Extended Abstract Journal of Molecular Microbiology 2021
Vol.5 No.4

Evaluation of antibacterial properties and the rate of killing of cashew (Anacardium occidentale) nut shell oil on selected bacteria

Jesse Innocent Apameio
Nigeria
Email - jesseinnocent75@gmail.com

1.0 INTRODUCTION

Anacardium occidentale popularly known as cashew tree is native of Brazil. Nigeria is the world's sixth largest producer of cashew. The cashew tree has been cultivated for food and medicine for over 400 years (Balaji et al., 2016). The various parts of the cashew fruits are of economic value. This include: the apple, nut and kernel. Cashew nut is a value edible nut which yields two oil. The first type is the oil found in the kernel of the cashew nut. This is called the cashew kernel oil (CKO) while the second oil is in the seed coat or pericarp or shell, is called the Cashew Nut Shell Oil (CNSO). It contains a high proportion of phenolic compound. It is viscous and dark oil, which is extremely caustic. There are more than 200 patents for its industrial application. It is used in industry as a raw material for brake lining compounds, as a water proofing agent, a preservative and in the manufacturing of paints and plastics (Alia et al., 2016)

The main medicinal or ethnopharmacological applications of the CNSO include the treatment of infectious and inflammatory diseases and pain conditions, such as venereal diseases, skin diseases, diarrhea, stomatitis, bronchitis, intestinal cramps, muscle weakness, diabetes, tooth pain, weakness, inflammation, psoriasis, cough, ringworm, warts, corns use in the treatment of malaria (Amaral et al., 2016; Hesham et al., 2016).

The present study is therefore designed to determine the susceptibility and the rate of killing of cashew (Anacardium occidentale) nutshell oil on selected pathogens

2.0 MATERIALS AND METHODS

2.1 Collection and processing of sample

Cashew nuts were obtained from Wukari, Taraba state of Nigeria. Wukari is situated at 7.85º North latitude, 9.78º East longitude and 152 meters elevation above the sea level (Andrew et al., 2017). They nuts were air dried for a period of four weeks. They nuts were decorticated to obtained shell and kernel separately. This was done manually by placing the nut on a flat stone and cracked with a wooden mallet. The resulted products are the kernels and the shell. The shell was dried and pulverized using mortar and pestle (Idah et al., 2014).

2.2 Extraction procedure

Extraction of the oil content of Anacardium occidentale shell was done using the Soxhlet extractor. Digital weighing balance was used to weigh 50g of sample. The sample was introduced into a thimble and place in the Soxhlet column and 250ml of methanol was place in the round button flask at 65ºc. After three hours of extraction, the experiment was stopped and the trapped oil substance was concentrated using rotary evaporator under vacuum at temperature of 70ºc (Hesham et al., 2016; Idah et al., 2014).

2.3 Collection of test organisms

The selected pathogens used for this study include Pseudomunas aeruginosa, Escherichia coli, Staphylococcus aureus and Streptococcus pyogeness were obtained from the culture bank of Microbiology lab, Microbiology Department, Federal University of Technology, Minna, Niger State, Nigeria. The selected pathogens were screen to ascertain there viability using various morphological and biochemical identification techniques of Abalaka et al. (2009).

2.4 Determination of antibacterial susceptibility test

Selected pathogens were inoculated into the broth and incubate for 18-24hours. The resulting turbidity was adjusted to 0.5 McFarlarnd turbidity standard, using the same broth. Swap stick was used to inoculate it on Muller-Hinton agar. Sterile cork borer was used to bore hole on the agar aseptically. A know concentration of the CNSO extract was dispensed into the hole. Plates were allowed to stay for 1hour before they were inverted and incubated at 37ºc for 18-24hours (Ochei & Kolhatkar, 2010). Dimethyl sulphoxide (DMSO) was used as negative control and chloraphenicol was used as positive control. The antibacterial activity was assessed by the diameter of the zone of inhibition and results were recorded. This assay was performed in duplicate.

2.5 Determination of minimum inhibitory concentration (MIC) of the CNSO

The MIC of the crude extracts was determined by broth dilution method (Ewansiha et al., 2016). Nutrient Broths were prepared in five test tubes. Two fold serial dilutions of the crude extracts were prepared to give a decrease in concentration ranging from 50, 25, 12.5, 6.25, and 3.12mg/ml respectively. Two milliliter (2 ml) was transferred from test tube containing the stock solution to first test tube containing 2 ml of Nutrient Broth to give 50 mg/ml. This procedure continued until a concentration of 3.12mg/ml was obtained in the fifth test tube. Homogenous mixture was obtained by vortexing each tube for at least 5 seconds. Zero point two millilitre (0.2 ml) of bacteria suspension (1.5 x108) was inoculated in each of the test tubes containing 2 ml sterile Nutrient Broth. In the control tube, the test crude extract was not added. The test tube not inoculated was used to check the sterility of the medium and as negative control while the positive control tube was used to check the suitability of the medium for growth of the microorganisms and the viability of the inoculums. All the test tubes were properly shaken and then incubated at 37oc for 24 h and the change(s) in turbidity were observed. The MIC was determined by the lowest concentration of the crude extract that prevented visible growth (Ewansiha et al., 2016).

2.6 Determination of the Minimum Bactericidal Concentration (MBC) of the CNSO Extracts

The MBC of the crude extracts was determined from the MIC tubes that showed no visible growth. Zero point one millilitre (0.1 ml) from these concentrations that showed no visible growth was inoculated into 9ml recovery Nutrient Broth. These were incubated at 37°c for another 24 hour. The least concentration of the extracts that showed no bacterial growth in the recovery liquid medium was taken as the MBC (Bergen et

al., 2010).

2.7 Qualitative analysis for phytochemical constituents in CNSO

Phytochemical screening of CNSO was done according to the methods previously described by Abalaka et al. (2009) for the detection of bioactive components present.

2.8 Determination of the Rate of Killing

The rate of killing of CNSO against the selected pathogens was determined by the modified method of Hugo and Russell (2000) and Oloninefa et al. (2016) with minimal modifications. A standardized overnight culture with a population of 1.5 × 108 cfu/ml was used. Zero point two millilitres (0.20 ml) of the standardized inoculum were added to 10ml of CNSO. One millilitre (1.0 ml) of the suspension was withdrawn at various time intervals of 1, 2, 3, 4 and 5 hours respectively and would be transfer to 9 mL of nutrient broth recovery medium containing 5% "Tween 80" to neutralize the effect of the antimicrobial compound carry-overs from the test organisms (Gerit et al., 2016; Tang et al., 2016). The suspension was then successively diluted tenfold (10-1) and then plated for viable counts on Mueller-Hilton Agar in duplicates and incubated at 37oc for 18-24hours. Rate of killing for the control was determined by adding 0.20ml of the standardized inoculum to 10ml of Mueller-Hilton Broth. One millilitre (1.0 ml) of the mixture was withdrawn at various time intervals of 1, 2, 3, 4 and 5 hours respectively and diluted tenfold (10-1) then plated on Mueller Hilton agar in duplicates and incubated at 37ºc for 24 hours. The population of the colonies were counted and expressed in log10 CFU/ml.

2.9 Data analysis

Results were expressed as the mean values ± standard error of mean (SEM) by measuring three independent replicates. Analysis of variance (ANOVA) using one-way was done and Duncan's test was performed to test the significance difference between means values obtained among the treatments at the 5% level of significance using SPSS software (version 21, IBM SPSS) (Oloninefa et al., 2016).

3.0 RESULTS

3.1 Antibacterial susceptibility tests to CNSO

Table 1 showed the results of susceptibility tests of Escherichia coli, Pseudomunas aeruginosa, Streptococcus pyogeness and Staphylococcus aureus to CNSO. Concentration range of 25-100mg/ml were used, ciprofloxacin serve as positive control while DMSO serve as negative control

CNSO/ Control (mg/ml)	Selected bacterial pathogens and zone of inhibition (mm)			
	E. coli	*P. aeruginosa*	*S. pyogenes*	*S. aureus*
25	14±1.73[b]	14±0.57[b]	18±1.15[b]	20±0.57[b]
50	14±1.52[b]	20±0.57[c]	20±1.15[b]	30±0.00[c]
75	15±1.00[b]	22±0.57[c]	21±1.52[b]	30±0.57[c]
100	16±2.16[b]	25±1.15[d]	23±1.73[c]	32±0.57[c]
CPX	32±1.52[c]	30±0.88[e]	35±1.00[c]	35±0.57[c]
DMSO	0±0.00[a]	0±0.00[a]	0±0.00[a]	0±0.00[a]

Table 1. Susceptibility test of selected pathogens to CNSO

Key:

CXP = Ciprofloxacin

DMSO = Dimethyl sulphuroxide

Bacterial Pathogens	MIC (mg/ml)	MBC(mg/ml)
E. coli	12.5	NA
P. aeruginosa	6.25	NA
S. pyogenes	3.12	12.5
S. aureus	3.12	6.25

Table 2. MIC and MBC of test bacterial pathogens

Key:

NA = Not applicable

Phytochemical	Qualitative
Saponin	+
Flavonoid	+
Tanins	+
Phenol	+
Reducing sugar	+

Glycoside	-
Alkaloid	+
Steroid	+
Phlabotanins	+
Terpenoids	+

Table 3. Phytochemical analysis of CNSO

Key:

+ = Present

- = Absent

3.2 Rate of killing of selected pathogens by CNSO and Control

Figure 1 showed the rate of killing of Escherichia coli, Pseudomunas aeruginosa, Streptococcus pyogeness and Staphylococcus aureus by CNSO and the control in graphical representation when the log transform population density of selected pathogens was plotted against time in hours.

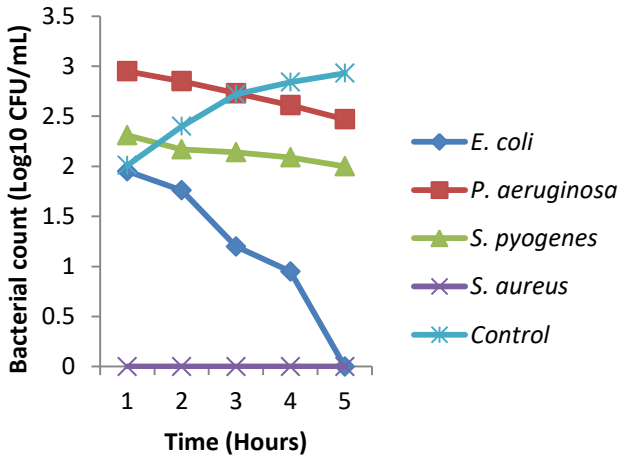

Figure 1: Log transform (Log10cfu/ml) population density of the rate of killing of bacterial pathogens by CNSO with respect to time

4.0 DISCUSSION

Antibacterial activity was recorded with methanol CNSO at varying concentrations of the crude extracts. The zones of inhibition increased as the concentrations of the extract increased. The activity of antimicrobial agent is concentration dependent (Willey et al., 2011). The size of zone of inhibition is determined by the initial population density of the test organisms, there growth rate and the rate of diffusion of the antimicrobial agent (Ochei and Kolhatkat, 2010). This is in agreement with the studies conducted by Abalaka et al. (2010) and Olonifade et al. (2018) where at higher concentration gives a larger zone of inhibition (Table 1).

The methanol extracts of CNSO record the highest zone of inhibitions in this study against S. aureus at different concentration. The antibacterial activity recorded with methanol CNSO in this study might be because of the amphiphilic nature of methanol to dissolve both polar and non-polar phyto-constituents of cashew nut shell. The result obtained in this study is in line with the work of Jebapritha and Karpagam (2017) where methanol was used as solvent of extraction and the extract tested against some selected bacteria.

The results recorded for ciprofloxacin against bacterial pathogens were as expected, since it's known to have antibacterial activity. Ciprofloxacin was found to have a larger diameter of inhibition than the crude oil extract. The wideness in the zones of inhibition recorded with ciprofloxacin as compared to the crude oil extracts show the level of its purity. There is high possibility that the crude oil extracts might perform better than ciprofloxacin if they are refined and purity further. Therefore, the secondary metabolites present in Annacadium occidental nut shell can be used as chemotherapeutic agent as earlier reported by Parasa et al. (2011).

The results of MIC and MBC recorded for methanol oil extract was bacteriostatic on the Gram negative bacteria namely E. coli and P. aeruginosa while on Gram positive bacteria namely S. pyogenes and S. aureus to be bactericidal. This finding is not in agreement with the works of Parasa et al. (2011) in that study MIC and MBC was recorded at a lower concentration. This might be due to the differences in the bacterial pathogens investigated or differences in laboratory procedures (Calixto, 2000; Silva et al., 2016).

Analysis of the phytochemical constituents of CNSO shows that the oil extracts is rich in phyto-constituents; saponins, flavonoid, reducing sugar, terpenoids, phenols, alkaloids, steroids, phlobotannins and terpenoids were all present with the exception of glycoside. This could be that this plant synthesizes these phyto-constituents from their normal metabolic processes for protection from excess sun light, herbivores and defence against they attach of microorganisms. This result is in agreement with the findings of Jebapritha and Karpagam (2017). These phyto-constituents have been known to exhibit medicinal physiological activities as reported by Oloninefa et al. (2018) and Chandra (2017).

The outcome of the rate of killing on selected bacterial pathogens by methanolic CNSO revealed that the oil extract had a bacteriostatic activity on P. aeruginosa and S. pyogenes up till the fifth hour of treatment. The extract was bacteriocidal for E. coli at the fifth hour and had a definitive bacteriocidal activity on S. aureus. This might be that the methanol extract contains bioactive components that can inhibit the growth of these bacteria pathogens or might be that the pathogens do not have the ability to resist these bioactive components. This is in line with the work of Peter et al. (2007) that vancomycin had a definitive bactericidal activity against S. aureus strains (Figure 1). Furthermore, this research is in agreement with the finding of Gerits et al. (2016) and Oloninefa et al. (2015) in a similar study on Cronobacter sakazaki.

There was a steady increment in the bacteria populations from the first to fifth hours in the control. These increments were expected because methanol CNSO was absent in the control treatment. It was observed that methanol CNSO extract might be responsible for inhibiting and killing the selected bacterial pathogens when compared with the extract control there was a steady increment in the bacterial population with increase in time.

5.0 Conclusion

The cashew nut shell oil (CNSO) is very rich in phytoconstituents and contain a large proportion of phenolic compounds, hence its antibacterial. It should be use in the treatment of bacterial infections.

Reference

Abalaka, M. E., Onaolapo, J.A., Inabo, H.I. and Olonitola, O.S. (2009). Extraction of Active Components of Mormodica Charantia L (Cucurbitaceae) for Medicinal Use. African Journal of Biomedical Engineering and Sciences, 1(1), 38-46.

Abalaka, M.E., Onaolapo, J.A., Inabo, H.I. and Olonitola, O.S. (2010). Antibacterial Activity of Chromatographically Separated Pure Fractions of Whole Plant of Momordica Charantia L (Cucurbitaceae). Advances in Environmental Biology, 1(1), 44-52.

Aderiye, B. I., David, O. M., and Atere, V. A. (2015). Administration of cashew extracts in the treatment of some infections and diseases. Advancement in Medicinal Plant Research, 3(3), 75-86, 75–86.

Alia, A. H. N. A., Shukri, M. A. M., and Razali, M. (2016). Antimicrobial potency of essential oil from cashew (Anacardium occidentale Linn .) clones. Journal of Tropical Agriculture and Food Science, 44(1), 73–80

Amaral, R., Liberio, S. A., Amaral, F. M. M., Raquel, F., Maria, L., Torres, B. and Luis, S. (2016). Antimicrobial and Antioxidant Activity of Anacardium occidentale L. Flowers in Comparison to Bark and Leaves Extracts. Journal of Biosciences and Medicines, 4, 87-99.

Andrew, C., Aremu, M. O., Oko, O. J. and Shenge, G. A. (2017) Seasonal analysis of water quality in two settlements of Wukari Local Government Area, Taraba State, Nigeria, FUW Trends in Science & Technology Journal, 2(1): 613 – 617.

Balaji, B., Khaja, M. S., Senthil, K. K., and Karthikeyan, M. (2016). Cashew nut shell liquid poisoning. International Journal of Critical Care Medicine, 20(1): 57–58.

Bergen, P. J., Bulitta, J. B., Forrest, A., Tsuji, B. T., Li, J. and Nation, R. L. (2010). Pharmacokinetic/pharmacodynamic investigation of colistin against Pseudomonas aeruginosa using an in vitro model. Antimicrobial Agents Chemotherapy, 54: 3783- 3789.

Calixto, J. B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines. Brazilian Journal of Medicinal and Biological Research, 33:179-189.

Chandra, P. K. (2017). Traditional Health Care systems and herbal medicines. European Journal of Environment and public Health, 1(1)3:37-47.

Ewansiha, J. U., Garba, S. A., Musa, G., Daniyan, S. Y., Busari, M. B., Innalegwu, D. A. and J. H. Doughari, J. H. (2016). Preliminary Phytochemical and Antimicrobial Activity of Citrus limon (L) Burm. f. (lemon) Leaf Extract against Some Pathogenic Microorganisms. Journal of Applied Life Sciences International, 6(4): 1-10.

Gerits, E., Eline B., Lippell, A., Alex J. O., Bram, W., Maeyer, D., Fierro, C. A., Kathleen, M., Arnaud, M., Patrick, C., Pieter, S, Katrijn, B., Karin, T. and Jan, M. (2016). Elucidation of the Mode of Action of a New Antibacterial Compound Active against Staphylococcus aureus and Pseudomonas aeruginosa. Journal of Microbiology.11(5), 75-79.

Hemshekhar M, Santhosh SM, Kemparaju K, Girish KS (2011) Emerging roles of anacardic acid and its derivatives: a pharmacological overview. Basic Clinical Pharmacology Toxicology, 110(2):122–132.

Hesham, H. A. R., Abdurahman, H. N. and Rosli, M. Y. (2016). Techniques For Extraction of Essential Oils From Plants: A Review. Australian Journal of Basic and Applied Sciences, 10(16), 117-127.

Hugo, W. B. & Russell, A. D. (2000). Determination of rate of killing. In: Pharmaceutical Microbiology. Sixth edition (pp. 229-244). Oxford, London: Blackwell Science.

Idah, P. A., Simeon, M. I., & Mohammed, M. A. (2014). Extraction and characterization of cashew nut (Anacardium occidentale) oil and cashew shell liquid oil. Academic Research International, 5(3), 50

Jebapritha, S. D. S., and Karpagam, S. (2017). Phytochemical Content And Antimicrobial Activity of Cashew Nut Shell Oil, Journal of Pharmacy and Biological Sciences,12(4), 61–64.

Kannan, V. R., Sumathi, C. S., Balasubramanian, V., and Ramesh, N. (2009). Elementary Chemical Profiling and Antifungal Properties of Cashew (Anacardium occidentale L .) Nuts, Botany Research International, 2(4), 253–257.

Ochei, J. and Kolhatkar, A. (2000). Medical Laboratory Science, Theory and Practical. Bacteriology antimicrobial susceptibility test. Tata McGram-Hill Publishing company limited, New Delhi, 801-804.

Oloninefa, S. D, Abalaka, M. E, Daniyan, S. Y & Mann, A. (2018). Phytochemical screening and antibacterial susceptibilty of whole plant of Euphorbia heterophylla crude extracts against selected bacteria pathogens, Bayero Journal of Pure and Applied Science, 11(1); 211-220.

Oloninefa, S. D., Abalaka, M. E. & Daniyan, S. Y. (2016). Determination of the Rate of Killing of the Antibiotics Effective against C. sakazakii. In: Antibacterial Susceptibility of C. sakazakii to Known Antibiotics. 1st edition (pp. 65), Saarbrucken, Deutschland, Germany: Lambert Academic Publishing Omniscriptum GmbH & Co. KG, BahnhofstraBe, 28, 66111.

Parasa, L. S., Srinivasa, R. T., Cyril, A. K., Srinivasa, P. C., and Rao, G.S. (2011). In vitro – antimicrobial activity of cashew (Anacardium occidentale, L.) nuts shell liquid against methicillin resistant Staphylococcus aureus (MRSA) clinical isolates. International Journal of Pharmacy and Pharmaceutical Sciences, 3(4), 436-440

Silva, R.A., Silvana, A. L., Flavia, M. M., Amaral, F. R., Luce, M. B. T., Valério, M. N. & Rosane, N. M. G. (2016). Antimicrobial and Antioxidant Activity of Anacardium occidentale L. 4(6)87-95.

Tang, Q., Ai-Rong, K. and Cong, L. (2015). Phytochemical Analysis, Antibacterial Activity and Mode of Action of the Methanolic Extract of Scutellaria barbata Against Various Clinically Important Bacterial Pathogens. International Journal of Pharmacology, 12: 116-125.

Willey J. M., Sherwood L. M., and Woolverton C. J., (2011). Prescott's Microbiology, Eight edition, McGraw Hill; 546-558.