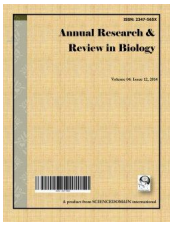

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Effect of Sodium Chloride Extract of *Dacryodes edulis* and *Chrysophyllum albidum* Seeds on Enteric Pathogens

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Authors' contributions

This work was carried out in collaboration between all authors. Author AS conceived the study, designed and performed the experiment, performed the statistical analysis and prepared the draft of the manuscript. Authors ABE and JAMA supervised the work and edited the manuscript. Authors SOK and RB supervised the work. Author OO collected plant materials. All authors read and approved the final manuscript.

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ABSTRACT

Effect of sodium chloride extract of *Dacryodes edulis* (African pear) and *Chrysophyllum albidum* (African star apple) seeds on enteric pathogens (*Escherichia coli* (ATCC25922); *Salmonella typhi* (clinical strain); *Klebsiella pneumoniae* (clinical strain); *Pseudomonas spp.* (ATCC4853); *Enterococcus faecalis* (ATCC29212) and *Staphylococcus aureus* (ATCC25923) were investigated using agar well diffusion and micro broth dilution methods. Results revealed that the extracts have antimicrobial activity against the test organisms. In agar well diffusion method, the extracts were

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most effective at concentration 100 mg/ml as inhibition zone diameter (IZD) values ranges from 16.5 mm to 23 mm for African pear seed extract and 16.5 mm to 21.9 mm for African star apple seed extract. In the broth dilution method, the extracts were bacteriostatic at lower concentration and bactericidal at higher concentration against all test organisms. Sodium chloride extract of African pear seed shows minimum inhibitory concentration (MIC) values ranges from 1.5625 mg/ml to 50 mg/ml and minimum bactericidal concentration (MBC) values ranges from 6.25 mg/ml to 50 mg/ml respectively while sodium chloride extract of African star apple seed shows MIC values ranges from 6.25 mg/ml to 50 mg/ml and MBC values ranges from 25 mg/ml to 100 mg/ml respectively. In liquid broth medium, sodium chloride extract of African pear seed exhibited the highest activity against *Pseudomonas* as the least MIC (1.5625 mg/ml) and MBC (6.25 mg/ml) were recorded against the test organism. It is concluded that the sodium chloride extract of African pear and African star apple seeds showed potential antimicrobial activity of MIC and MBC ≤ 100 mg/ml, thus they have antimicrobial activity against enteric pathogens. Hence, sodium chloride will be useful for extracting bioactive agents in African pear and African star apple seeds, thus this will help reduce the cost of extraction and incidence of intestinal diseases.

Keywords: Sodium chloride; seed extracts; Dacryodes edulis; Chrysophyllum albidum; seeds; enteric pathogens; antimicrobial activity.

1. INTRODUCTION

A large number of pathogens such as bacteria, viruses, protozoans and fungi can cause intestinal disease. Some of the leading causes of intestinal infections are viruses (rotavirus and Norwalk agent), *Escherichia coli* (*E.coli*), *Campylobacter jejuni* and *Salmonella spp.* which are mainly spread by contamination of food and water [1]. Contaminated food and water can transmit diseases such as cholera, diarrhoea, dysentery, typhoid and polio and it has been estimated that contaminated water cause 502,000 worldwide diarrhoeal deaths annually [2]. The prevalence of these diseases is continuously increasing as some pathogens are now beginning to resist frequently used antibiotics [3] and this has been classified as a serious threat facing human existence [4]. Hence, there is a growing interest in the research of new antimicrobials which could effectively fight these pathogens.

Due to the emergence of synthetic antimicrobials resistance pathogens and some health issues associated with the use of synthetic compounds, new approach are being evaluated for finding new antimicrobials. Some plant secondary metabolites (phenols, polyphenols (tannins, flavonoids, coumarins), alkaloids, lectins and polypeptides) have been found to have antimicrobial properties [5,6,7] and as such the effect of plant materials on pathogenic organisms are now being evaluated. These materials are presumed to be safe because they are of natural origin [3,8,7].

There are numerous number of plant species and only a few have been investigated for antimicrobial activity [9,5,3,10,6], thus the need to investigate more plants for antimicrobial activity.

African or bush pear (*Dacryodes edulis)* seeds are surrounded by a pulpy butyraceous pericarp, which is the edible portion consumed either raw or cooked. African pear has long been use for the treatment of various ailments such as wound, skin diseases, dysentery and fever [11,12]. The seeds are rich in carbohydrates, protein, minerals and crude fibres, thus may be used as excellent sources of nutrition to consumers or as coagulant aids in water treatment [13], thus encouraging their high rate of production and commercialization for decades [14]. The seed also contained secondary metabolites (tannins, glycoside, flavonoids, coumarins and phenols) [13] that have antimicrobial properties .The seed oil has been found to have both domestic and industrial potentials [15,16].

African star apple (*Chrysophyllum albidum*) seeds have a shiny hard brown casing which feels like plastic that allows them to be viable for years and used for local games [17]. African star apple has been used in folk medicine for treatment of diseases. Cotyledons from the seeds are used to treat type two diabetes and as ointment in the treatment of vaginal and dermatological infections in Sub-Sahara Africa [18]. The seeds contain carbohydrates, protein, minerals, crude fibres and phytochemicals such as tannins, glycoside, saponins, alkaloids, flavonoids, coumarins and phenols [13].

African pear and African star apple seeds have multiple uses and could be cultivated intensively and help to improve the quality of life of both the rural and urban people.

Extracts from plant materials used for antimicrobial activity are most often prepared using the solvent extractions method due to their ease of use, effectiveness and wide applicability [19]. Although extracts prepared from plant materials using solvents such as methanol, ethanol, chloroform, petroleum ether, ethyl acetate and water have all shown promising antimicrobial activities, however organic solvent (methanol and ethanol) extract showed better antimicrobial activity than aqueous extract. For example, methanol extract of all parts (fruit, leave, seed and stem) of *Ziziphus spina-christi* and ethanol extract of banana fruit peel showed better antimicrobial activity than aqueous extract [20,21]. Hence, they are the most frequently used solvents. However these solvents have some draw backs associated with their use, for instance, when methanol and ethanol are used to prepare extracts from plant materials, they may cause artefact methylated and ethyl derivatives and these compounds are known as carcinogens and neurotoxins [22]. Also with the use of these solvents, several extractions need to be carried out on the same plant material to ensure that natural compounds are extracted and this increase the amount of organic solvent to be used and thus increase the cost of extraction. Hence there is the need to improve the extraction method of inorganic solvents (water) with inorganic compounds.

Sodium chloride, also known as salt (inorganic compound) is an essential compound our body need to function properly, when consumed moderately and as such when used for extraction of plant materials, the isolated compounds will pose no threat to human health. Also, sodium chloride extract natural compound from plant materials due to the salt aggressiveness in separating the plant cells or tissues [23]. For example this may be explained by the salting-in effect of protein at higher ionic strength [24]. Hence extraction with sodium chloride will not involve several extractions stages and as such will reduce the amount of solvent to be used for extraction as well as the cost of extraction.

Limited studies have investigated the antimicrobial activity of African pear and African star apple seed extracts, most of which have focus on crude extracted with water, organic solvents (hexane, ethanol and methanol) and their combinations, thus there is little or no information relating to the extracts with sodium chloride. Hence, this study aimed at investigating the effect of sodium chloride extract of *Dacryodes edulis* (African pear) and *Chrysophyllum albidum* (African star apple) seeds on enteric pathogens.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh ripe fruits of African pear and African star apple were purchased from a local market (Uselu) in Benin City, Edo State, Nigeria. The fruits were identified and authenticated by a Botanist in the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

2.2 Processing of the Plant Materials

The fruits were washed thoroughly with distilled water and air-dried, after which they were sliced open manually using a stainless steel knife to obtain their seeds. These seeds were sun dried and then further shelled manually by hand squeezing and stone cracking to obtain their seed kernels which were air-dried at room temperature. The seed kernels were then pulverised mechanically and sieved manually into fine powders.

2.3 Preparation of Extracts

The seed kernel powders were extracted with sodium chloride. The extracts were prepared to obtained concentrations of 50 mg/ml and 100 mg/ml. These concentrations were prepared by adding 0.5 g and 1 g separately of each seed kernel powder into 0.1 M sodium chloride solution (0.58g of sodium chloride powder was added to 10ml of distilled water and stirred properly using a magnetic stirrer for 10 minutes to completely dissolve the salt powder), the suspensions were mixed using a magnetic stirrer for 10 minutes and then filtered using WhatMan Number 1 equivalent filter paper. The filtrates were used as the sodium chloride seed extracts. Fresh sodium chloride seed extracts were prepared at every day of use.

2.4 Preparation of the Culture Media

The culture media used in the study are the CM0003 nutrient agar (Oxoid) and the CM0001

nutrient broth (Oxoid) media. They were prepared according to manufacturer's specifications and for the nutrient broth media, both the single and double strength were prepared.

2.5 Test Micro-organisms Used

The test micro-organisms used in the study were obtained from Microbiology Laboratory, Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi and they are; *Escherichia coli* (ATCC25922), *Salmonella typhi* (clinical strain), *Klebsiella pneumoniae* (clinical strain), *Pseudomonas spp.* (ATCC4853), *Enterococcus faecalis* (ATCC29212) and *Staphylococcus aureus* (ATCC25923). The stock solutions of the test micro-organisms were prepared as follow;

2.5.1 Preparation of stock solutions

Test organisms were cultured in a single strength nutrient broth media to obtain stock solutions; 0.1 ml (100 µl) of individual test organism was inoculated into 10 ml of the liquid broth inside separate test tubes, the test tubes were plugged with cotton wool and incubated at 37°C for 24 hours. These microbial suspensions were further prepared to 0.5 McFarland standard by double fold serial dilution to obtain standardized microbial suspensions.

2.6 Antimicrobial Activity of Extracts

The extracts were initially screened for Antimicrobial activity using Kirby-Bauer agar well diffusion method. Then, the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) testing was carried out using the micro broth dilution method.

2.6.1 Antimicrobial susceptibility testing

The antimicrobial activity for each type of seed extract against individual test organism; *S.aureus*, *E. faecalis*, *E. coli*, *K. pneumoniae, Pseudomonas* and *S. typhi* was carried out using Kirby-Bauer agar well diffusion method. Growth medium (about 22ml) was poured into sterile plates (90 mm diameter) and then allowed to solidify at room temperature. Wells were bored in the agar plates using a sterile cork borer (no. 7). The wells were filled with 0.2 ml of 50 mg/ml or 100 mg/ml concentrations of the seed extracts. The plates were left for 30 minutes for effective diffusion of the extracts into the agar and then incubated at 37°C for 24 hours. The diameters of the zone of growth inhibition if any were measured with a transparent ruler. The standard mean error was calculated from the standard deviation of the diameter zone of inhibition determined. The experiments were done in replicates to ensure consistency.

2.6.2 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations of the extracts were determined by the micro broth dilution method using 96 well micro-titre plates [25,26]. A double fold serial dilution of the most effective concentration (100mg/ml) from previous initial screening of the seed extracts was made using distilled water to obtain further concentrations; 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml,1.5625 mg/ml, 0.78125 mg/ml. Each well in the micro-titre plate was inoculated with equal volume of each type of extract and double strength nutrient broth (100 µl), followed by the standardized micro-organisms (10 µl) making a total volume of 210 µl on each well. A micro-titre plate with wells containing double strength nutrient broth and standardized micro-organisms; double strength nutrient broth without organisms serves as positive and negative controls. The micro-titre plates were immediately incubated at 37°C for 24 hours. The lowest concentration of each type of extract that inhibited microbial growth was observed and recorded as the MIC. This was indicated by the absence of purple colouration after 30 minutes upon the addition of 3-(4, 5-dimethylthiazol -2-yl) -2, 5-diphenyltetrazoliumbromide (MTT) solution to the micro-titre wells after the 24 hours incubation period. The experiments were also done in replicates to ensure consistency.

2.6.3 Minimum bactericidal concentration (MBC)

New micro-titre plates were inoculated with fresh double strength nutrient broth and samples from each well in the micro-titre plates that showed no visible growth from the MIC test. Micro-titre wells were also prepared to contain only double strength nutrient broth which serves as negative control to check the sterility of the media. These micro- titre plates were immediately incubated at 37°C for 24 hours. The minimum bactericidal concentration (MBC) is the lowest concentration

of an antimicrobial agent that results in the death of the microorganisms. Therefore, the lowest concentrations of each type of extracts yielding no growth were considered as the MBC. Experiments were done in replicates to ensure consistency.

3. RESULTS AND DISCUSSIONS

Results of the antimicrobial susceptibility testing, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are presented in Tables 1, 2, 3, 4 and 5.

Table 1 shows result of antimicrobial activity of sodium chloride seed extracts against test organisms. The seed extracts showed antimicrobial activity against most of the test organisms during initial screening. Result also revealed that as concentration of the extracts increases, the inhibitory effect also increases, thus indicating more active components in the extracts. This is in agreement with results reported from previous findings on antibacterial effect of plant extracts (*Citrus sinensis*) against some pathogenic organisms (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*) [27], hence the extracts were more effective at concentration of 100 mg/ml. At this concentration, values of inhibitory zone diameter of African pear seed extract ranges from 16.5 to 23 mm and that of African star apple seed extract ranges from 16.5 to 21.9 mm. *S. typhi*, *Pseudomonas* and *E. faecalis* showed sensitivity to both extracts. *E. coli, K. pneumoniae.* and *S. aureus* showed no activity against African star apple seed extract. This may be attributed to the fact that on solid medium bacteria grow on the surface and concentration gradient could develop during incubation leading to pseudo resistance of bacteria [28]. Largest inhibition zone diameter was showed by African pear seed extract against *S. typhi*. Studies have revealed that certain bioactive components in plants are known to exert antimicrobial activity [29].

Table 2 and Table 3 showed result of minimum inhibitory concentration assay and minimum inhibitory concentration (MIC) of sodium chloride seed extracts. Results indicated that the activities of the both extracts were concentration dependent. Positive control showed that the media without the extracts had no inhibitory effect on bacterial growth and negative control showed that the media was not contaminated with bacteria. From Table 3, result revealed that both extracts were bacteriostatic against all test organisms. This might be due to the fact that in a liquid broth medium, there are more cells to antimicrobial compound contact as the bacteria is submerged in the plant extracts-containing medium [28]. MIC values ranges from 1.5625 to 50 mg/ml for African pear seed extract and 6.25 to 50mg/ml for African star apple seed extract. Notably, the lowest MIC value (1.5625mg/ml) was recorded for African pear seed extract against *Pseudomonas*, thus the extract exhibited highest antimicrobial activity against the organism and the highest MIC value (50mg/ml) was recorded for both extracts against *K. pneumonia*.

Aqueous and methanol extracts of African star apple leave have also exhibited antimicrobial activity against microorganisms (*E. coli, S. typhi, Shigella spp.*) [18]. Some plant seed powders (*Mangifera indica* and *Citrus aurantiifolia*) extracted with sodium chloride have also shown antibacterial activity against some pathogens (*E. coli*, *S. typhi*, *K. pneumoniae*, *Pseudomonas*, *E. faecalis* and *S. aureus*) [30].

Table 4 and Table 5 showed results of minimum bactericidal concentration (MBC) assay and minimum bactericidal concentration (MBC) of sodium chloride seed extracts. Negative control further showed that the media was not contaminated with bacteria. Result from Table 5 indicated that the extracts were bactericidal against all test organisms with concentrations (MBC) ranging from 6.25 to 100 mg/ml for African pear seed extract and 25 to 100mg/ml for

Table 1. Antimicrobial activity of sodium chloride seed extracts against test organisms

Seed extracts/ concentration (mg/ml)	Test organisms/ Mean diameter zone of inhibition (mm)±SEM against					
	E. coli	***S. typhi***	***K. pneumoniae***	***Pseudomonas***	***E.faecalis***	***S.aureus***
APS						
100	18.5±0.87	23±0.0	18±0.58	21±0.58	21.9±0.64	16.5±0.58
50	na	na	na	17.6±0.23	na	na
ASAS						
100	na	17±0.0	na	16.5±0.87	21.9±0.64	na
50	na	na	na	na	na	na

na=No activity; SEM=Standard error mean; APS= African pear seed; ASAS= African star apple seed

Table 2. Minimum inhibitory concentration assay of sodium chloride seed extracts

Seed extracts	Test organisms	Concentrations (mg/ml)							
		100	50	25	12.5	6.25	3.125	1.5625	0.78125
APS	*E. coli*	-	-	-	+	+	+	+	+
	S. typhi	-	-	-	-	+	+	+	+
	K. pneumoniae	-	-	+	+	+	+	+	+
	Pseudomonas	-	-	-	-	-	-	-	+
	E. faecalis	-	-	-	+	+	+	+	+
	S. aureus	-	-	-	+	+	+	+	+
ASAS	*E. coli*	-	-	+	+	+	+	+	+
	S. typhi	-	-	-	+	+	+	+	+
	K. pneumoniae	-	-	+	+	+	+	+	+
	Pseudomonas	-	-	-	-	-	+	+	+
	E. faecalis	-	-	-	-	+	+	+	+
	S. aureus	-	-	+	+	+	+	+	+

+= Indicate growth; -= Indicate no growth

Table 3. Minimum inhibitory concentration (MIC) of sodium chloride seed extracts

Seed extracts	Test organisms/ MIC (mg/ml)					
	E. coli	***S. typhi***	***K. pneumoniae***	***Pseudomonas***	***E. faecalis***	***S. aureus***
APS	25	12.5	50	1.5625	25	25
ASAS	50	25	50	6.25	12.5	50

Table 4. Minimum bactericidal concentration assay of sodium chloride seed extracts

Seed extracts	Test organisms	Concentrations (mg/ml)							
		100	50	25	12.5	6.25	3.125	1.5625	0.78125
APS	*E. coli*	-	-	nd	nd	nd	nd	nd	nd
	S. typhi	-	-	nd	nd	nd	nd	nd	nd
	K. pneumonia	-	+	nd	nd	nd	nd	nd	nd
	Pseudomonas	-	-	-	-	-	+	+	nd
	E. faecalis	-	-	+	nd	nd	nd	nd	nd
	S. aureus	-	-	nd	nd	nd	nd	nd	nd
ASAS	*E. coli*	-	nd	nd	nd	nd	nd	nd	nd
	S. typhi	-	-	nd	nd	nd	nd	nd	nd
	K. pneumonia	-	nd	nd	nd	nd	nd	nd	nd
	Pseudomonas	-	-	-	+	+	nd	nd	nd
	E. faecalis	-	-	-	+	nd	nd	nd	nd
	S. aureus	-	+	nd	nd	nd	nd	nd	nd

+= Indicate growth; -= Indicate no growth; nd= not determined

Table 5. Minimum bactericidal concentration (MBC) of sodium chloride seed extracts

Seed extracts	Test organisms/MBC(mg/ml)					
	E. coli	***S. typhi***	***K. pneumoniae***	***pseudomonas***	***E. faecalis***	***S. aureus***
APS	50	50	100	6.25	50	50
ASAS	100	50	100	25	25	100

African star apple seed extract. Again, the least MBC value (6.25mg/ml) was recorded for African pear seed extract against *Pseudomonas* and the highest MBC value (100mg/ml) was recorded for both extracts against *K. pneumoniae*.

Results (Table 3 and 5) clearly showed that MIC values were lower than MBC values, suggesting that both extracts were bacteriostatic at lower concentration and bactericidal at higher concentration. These extracts may be working with cytotoxicity mechanism as the cell of the test

organisms may have undergoes necrosis in which they might have lost their membrane integrity and died rapidly as a result of cell lysis. A combination of aqueous and ethanol extract of African pear seed has been reported to show antimicrobial activity against pathogenic organisms (*S. aureus, Streptococcus faecalis, Candida albicans, E. coli, Coliform bacilli* and *S. typhi*) [31]. It was found that the extract was bactericidal against *C. albicans* only whereas it was bacteriostatic against all other tested organisms (*S. aureus, S. faecalis, E. coli, C. bacilli* and *S. typhi*). Hence, sodium chloride extract of African pear seed showed better antimicrobial activity, since it was both bacteriostatic and bactericidal against all the tested organisms (*E. coli*, *S. typhi*, *K. pneumoniae*, *Pseudomonas*, *E. faecalis* and *S. aureus*).

4. CONCLUSION

This study demonstrated that the sodium chloride extract of African pear and African star apple seeds showed potential antimicrobial activity of MIC and MBC ≤ 100 mg/ml, thus they have antimicrobial activity against enteric pathogens (*E. coli*, *S. typhi*, *K. pneumoniae*, *Pseudomonas*, *E. faecalis* and *S. aureus*). Hence, sodium chloride will be useful for extracting bioactive agents in African pear and African star apple seeds, thus the compounds isolated will pose no threat to human health, the cost of extraction will be reduced (since several stages of extraction are not required to obtained natural compounds) and this will lead to reducing the incidence of intestinal diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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REFERENCES

1. Anonymous. Infection and intoxications of the intestines diseases. [Online] Accessed; 2017.
Available:https://www.atsu.edu/faculty/chamberlain/Website/lectures/lecture/GI4.htm
2. WHO/UNICEF. Joint Monitoring Programme for Water Supply and Sanitation: Progress on Drinking Water and Sanitation; 2015.
3. Ginovyan M, Petrosyan M, Trchounian A. Antimicrobial activity of some plant materials used in Armenian traditional medicine. BMC Complement Altern Med. 2017;17:50.
DOI: 10.1186/s12906-017-1573-y
PMCID: PMC5240328
4. World Health Organization. Antimicrobial resistance. Antimicrob Resist Glob Rep surveillance. Geneva WHO Press; 2014.
5. Cowan MM. Plant Products as Antimicrobial Agents. Clin Microbiol Rev. 1999;12:564–582.
PMID: 10515903
PMCID: PMC88925
6. Savoia D. Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiol. 2012;7(8):979–990.
DOI: 10.2217/fmb.12.68
PMID: 22913356
7. Upadhyay A, Upadhyaya I, Kollanoor-Johny A, Venkitanarayanan K. Combating Pathogenic Microorganisms Using Plant-Derived Antimicrobials: A Minireview of the Mechanistic Basis. BioMed Research International. 2014;18.
DOI: ttp://dx.doi.org/10.1155/2014/761741
8. Rajeh MAB, Zuraini Z, Sasidharan S, Latha LY, Amutha S. Assessment of Euphorbia hirta L. leaf, flower, stem and root extracts for their antibacterial and antifungal activity and brine shrimp lethality. Molecules. 2010;15:6008–6018.
DOI: 10.3390/molecules15096008
PMID: 20877206
9. Borris RP. Natural products research: perspectives from a major pharmaceutical company. J Ethnopharmacol. 1996;51:29–38.
DOI: 10.1016/0378-8741(95)01347-4
10. Petrosyan M, Shcherbakova Y, Sahakyan N, Vardanyan Z, Poladyan A, Popov Y, et al. Alkanna orientalis (L.) Boiss. plant isolated cultures and antimicrobial activity of their extracts: phenomenon, dependence on different factors and

effects on some membrane-associated properties of bacteria. Plant Cell Tissue Organ Cult. 2015;122:727–738.
DOI: 10.1007/s11240-015-0806-3

11. Okunomo K, Egho EO. Economic importance of some underexploited tree species in Nigeria: urgent need for separate research centres. Continental J. Biol. Sci. 2010;3:16-32.
12. Okwu DE, Nnamdi FU. Evalution of the chemical composition of *Dacryodes edulis* and *Raphia hookeri mann* exudates used in herbal medicine in Southern Eastern Nigeria. Afri. J. Traditional Complex Altima Med. 2008;5:194-200.
13. Seghosime A, Awudza JAM, Buamah R, Kwarteng SO. Comparative studies on proximate composition and phytochemical screening of mango, key lime, African star apple and African pear seeds as possible coagulant aids for water treatment. American Journal of Environmental Sciences. 2017;13(4):325-333.
DOI: 10.3844/ajessp.2017.325.333
14. Ogunmoyole T, Kade IJ, Johnson OD, Makun OJ. Effect of boiling on the phytochemical constituents and antioxidant properties of African Pear (*Dacryodes edulis)* seed in *vitro.* African Journal of Biochemistry Research. 2012;6(8):105-114.
DOI: 10.5897/AJBR12.023
15. Ajayi IA, Adesanwo O. Comparative study of the mineral element and fatty acid composition of *Dacryodes edulis* pulp and seed. World J. Agric. Sci. 2009;5(3):279-283.
16. Isiuku B, Nwanjo H, Asimole C. A comparative study of the lipid protein and mineral contents of African pear *(Dacryodes edulis)* seed and avocado pear seeds. The Internet Journal of Nutrition and Wellness. 2008;8(2).
17. Akpabio UD, Aniekan EA, Godwin NE. Evaluation of proximate compositions and mineral elements in the star apple peel, pulp and seed. J. Basic. Appl. Sci. Res. 2012;2(5):4839-4843.
18. Muanya C. Researchers discover African star apple; agbalumo to treat diabetes, heart disease, infections. Africa Information; 2011.
(Accessed 27 November 2017)
Available:http://www.informafrica.com/blog/researchers-discover-african-star-apple-agbalumo-to-treat-diabetes-heart-disease-infections
19. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 2010; 15:7313-7352.
DOI: 10.3390/molecules15107313
20. Ali AB, Almagbout AZ, Mohammed OM. Antimicrobial activity of fruits, leaves, seeds and stems extracts of *ziziphus spina-christi*. Arabian Journal of Medicinal and Aromatic Plants. 2015;1(2):94-107.
21. Ehiowemwenguan G, Emoghene AO, Inetianbor JE. Antibacterial and phytochemical analysis of banana fruit peel. Journal of Pharmacy. 2014;4(8):18-25.
22. The National Institute for Occupational Safety and Health (NIOSH). National Institute for Occupational Safety and Health Education and Informative division; 2017.
23. Alfred NJ, Bridgeman J. Characterisation of natural organic matter (NOM) in water treatment using seed extracts. Journal of Water Management and Research. 2015; 71:239–245.
24. Okuda T, Baes UA, Nishijima W, Okada M. Improvement of extraction method of coagulation active components from *moringa oleifera* seed. Wat. Res. 1999;33(15):3373-3378.
25. Eloff JN. A sensitive and quick micro-plate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998;64(8):711-713.
DOI: 10.1055/s-2006-957563
PMID: 9933989
26. Wiegand I, Hilpert K, Hancock RW. Agar and broth dilution methods to determine the minimal inhibitory concentration of antimicrobial substances. Nat. Protoc. 2008;3(2):163-175.
DOI: 10.1038/nprot.2007.521
PMID: 18274517
27. Adu F, Apenteng JA, Kuntworbe N, Akanwariwiak WG, Appiah T, Mintah DN. Antimicrobial and antioxidant activities of *citrus sinensis* var. late Valencia fruit at various stages of development. African Journal of Microbiology Research. 2016; 10(3):73-78.
DOI: 10.5897/AJMR2015.7725
28. Mushore J, Matuvhunye M. Antibacterial properties of *mangifera indica* on *staphylococcus aureus*. African Journal of Clinical and Experimental Microbiology. 2013;14(2):62-74.
Doi:http://dx.doi.org/10.4314/ajcem.v14i2.4

29. Chung PY, Navaratnam P, Chung LY. Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against *staphylococcus aureus* strains. Annals of Clinical Microbiology and Antimicrobials 2011;10(25):1–6. DOI:10.1186/1476-0711-10-25 PMID: 21658242 PMCID: PMC3127748
30. Seghosime A, Ebeigbe AB, Awudza JAM. Potential use of *mangifera indica* seed kernel and *citrus aurantiifolia* seed in water disinfection. Nigerian Journal of Technology. 2017;36(4):1303-1310. DOI: http//dx.doi.org/10.4314/njt.v36i4.41
31. Nwokonkwo DC. The phytochemical study and antibacterial activities of the seed extract of *Dacryodes edulis* (African native pear). American Journal of Scientific and Industrial Research. 2014;5(1):7-12.

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