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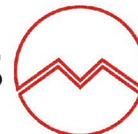

Antibacterial Effect Of *Jatropha Curcas* And *Ricinus Communis* Oils Extracts Against Selected Bacteria

[1]Etu Emmanuela and [2]Isu, Nnena Rosemary

Department of Microbiology, University of Auja, P.M.B. 117, Abuja, Nigeria
[1]josephella2014@gmail.com, [2]nnennaya.isu@uniabuja.edu.ng

Abstract: The antibacterial effect of *Jatropha curcas* and *Ricinus communis oils* against *Staphylococcus aureus* (ATCC 6538)*, Bacillus subtilis* (ATCC 6633)*, Escherichia coli* (LMG 21766) and *Pseudomonas aeruginosa* (ATCC 9027) was carried out. The crude extracts were obtained by mechanical exhaustive extraction using the hydraulic press from the seeds. Antibacterial activities of the oil extracts were determined against the bacterial strains using agar well diffusion technique. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were investigated by broth dilution technique. The activities of two commonly used antibiotics served as controls against the test organisms. Oil yield for both seeds was 10%. The two oil extracts were effective against all the test organisms but significantly lower ($p<0.05$) than the control antibiotics (26.0±1.00-34.0±0.00 mm). The antibacterial effect of *J. curcas* oil was significantly higher ($P<0.05$) with zone diameter of inhibition of 12.5±0.50 mm than *R. communis* 10.5±0.50 mm against *P. aeruginosa* at a concentration of 100mg/ml. The MIC for *J. curcas* (50 mg/ml) was significantly lower ($P<0.05$) than that of *R. communis* (100 mg/ml) against *P. aeruginosa.* Similarly, the MBC for *J. curcas* and *R. communis* against *P. aeruginosa* were (50 mg/ml) and (100 mg/ml) which were effective against the test organisms but significantly lower ($P<0.05$) than the MBC of the control antibiotics (12.50 mg/ml). The antibacterial effect of the oil extracts increased with concentrations.

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Keywords: *Jatropha curcas, Ricinus communis* oils, Antibacterial Effect, MIC, MBC

1. Introduction

The oils of medicinal plants have been used for treatment of various ailments since men learnt the art of extraction (Dan *et al.,* 2004). Clove oil for instance, has been used for dental pain as an anodyne (painkiller), as antihelmintic and as aromatherapy when warming of the digestive system is needed as far back as 1721 BC (Weiss and Fintelmann, 2010). *Jatropha curcas*, is a drought resistant tropical tree and Kumar and Sharma (2008) reported that the oil from its seeds has been found useful for medicinal and veterinary purposes, as insecticide, for cosmetics production as a fuel substitute.

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*., 2009). 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*., 2009).

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*., 2007).

The oil is fixed and dries very slowly, having a specific gravity of 0.958, a refractive index of 1.4790 to 1.4805 and solidifies at -10° C to - 18°C (Odungbemi, 2006). Its acidity is expressed as oleic acid which is 1.5 percent. Ethnomedically, the oil extracted from the seed has been used in small doses for numerous health conditions such as liver and gallbladder disturbances, abscesses, headaches, appendicitis, epilepsy, hemorrhoids, constipation, diarrhea, intestinal obstructions, skin diseases, hyper activity in children and to avert threatened abortion in pregnant women (Odungbemi, 2006). The development and spread of bacterial resistance to commonly used antibiotics by microbial cells has increased efforts in the search for new antibiotics for the treatment of microbial infections and diseases

(Prminik, 2002). Therefore, there is need to evaluate mechanically extracted *Jatropha curcas* and *Ricinus communis* oils for their antibacterial effects against *Escherechia coli*, *Pseudomonas aeruginosa Bacillus subtilis* and *Staphylococcus aureus*.

2. Materials and Method

2.1 Study Area

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

2.2 Preparation and sterilization of media

The media used in this study are: Nutrient agar (Himedia M001-500G), MacConkey agar (Himedia M001-500G), Eosin methylene blue agar (Titan), Mannitol salt agar (Himedia), Mueller Hinton agar (Himedia) and peptone water (Himedia) and they were prepared according to the manufacturers' instructions.

2.3 Sample collection and identification

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

2.4 Extraction and sterilization of *Jatrophacurcas* and *Ricinuscommunis* 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 sterilized using membrane filtration according to Muzenda *et al.* (2012) and stored in sterile bijoux bottles at 4°C.

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. They were then 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) and then subjected to catalase and coagulase tests while spore staining was carried out 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 subcultured 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 stock 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) millilitre 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 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 surface of Mueller Hinton Agar plate 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 borne 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 as well as the diluent (DMSO) were used controls. 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 24hrs, 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.

2.9 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 minimuminhibitory 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. Results

The results are presented as follows:

3.1 Percentage oil yield of *Jatropha curcas* and *Ricinus communis*

From the data, the oil yields of *Jatropha curcas* and *Ricinus communis* are not significantly ($P>0.05$) different (Table 1).

Table 1: Physical characteristics of the *Jatropha curcas* and *Ricinus communis* oil extracts

Physical characteristics	Plant Extracts	
	JCO	RCO
Weight of seeds (g)	1000	1000
Weight of oil (g)	100	100
Yield (%)	10	10
Colour	Yellow	Pale yellow
Texture	Liquid	Liquid

Keys: J.C.O = *Jatropha curcas oil*, R.C.O = *Ricinus communis oil*, 1 litre of oil= 1000g, Hence, 100ml of oil = 100g.

3.2 Antibacterial activity of *Jatropha curcas* and *Ricinus communis* oil extracts

Table 2 shows the antibacterial 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 antibacterial effects on all the test organisms but the effect of *J. curcas* oil is significantly higher (P< 0.05) than *R. Communis* oil. The antibacterial 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. The 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 2.

Table 2: Zone Diameter of Inhibition of *Jatropha curcas* and *Ricinus communis* oil extracts showing mean zones of inhibition in millimeter (mm)

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.*

3.3 Minimum inhibitory concentration of *Jatropha curcas* and *Ricinus communis* oil extracts

Table 3 shows 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) respectively. 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 3).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 3: 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.

3.4 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) respectively are shown in Table 4. 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% respectively which were significantly different (P<0.05) from the minimum bactericidal concentration of *Psuedomonas aeruginosa* (50 %).Furthermore, from the same Table 4, the minimum bactericidal concentration of *Ricinus communis* oil extract against *Staphylococcus aureus* (ATCC 6538) was 25 %, *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 4: 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. Discussions

The oil extracts of *Jatropha curcas* and *Ricinus communis* used in this study show promising antimicrobial properties against both Gram positive and Gram negative bacteria. The activity of the *Jatropha curcas* and *Ricinus communis* against both Gram-positive and Gram-negative bacteria suggest the potential of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. The antibacterial activities varied against different bacteria were varied with the concentration of extracts tested. High concentrations inhibited the growth of the bacterial species *Bacillus subtilis, Staphylococcus aureus,*and *Escherichia coli,* however, the inhibitory activities decreased with the concominent decrease of extract concentration.

The antibacterial activities of *Jatropha curcas* and *Ricinus communis* oil extracts mean zones of inhibition against *Pseudomonas aeruginosa* (ATCC 9027) were not significant ($P > 0.05$).Verma *et al.* (2011) reported similar result on antimicrobial potential of roots of *Ricinus communis* against pathogenic *Pseudomonas aeruginosa* and *Escherichia coli*. Among the four microorganisms tested, two bacterial species, *Staphylococcus aureus* and *Bacillus subtilis* were susceptible to the high concentration of both *Jatropha curcas* and *Ricinus communis*.

The antimicrobial effects of both *Jatropha curcas* and *Ricinus communis* oil against all the test organisms also increased with concentration. This showed that inhibitory activity of plant extracts probably depends upon the concentration, type of parts used and microbes tested. The accumulation and concentration of secondary metabolites 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 explored as antimicrobial agents in new phermaceuticals for the therapy of infectious diseases caused by bacterial 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 malad, coxalgia, colic, and lumbago. The large zone of inhibition exhibited by the extracts on *S.aureus* and *E. coli* justify their use by trado-medical practitioners in the treatment of sores and control of diarrhea and dysentery.

The *Jatropha curcas* and *Ricinus communis* extract (oil) was very effective against *Staphylococcus aureus* this confirmed its use traditionally in the treatment of skin ailments (scabies, trachoma, etc.). The low minimum inhibitory concentration (MIC) exhibited by the oil extracts on *S. aureus* is interesting and might be of great significance in the health care delivery system since it could be used as an alternative to orthodox antibiotics in the treatment of infections due to these bacteria 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 a standardized and effective dosage can be prepared for the control and eradication of these pathogens.

4.1 Conclusion

Data from this study lead credence to the folkloric use of these plants in treating bacterial infections and suggest that *Jatropha curcas* and *Ricinus communis* could be exploited for new potent pharmaceuticals against Gram positive and Gram negative bacteria.

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