R. communis* oil, *Bacillus subtilis* was 14.0±1.00mm, *Staphylococcus aureus* 16.5±0.00mm, *Escherichia coli* 12.0±2.00mm and *Pseudomonas aeruginosa* 10.5±0.50mm. zone diameter of inhibition of the controls (Ampicillin against *Bacillus subtilis* was 30.0±2.00 mm, *Staphylococcus aureus*34.0±0.00 mm and Chloramphenicol against *Escherichia coli* was 28.0±1.00 mm, *Pseudomonas aeruginosa* 26.0±1.00 mm) were significantly (P<0.05) higher than that of the extracts of both *Jatropha curcas* and *Ricinus communis* oils at all concentrations of 3.13%, 6.25%, 25%, 50% and 100 % as shown in Table 1.

Table 1: In-vitro Activity of *Jatropha curcas* and *Ricinus communis* Oil Extracts Showing Mean Zones of Inhibition in Millimeter

Test Organisms	Concentrations in %					
	100	50	25	12.50	6.25	3.13
J. curcas						
Ps	12.5±0.50	11.0±1.00	9.5±0.00	9.0±1.00	7.5±0.50	4.0±0.00
Bs	17.0±0.00	15.5±1.50	14.0±2.00	12.5±1.50	10.0±1.00	9.5±0.50
Sa	21.0±0.00	19.0±3.00	18.5±0.50	15.0±2.00	13.0±1.00	11.0±2.00
Ec	15.0±3.00	13.0±2.00	11.5±1.50	10.0±1.00	8.5±0.50	7.0±1.00
R. communis						
Ps	10.5±0.50	9.5±0.50	8.0±0.00	6.0±1.00	2.5±0.50	2.0±0.00
Bs	14.0±1.00	13.0±1.00	12.5±0.50	10.5±1.50	8.0±2.00	7.0±1.00
Sa	16.5±0.00	16.0±2.00	14.0±1.00	12.0±2.00	10.0±2.00	9.0±1.00
Ec	12.0±2.00	10.0±1.00	8.5±0.50	7.0±1.00	5.5±0.50	5.0±1.00
Controls						
Ps (CH)	26.0±1.00	24.0±2.00	22.0±1.00	18.5±2.00	16.0±1.00	14.0±2.00
Bs (AMP)	30.0±2.00	28.0±1.00	26.0±1.00	21.0±0.00	17.0±1.00	16.0±0.00
Sa (AMP)	34.0±0.00	30.0±2.00	28.0±2.00	23.0±1.00	19.0±2.00	18.0±0.00
Ec (CH)	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: CH= Chloramphenicol, AMP= Ampicillin, Ps= *Pseudomonas aeruginosa*, Bs= *Bacillus subtilis*, Sa= *Staphylococcus aureus*, Ec= *Escherichia coli.*

CLINICAL AND LABORATORY STANDARDS INSTITUTE GUIDELINES (CLSI, 2014), ZONE ≥ 18mm = Active, ≤ 6mm = Inactive, ≤ 15mm = Moderate.

3.2 Minimum inhibitory Concentration of *Jatropha curcas* and *Ricinus communis* Oil Extracts

The minimum inhibitory concentrations of *Jatropha curcas* and *Ricinus communis* oil extract against *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (LMG 21766) and *Pseudomonas aeruginosa* (ATCC 9027) as represented on Table 2. The minimum inhibitory concentration of *Jatropha curcas* oil extract against *Staphylococcus aureus* (ATCC 6538) was 12.50 %,*Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (LMG 21766) were 25 % respectively whereas *Pseudomonas aeruginosa,* was 50% which was significantly higher (P<0.05). The minimum inhibitory concentration of *R. communis* oil extract against *Staphylococcus aureus* (ATCC 6538) was 25%, *Bacillus subtilis* (ATCC 6633) and

Escherichia coli (LMG 21766) were 50% while *Pseudomonas aeruginosa* (ATCC 9027) was 100 % (Table 2). The minimum inhibitory concentration of *J. curcas* and *R. communis* oil extracts against all the test organisms were significantly different (P< 0.05) from the controls.

Table 2: Minimum Inhibitory Concentration (MIC) Of *Jatropha curcas* and *Ricinus communis* Oil Extracts Against Test Organisms

Test Organisms	Concentrations in %					
	100	50	25	12.50	6.25	3.13
J. curcas						
Ps	-	-*	+	+	+	+
Bs	-	-	-*	+	+	+
Sa	-	-	-	-*	+	+
Ec	-	-	-*	+	+	+
R. communis						
Ps	-*	+	+	+	+	+
Bs	-	-*	+	+	+	+
Sa	-	-	-*	+	+	+
Ec	-	-*	+	+	+	+
Controls						
Ps (CH)	-	-	-	-	-	+
Bs (AMP)	-	-	-	-	-	+
Sa (AMP)	-	-	-	-	-	+
Ec (CH)	-	-	-	-	-	+

Keys: CH= Chloramphenicol, AMP= Ampicillin, Ps= *Pseudomonas aeruginosa*, Bs= *Bacillus subtilis*, Sa= *Staphylococcus aureus*, Ec= *Escherichia coli*, + = Growth, - = No growth, -* = MIC.

3.3 Minimum Bactericidal Concentration of *Jatropha curcas* and *Ricinus communis* Oil Extracts

The minimum bactericidal concentrations (MBC) of *Jatropha curcas* and *Ricinus communis* oil extracts against *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (LMG 21766) and *Pseudomonas aeruginosa* (ATCC 9027) as represented on Table 3. The minimum bactericidal concentration of *J. curcas* oil extract against *Staphylococcus aureus* (ATCC 6538) was 12.50 %, *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (LMG 21766) were 25% and they were significantly different (P<0.05) from the minimum bactericidal concentration of *Psuedomonas aeruginosa* (50 %). Furthermore, from the same Table 5, the minimum bactericidal concentration of *Ricinus communis* oil extract against *Staphylococcus aureus* (ATCC 6538) was25 %, *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (LMG 21766) were 50 % while *Psuedomonas aeruginosa* (ATCC 9027) had a significantly higher MBC of (100%). The minimum bactericidal concentration of *J. curcas* and *R. communis* oil extracts against all the test organisms were significantly different (P< 0.05) from the controls.

Table 3: Minimum Bactericidal Concentration (MBC) Of *Jatropha curcas* and *Ricinus communis* Oil Extract Against Test Organisms

Test Organisms	Concentrations in %					
	100	50	25	12.50	6.25	3.13
J. curcas						
Ps	-	-*	+	+	+	+
Bs	-	-	-*	+	+	+
Sa	-	-	-	-*	+	+
Ec	-	-	-*	+	+	+
R.communis						
Ps	-*	+	+	+	+	+
Bs	-	-*	+	+	+	+
Sa	-	-	-*	+	+	+
Ec	-	-*	+	+	+	+
Controls						
Ps (CH)	-	-	-	-	-	+
Bs (AMP)	-	-	-	-	-	+
Sa (AMP)	-	-	-	-	-	+
Ec (CH)	-	-	-	-	-	+

Keys: CH= Chloramphenicol, AMP= Ampicillin, Ps= *Pseudomonas aeruginosa*, Bs= *Bacillus subtilis*, Sa= *Staphylococcus aureus*, Ec= *Escherichia coli*, + = Growth, - = No growth, -* = MBC.

4.0 Discussions

It appears from this study that both *Jatropha curcas* and *Ricinus communis* oil had in-vitro effects on *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (LMG 21766) and *Pseudomonas aeruginosa* (ATCC 9027). Although, the extracts 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). Antimicrobial properties of the different parts of castor plant have been reported by Ramos-L´opez *et al.* (2010) which is also in agreement with the findings in this study. Generally, the extracts of *Jatropha curcas* and *Ricinus communis* used in this study show promising antimicrobial properties. The in-vitro effects of the fractions of *Jatropha curcas* oil 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* combined fractions had antimicrobial effects on all the selected microorganisms but *Jatropha curcas* had higher antimicrobial activity (32±0.20 mm) than the *Ricinus communis* (28.0±0.11 mm). The broader spectrum of in-vitro activities observed in this study is interesting and could be due to the synergistic effects of the various phytochemical components in the *Jatropha curcas and Ricinus communis* oil extracts. The results of this study are robust and further promote the efficacy plant materials for the management of bacterial infections especially in remote rural areas where standard drugs are either not available or not affordable to the poor.

4.1 Conclusion

From this study, it can be concluded that both *Jatropha curcas* and *Ricinus communis* combined fractions had antimicrobial effects on all the selected microorganisms. Since this oil is inexpensive, environmentally friendly and also a naturally-occurring resource it is therefore recommended as a good source of alternative

medicine for the treatment of diseases caused by these test organisms.

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