In vitro antibacterial activity of acetone crude extract, partitioned and chromatographic fractions of *Dacryodes edulis* leaf

Hassan-Olajokun, R. E[1], Ajayi, O. M[2], Olasunkanmi, O. O[3], Olaniran, O[1], Awoyeni, A. E[1] Odetoyin, B. W[1], Makanjuola, S. O[4] and Folorunso, J. B[5]

[1]Department of Medical Microbiology and Parasitology, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
[2]Department of Family Medicine and Primary Care, Faculty of Health Sciences, University of the Witwatersrand, South Africa
[3]Department of Microbiology, Faculty of Science, Awolowo University, Ile-Ife, Osun State, Nigeria.
[4]Department of Medical Microbiology and Parasitology, College of Health Sciences, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria.
[5]Medical Laboratory Department, Directorate of Health Services, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.
Author Correspondence: racheloghogho@gmail.com , +2348034037587

Abstract

The rise in the need for the development of alternative antibiotics is a very crucial and urgent matter. This is as a result of abuse of antibiotics use which resulted in the emergence of multi-drug resistance microorganisms.The objective of this study is to determine the antimicrobial activities of the leaf extract of African pear Dacryodes edulis on bacterial isolates.The leaf of D. edulis was extracted with absolute acetone, partitioned into Dichloromethane and water, and fractionized with thin-layer chromatography. The antibacterial activities of the crude extract, partitioned and chromatographic fractions were determined by agar well diffusion test, while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts and fractions were determined by broth dilution and agar diffusion methods respectively. Partitioning resulted in two major fractions; aqueous (AQUpf) and dichloromethane (DCMcf), and chromatography produced three column fractions by column chromatography; AQU1cf, AQU2cf and DCMcf. Both crude extract and fractions had antibacterial activities against all tested bacteria while the control standard antibiotic (ampicillin) was not active against two bacteria, Pseudomonas aeruginosa and Bacillus subtilis. The results showed that crude extract and fractions exhibited broad-spectrum antibacterial efficacy but DCMpf and AQUpf fractions had greater activities. There was a significant difference between the activities of DCMpf (19-32mm) and AQUpf (17-30mm) at 95% confidence interval ($p<0.05$), which indicated that DCM is a better solvent for extraction than water. Both AQU1cf and DCMcf had significantly greater zones of inhibitions and therefore greater activity compared to AQU2cf ($p<0.05$). All the fractions showed greater antimicrobial efficacy than the crude extract hence proving that the fractions contain substantial bioactives. The MIC index of all extracts and fractions indicates the bactericidal activity of the plant. From this study, D. edulis leaf appears to be a promising source of antimicrobial agents as it retained bioactive in a partially purified state.

Keywords: *Dacryodes edulis,* Antimicrobial susceptibility test, Acetone crude Extract. Column fraction

Activité antibactérienne In Vitro de l'Extrait Brut à l'Acétone, fractions Partitionnées et Chromatographiques de la Feuille de *Dacryodes edulis*

Resume

L'augmentation du besoin de développement d'antibiotiques alternatifs est une question très cruciale et urgente. Ceci est dû à l'utilisation abusive d'antibiotiques qui a entraîné l'émergence de micro-organismes multirésistants aux médicaments. L'objectif de cette étude est de déterminer les activités antimicrobiennes de

l'extrait de feuille de poirier africain Dacryodes edulis sur des isolats bactériens.La feuille de Dacryodes edulis a été extraite avec de l'acétone absolue, partagée dans du dichlorométhane et de l'eau, et fractionnée par chromatographie en couche mince. Les activités antibactériennes de l'extrait brut, des fractions partitionnées et chromatographiques ont été déterminées par un test de diffusion en puits d'agar, tandis que la concentration minimale inhibitrice (CMI) et la concentration bactéricide minimale (CBM) des extraits et des fractions ont été déterminées respectivement par les méthodes de dilution en bouillon et de diffusion sur gélose. Le partitionnement a abouti à deux fractions principales ; aqueux (AQUpf) et du dichlorométhane (DCMcf), et la chromatographie a produit trois fractions de colonne par chromatographie sur colonne ; AQU1cf, AQU2cf et DCMcf. L'extrait brut et les fractions avaient des activités antibactériennes contre toutes les bactéries testées, tandis que l'antibiotique standard de contrôle (ampicilline) n'était pas actif contre deux bactéries, Pseudomonas aeruginosa et Bacillus subtilis. Les résultats ont montré que l'extrait brut et les fractions présentaient une efficacité antibactérienne à large spectre, mais que les fractions DCMpf et AQUpf avaient des activités plus importantes. Il y avait une différence significative entre les activités du DCMpf (19-32 mm) et de l'AQUpf (17-30 mm) à un intervalle de confiance de 95 % ($p < 0,05$), ce qui indique que le DCM est un meilleur solvant d'extraction que l'eau. AQU1cf et DCMcf avaient des zones d'inhibition significativement plus grandes et donc une plus grande activité par rapport à AQU2cf ($p<0,05$). Toutes les fractions ont montré une plus grande efficacité antimicrobienne que l'extrait brut, prouvant ainsi que les fractions contiennent des substances bioactives substantielles. L'indice MIC de tous les extraits et fractions indique l'activité bactéricide de la plante. D'après cette étude, la feuille de D. edulis semble être une source prometteuse d'agents antimicrobiens car elle conserve son bioactivité dans un état partiellement purifié.

Mots-clés : *Dacryodes edulis*, test de sensibilité aux antimicrobiens, extrait brut d'acétone. Fraction de colonne

إن زيادة الحاجة إلى تطوير : *الخلفية* *In Vitro antibacterial activity Of acetone crude extract, partitioned and .مضادات حيوية بديلة أمر بالغ الأهمية هذا نتيجة تعاطي المضادات الحيوية التي أسفرت عن ظهور كائنات دقيقة مقاومة للأدوية المتعددة *Dacryodes*الهدف من هذه الدراسة هو تحديد الأنشطة المضادة للميكروبات لمستخلص الأوراق من الكمثرى الأفريقية :*هدف البحث* بالأسيتون المطلق مقسمة إلى ثنائي كلورو الميثان والماء، D. edulisتم استخراج ورقة :*منهج البحث* .على العزلات البكتيرية *edulis* ومقسمة إلى كروماتوغرافيا رقيقة الطبقة الأنشطة المضادة للبكتيريا في المستخلص الخام تم تحديد الكسور المقسمة والكروماتوغرافية من خلال اختبار انتشار آبار آجار، في حين أن الحد الأدنى للتركيز المثبط والحد الأدنى للتركيز البكتيري المستخلصات والكسور تحددها طرق وثنائي كلورو الميثان والكروماتوغرافيا (AQUpF)وأسفر التقسيم عن جزأين رئيسيين ؛ المائية :*نتيجة* .تخفيف المرق وانتشار الأغار كان لكل من المستخلص الخام والكسور *AQU1cf, AQU2cf and DCMcf* ;أنتجت ثلاثة أجزاء من الأعمدة حسب كروماتوغرافيا العمود لم يكن نشطًا ضد بكتيريا (الأمبيسيلين)أنشطة مضادة للبكتيريا ضد جميع البكتيريا المختبرة في حين أن المضاد الحيوي القياسي للتحكم أظهرت النتائج أن المستخلص الخام والكسور أظهرت مضادات البكتيريا واسعة *Bacillus subtilis*. و*Pseudomonas aeruginosa* لهما أنشطة كبيرة، كان هناك فرق كبير بين أنشطة AQUpf DCMpfالطيف لكن قسمة *DCMpf (19-32 mm)*و (ملم *AOFFpf (17-30* و*DCMcf* AQU1cfهو مذيب أفضل للاستخراج من الماء كان لكل من DCMالذي أشار إلى أن ($p < 0.05$)٪ فترة ثقة 95في (ملم أظهرت جميع الكسور فعالية مضادة للميكروبات أكبر من *AQU2cf (p<0.05)*مناطق أكبر بكثير من المثبطات وبالتالي أكبر قياسا ب لجميع المستخلصات والكسور إلى النشاط MICالمستخلص الخام وبالتالي إثبات أن الكسور تحتوي على مواد حيوية كبيرة يشير مؤشر مصدر واعد للعوامل المضادة للميكروبات حيث أنها تحتفظ بنشاط D. edulisمن هذه الدراسة، يبدو أن ورقة *ختاما* .البكتيري للنبات بيولوجي في حالة تنقية جزئية.*chromatographic fractions of dacryodes edulis leaf*

Introduction

Since the ancient times, mankind has been relying on plant as sources of therapy to alleviate or cure illness (Kamboj, 2000). In most parts of the world, especially in Africa and China, plant-derived medicines have been part of Ethnomedicine for thousands of years and there is increasing interest in them as sources in the treatment of diseases (Ajayi , Akintola, 2010). The advantages of natural agents are that they are cheaper, readily available, believed to be relatively safe with lesser "antibiotic resistance", a phenomenon commonly developed with the long-term use of synthetic antibiotics (Vukovic et

al 2007). Medicinal plants have been highly esteemed and regarded as the most exclusive source of life-saving therapy for the majority [80%] of the world's population. Over time, bacterial pathogens have persisted as a major health problem with the emergence of multi-drug resistance (MDR) strains (Okoye, 2006). Currently, there are several ongoing intensive studies for the possible discovery of new agents with antibacterial potentials especially as microorganisms continue to have unfavourable impact on the quality and safety of life (Okoye et al 2010). According to the World Health Organization (WHO) medicinal plants would be the best source to lead for a variety of drug compounds since the majority of individuals worldwide use traditional medicine which contain several phytochemicals (Santos et al 1995). These pieces of evidence contribute to supporting and quantifying the importance of screening natural products. Therefore, researches are necessary to discover new antibiotics, especially from the plant source. *Dacryodes edulis* known as African pear is an annual fruiting tree, consumed traditionally either as raw, roasted or boiled in hot water and can be eaten alone or used in garnishing fresh maize. It is widely found in many sub-Saharan countries including Nigeria, Liberia, Cameroons and Zaire (Boungou et al 1991). Usually, the trees are grown around homesteads and flowering always takes place from January to April, with fruiting season between May and October. It may be available for up to 6 months of the year according to (Omoti and Okiy , 1987)and (Lam 1985). In African ethno medicine the different parts of the plant are useful in the treatment of several diseases from minor ailments to severe ones including wounds, skin diseases, dysentery and fevers (Lampinen, 2005). The parts of the plant for medicinal use are the bark, roots leaves, bark resin and leaf resin. Each part has a definite way of preparation which includes methods like decoction, boiling, aroma and sap. *D. edulis* has also been found to be effective synergistic property when combined with other plants like *Lanata camara* and *Persea americana* (EUCAST, 2003). In the Democratic Republic of Congo, a decoction of bark is used for gargle, mouth wash and for tonsillitis (CLSI, 2006). A decoction of the root bark is taken for leprosy, with combination of palm oil, is applied topically to relieve pains, fatigue, and stiffness and cures skin disease (Cao, Xue and Liu, 2009). Resins from the bark have been reported to heal scars and other skin problems in Nigeria (Mayachiew, et al 2010) including parasitic skin diseases and jiggers (Valgas et al, 2007). The effective topical application has been reported by Ekpa when the resin mixed with lotion and creams smoothen and protect the skin. Antibaterial activity of *D. edulis* seed of food-borne pathogens have been reported (Irobi et al, 1994) and Hexane extract of the fruits is reported to be anti-diabetic and hypolipidaemic potentials in Alloxan Diabetic of rats. (Olasunkanmi et al, 2017). Clinical trials are focused on understanding among others the bio-availability, efficacy, safety and drug synergism of newly discovered bioactive components and their formulations (extracts). This study is therefore aimed at *D. edulis* plant crude extract, partitioned and chromatographic fractions for in vitro antibacterial activities against different bacteria as well as to determine the minimum inhibitory and minimum bactericidal concentrations of the crude extract and fractions.

Materials and methods

Bacterial isolates

Locally isolated organisms (LIO) used were *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli, Corynebacterium pyogenes*, and *Proteus vulgaris.* These were obtained from the Department of Medical Microbiology and Parasitology of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Nigeria. Others were *Bacillus subtilis* (NCIB 3610), *E. coli* (NCIB 86), *S. aureus* (NCIB 8588), *Micrococcus luteus* (NCIB 196), *Klebsiella pneumoniae* (NCIB 418), *Pseudomonas aeruginosa* (NCIB 950), and *Pseudomonas fluorescens* (NCIB 3756).

Plant sample

The leaves of *D. edulis* were collected from Opa area, Ile-Ife, Osun State, Nigeria between October and December 2018, a period outside the fruiting season. The plant was identified and authenticated by Mr. G. A. Ademoriyo of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria and a voucher specimen (IFE-17653) was

deposited. The sample was oven-dried at 40 °C until a constant weight was observed. The dried leaf was powdered and stored in an air-tight container for further use.

Preparation of extract

About 1 kg of the powdered leaf of *D. edulis* was extracted at room temperature using absolute acetone for 3 days. The mixture was then filtered and the filtrate was dried in vacuo using a rotary evaporator. A glossy black yield of 122 g was collected.

Fractionation of crude acetone extract

The crude extract was successfully partitioned using organic solvents in order of their polarity index n-hexane (0.1), dichloromethane (3.1), ethyl acetate (4.4) and n-butanol (4.0). Exactly 90 g of the crude extract was dissolved in 100 mL of sterile distilled water in a 500 mL separatory funnel, extracted with n-hexane until a clear and colourless layer of n-hexane was obtained. The resulting n-hexane fraction was concentrated to dryness *in vacuo* and the fraction residue was kept in a freezer in an air-tight container. The resultant aqueous phase was re-concentrated *in vacuo* to remove traces of n-hexane. The residue was further extracted with dichloromethane (DCM) until a clear and colourless layer of DCM was obtained. The DCM fraction obtained was also concentrated *in vacuo* to dryness and the powder collected was kept in the freezer for further use. N-butanol and ethylacetate fractions were also obtained using a similar procedure. The aqueous fraction was freeze-dried and the resultant powder was kept in the freezer for further use.

Column chromatography

A glass column (650 x 40 mm) was clamped on the retort stand with the tap opened. This was rinsed with 100 % methanol to remove any contaminant that may be present followed by n-hexane. An adequate amount of cotton wool was decontaminated with methanol in a beaker to remove likely contaminants; the cotton wool was pressed against the side of the beaker with a clean spatula to drain and dry and then soaked in n-hexane. The decontaminated cotton wool was transferred into the glass column with a clean spatula, driven with a clean glass packing rod to the bottom of the glass column and filled with n-hexane up to three-quarter of the capacity. Exactly 20 g of silica gel was poured gently into the glass column with constant tapping of the glass column with a rubber ring and an adequate amount of n-hexane was allowed to flow through the silica gel in the glass column. Thereafter, exactly 8 g of the sample was dissolved in a small amount of methanol and adsorbed on 25 g of silica gel and allowed to dry in a cool dry place. This was carefully transferred into the glass column using a clean glass funnel such that it forms a proper layer on the silica gel slurry. An adequate amount of pre-extracted cotton wool was inserted and driven down with the glass rod until it was just above the top of the loaded sample. This was eluted with 100 % n-hexane and then the polarity was varied in an increasing gradient of ethylacetate and methanol. The eluent was collected in test tubes, analysed using thin-layer chromatography (TLC) to determine fractions with similar retention factors (Rf). Fractions with similar retention factors were bulked together, concentrated to dryness in *vacuo* and stored in an airtight container in the refrigerator for further use.

Determination of solvent system using thin-layer chromatography

The best solvent system for the elution of dichloromethane fraction on column chromatography was determined by eluting the fractions with different solvents system on thin layer chromatography (TLC) plates. The solvent was poured into the developing chamber to a depth of about 0.5 cm. Part of the inside of the chamber was lined with filter paper, covered with a lid, swirl gently and allowed to stand till the TLC plate was prepared. The pre-coated TLC plate was cut out into a convenient size without disturbing the coating on the adsorbent. A line that served as the origin was carefully drawn across the TLC plate at a 0.5 cm mark above the bottom of the plate with a pencil. The fraction (1 mg) was dissolved in 1 mL of dichloromethane and the resulting solution was carefully spotted on the prepared TLC plate at the origin with micro-capillary tube. The spotted plate was afterward developed in the TLC chamber. The TLC plate was allowed to dry. The coloured spot was cycled with pencil and plate visualized under UV light at 254 nm and 366 nm and in an iodine tank to view

any colourless spot. The solvent system determined for dichloromethane fraction elution based on the Retention factor (Rf) values was in the order of dichloromethane-methanol (4:1), dichloromethane-methanol (3:2), and dichloromethane-ethylacetate (3:2) while the aqueous fraction elution was dichloromethane-methanol (7:3), dichloromethane-ethylacetate (3:2) and n-hexane-ethylacetate (4:1). The Rf for each active compound was calculated for each fraction using the following formula:

$$Rf = \frac{\text{Distance moved by the solute/compound}}{\text{Distanced moved by the solvent (solvent front)}}$$

The AST of the extract was determined using the agar-well diffusion method as described by Irobi *et al 1994*. Freshly grown bacterial culture in nutrient broth for 18 hrs was used for this experiment. For standardization the broth culture was diluted until the bacterial suspension matched with the turbidity of 0.5 McFarland standards. Exactly 0.1 ml of the standardized test isolates were evenly spread on an agar medium using a sterile glass spreader. Wells were bored into the agar medium using a sterile 6 mm cork borer and carefully filled up with 35 mg/ml concentration of acetone crude extract (ACE) solution without spilling. One out of the wells in each plate was filled with 0.1ml of Acetone, Dichloromethane and Distilled sterile water as controls. The plates were allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the extract into the medium before incubation at 37ºC and zones of inhibition were recorded after 24 h. Three cultures were done for each organism and the mean values of the zone diameter of a replicate assay (as mean±SD of a triplicate assay) were taken. Standard ampicillin (AMP) antibiotic (0.1ml of 1 mg/ml) was tested alongside as controls. This procedure was also used for antibacterial activities of the partitioned (AQUpf, DCMpf) and column fractions (AQU1cf, AQU2cf and DCMcf).

Minimum inhibitory concentrations of D. edulis crude extract and fractions on susceptible bacterial strains

The crude extract and fractions minimum inhibitory concentrations were determined by applying the procedure of Olasunkanmi *et al, 2017*. Two millilitres of different concentrations of the solution obtained after two-fold dilution of the extract was mixed with 18 mL of already sterilized molten nutrient agar resulting in a mixture with a concentration range of 0.195 to 25.0 mg/mL. Thereafter, the mixture was emptied into sterile petri dishes and allowed to be set. The plates were oven-dried before streaking onto the set plates, 18 h old strains of bacteria whose turbidity had been adjusted to 0.5 McFarland. The petri dishes were then placed in the incubator for 72 h at 37ºC. Thereafter, the lines of streak on each plate were observed for the presence or absence of growth. The least concentration that had no growth of the bacterial strain was regarded as the minimum inhibitory concentration. The same procedure was followed for the fractions.

Minimum bactericidal concentrations of D. edulis crude extract and fractions on bacterial strains

The minimum bactericidal concentrations of the crude extract and its fractions were evaluated using the method of Okore, 2005. Inocula for this assay were obtained from the streak lines on MIC plates that had observable growth and then transferred by streaking onto freshly prepared nutrient agar plates devoid of the extract and then incubated for 48 h at 37^0C.

Statistical analysis

All the experiments were done in triplicates. Data were analysed by a 4 x 4 Latin square design with statistical program using the GLM model (Statistical Analysis Systems, SAS Institute, Cary, NC, USA, 2001). Results were contrasted with negative and positive control. The mean of the values was compared using an independent t-test of significance ($p < 0.05$) and ANOVA.

Results

Results of Antibacterial sensitivity testing

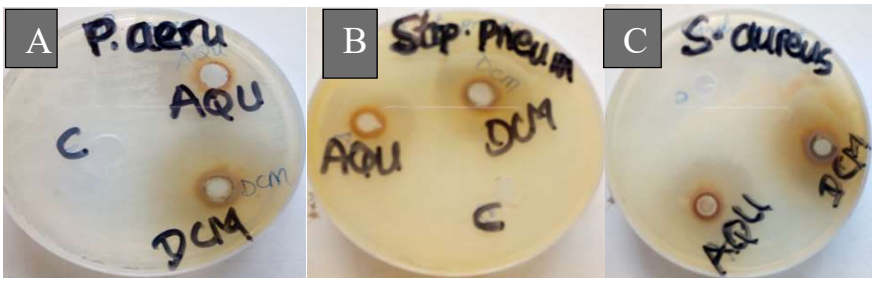

Plate 1: (A) *P. aeruginosa* (B) *S. pneumoniae* (C) *S. aureus* sensitivity to DCMpf and AQUpf partition fractions.

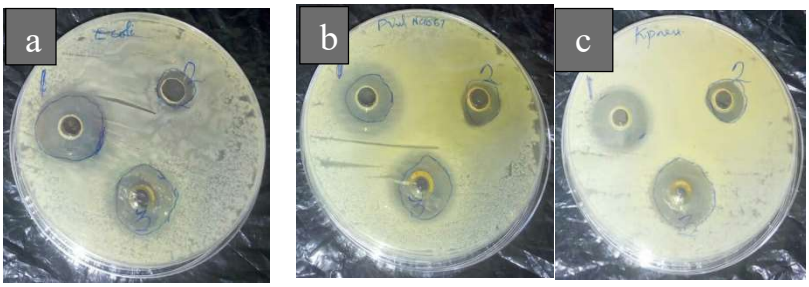

Plate 2: (a) *E. coli* (b) *P. vulgaris* (c*) K. pneumonia* sensitivity to *D. edulis* leaf column fractions (cf).
KEY: 1=AQU1cf, 2=AQU2cf, 3-DCMcfTable 1: Antibacterial activities of the mean values of the zone diameter of a replicate assay of acetone crude extract, fractions and ampicillin, determined by agar diffusion method

Test Organisms	**Zone of inhibition (mm)**						
	ACE (35 mg/mL)	**AQUpf (35 mg/mL)**	**DCMpf (35 mg/mL)**	**AQU1cf (35 mg/mL)**	**AQU2cf (35 mg/mL)**	**DCMcf (35 mg/mL)**	**AMP (1 mg/mL)**
S. aureus (LIO)	17	28	30	17	13	19	20
P. vulgaris (LIO)	15	30	32	19	14	16	15
C. pyogenes (LIO)	15	27	28	20	11	19	21
K. pneumoniae (NCIB 418)	16	25	30	21	15	20	10
E. coli (LIO)	17	20	22	20	13	23	22
M. luteus (NCIB 196)	10	22	24	19	18	20	19
P. aeruginosa (NCIB 950)	15	21	28	20	12	15	0.0
P. fluorescens (NCIB 3756)	14	25	28	17	11	18	20

B. subtilis (NCIB3610)	17	17	19	19	12	20	0.0
S. pneumoniae (LIO)	16	22	24	17	15	18	15

KEY: ACE=Acetone crude extract, (35 mg/mL) AQUpf=Aqueous partitioned fraction, DCMpf=Dichloromethane partitioned fraction, AQU1cf=Aqueous column fraction 1, AQU2cf=Aqueous column fraction 2, DCMcf=Dichloromethane column faction, AMP= Ampicillin. LIO=Locally Isolated Organisms, NCIB=National Collection for Industrial Bacterial.

As presented in Table 1, ACE (10 – 17 mm) at 35 mg/mL had significantly lower antibacterial activity than all the fractions (35 mg/mL) and the standard (control) antibiotic, Ampicillin (1 mg/mL) ($P < 0.05$). DCMpf showed the highest activity of all others and was closely followed by AQUpf

.

Table 2: Minimum Inhibitory and Minimum Bactericidal Concentrations of Acetone crude extract

Test Organisms	MIC (mg/mL)	MBC (mg/mL)	MIC index
Staphylococcus aureus (LIO)	1.56	3.13	2.0
Streptococcus pneumoniae (LIO)	6.25	12.5	2.0
Micrococcus luteus (NCIB 196)	1.56	3.13	2.0
Escherichia coli (LIO)	1.56	3.13	2.0
Klebsiella pneumoniae (NCIB 418)	3.13	6.25	2.0
Pseudomonas aeruginosa (NCIB 950)	3.13	6.25	2.0
Pseudomonas fluorescens (NCIB 3756)	3.13	6.25	2.0
Corynebacterium pyogenes (LIO)	3.13	6.25	2.0
Bacillus subtilis (NCIB 3610)	1.56	3.13	2.0
Proteus vulgaris (LIO)	1.56	3.13	2.0

KEY: LIO=Locally Isolated Organisms, NCIB= National Collection for Industrial Bacteria, MIC index = Ratio of MBC to MIC.

Results of Partitioning, Fractionation and Antibacterial Sensitivity Tests

The partitioning gave two main fractions (AQUpf and DCMpf). N- hexane, ethylacetate and butanol fractions were obtained in small quantities. Based on TLC procedure of fractionation, three fractions resulted (AQU1cf, AQU2cf and DCMcf) from Methanol: Ethylacetate (3:2), N-hexane: Ethylacetate (4:1) and N-hexane: Ethylacetate (4:1) respectively as shown in Tables 4 and 5. Antibacterial efficiencies of the two partition fractions (AQUpf and DCMpf) and three-column

fractions (AQU1cf, AQU2cf and DCMcf) were evaluated against the test organisms
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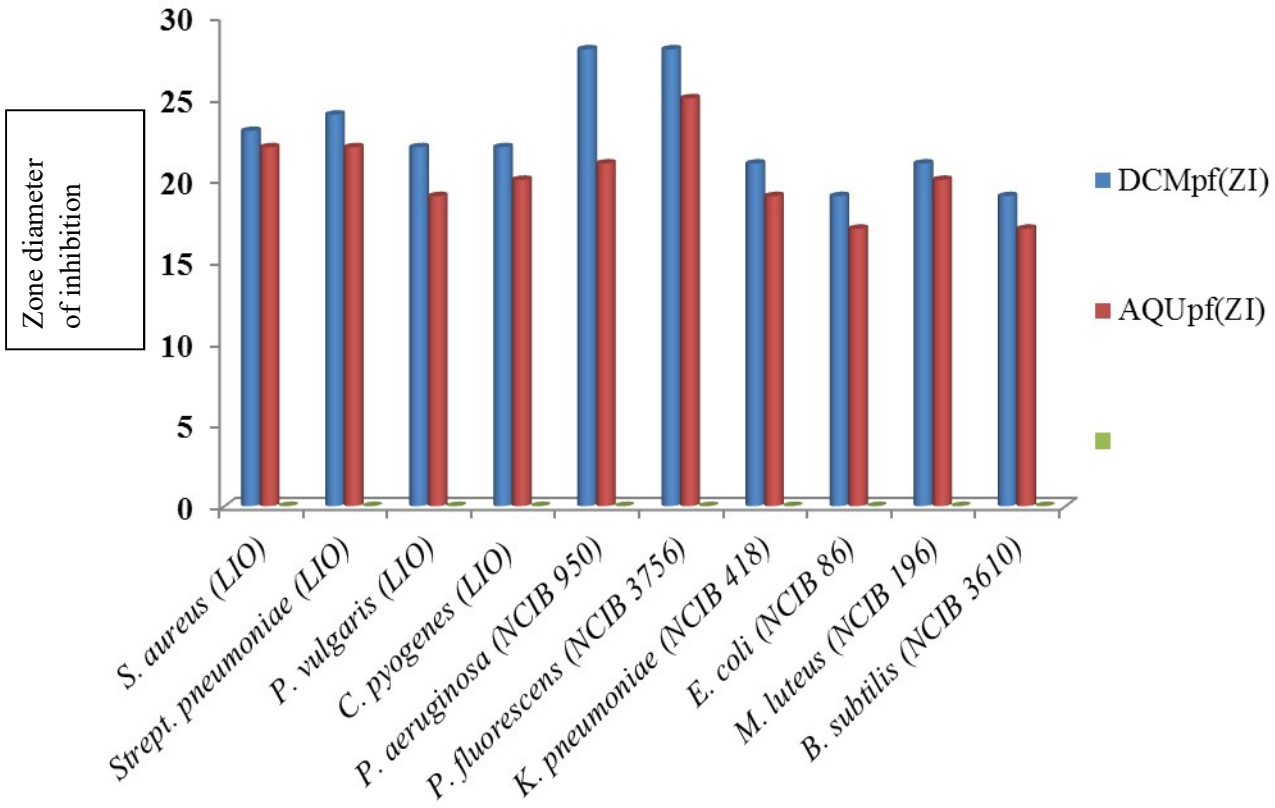

Figure 1: Sensitivity result of Dichloromethane (DCMpf) and Aqueous (AQUpf) funnel fractions at 35 mg/ml
Key: ZI=Zone of Inhibition, mm=Millimeter, LIO=Locally Isolated Organisms, NCIB=National Collection for Industrial Bacteria, AQUpf=Aqueous partition fraction, DCMpf=Dichloromethane partition extract.

Paired Sample Test shows that the zone diameter of inhibition produced by DCMpf (19-32mm) against the test bacteria was significantly greater than that produced by AQUpf (17-30mm) at 95% confidence interval (CI) (p=0.02 <0.05). This indicates that DCM is a better solvent for the extraction of antibacterial compounds in *D. edulis* than water.

Table 3: Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of Aqueous (AQUpf) and Dichloromethane (DCMpf) fractions

Test Organisms	AQUpf		DCMpf		MIC index
	MIC (mg/ml)	MBC (mg/ml	MIC (mg/ml	MBC (mg/ml	

		)		)	
Staphylococcus aureus (LIO)	1.56	3.13	0.78	1.56	2.0
Streptococcus pneumoniae (LIO)	3.13	6.25	3.13	6.25	2.0
Micrococcus luteus (NCIB 196)	1.56	3.13	0.78	1.56	2.0
Escherichia coli (LIO)	1.56	3.13	1.56	3.13	2.0
Klebsiella pneumoniae (NCIB 418)	1.56	3.13	1.56	3.13	2.0
Pseudomonas aeruginosa (NCIB 950)	3.13	6.25	1.56	6.25	2.0
Pseudomonas fluorescens (NCIB 3756)	0.78	1.56	0.78	1.56	2.0
Corynebacterium pyogenes (LIO)	1.56	3.13	1.56	3.13	2.0
Bacillus subtilis (NCIB 3610)	1.56	3.13	0.78	1.56	2.0
Proteus vulgaris (LIO)	0.78	1.56	1.56	3.13	2.0

AQU = Aqueous fraction; DCM = Dichloromethane fraction. LIO=Locally Isolated Organisms, NCIB= National Collection for Industrial Bacteria, MIC index =Ratio of MBC to MIC.

The MIC of both AQUpf and DCMpf ranged between 0.78 - 3.12 mg/mL while the MBC ranged from 1.56 mg/mL to 6.25 mg/mL for both fractions. The MIC_{index} (MBC/MIC) is 2 for both fractions showing bactericidal activity.

Table 4: R_f values of TLC solvent systems for aqueous (AQU) fraction of *Dacryodes edulis*

Serial nos.	AQU Test tubes	Nos. of spots	R_f values	Eluent code	Eluent
1	225-234	1	0.947	-	DCM -: Methanol (7:3)
2	260-263	1	0.892	-	DCM -: Ethylacetate (3:2)
3	201-211	1	0.853	-	DCM -: Methanol (7:3)
4	195-198	1	0.882	-	DCM -: Methanol (7:3)
5	146	1	0.8	AQU2cf	N-hexane-:Ethylacetate (4:1)
6	140	1	0.305	AQU1cf	N-hexane -: Ethylacetate (4:1)

AQU1cf and AQU2cf = Aqueous Column Fractions 1 and 2, Rf= Retention fraction. Aqueous fractions 1 and 2 in tubes 146 and 140 had lower Rf and gave one pure spot each.

Table 5: R_f values of TLC solvent systems for Dichloromethane (DCM) fraction of *Dacryodes edulis*

Serial nos.	DCMcf Test tubes	Nos. of spots	R_f values	Eluent code	Eluants
1	213-219	1	0.243	-	DCM -: Methanol (4:1)
2	223	2	0.216	-	DCM -: Methanol (4:1)
			0.622	-	DCM -: Methanol (4:1)
3	243	1	0.816	-	DCM -: Methanol (3:2)
4	245	1	0.842	-	DCM -: Methanol (3:2)
5	254	1	0.789	-	DCM -: Methanol (3:2)
6	117	1	0.943	DCMcf	Methanol:Ethylacetate (3:2)

DCMcf = Dichloromethane Column Fraction

All the fractions except those in tubes 213-219 had the lowest Rf with only one spot indicating purity.

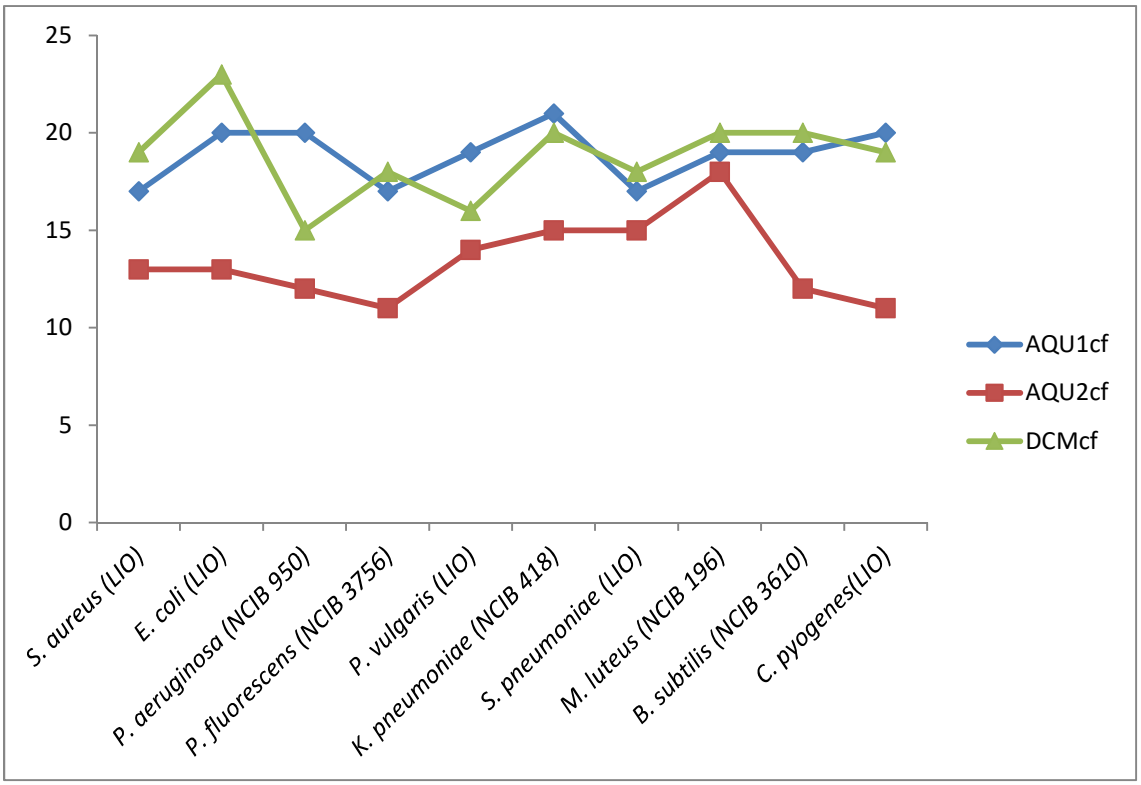

Figure 2: Antibacterial activities of Column Fractions at 35 mg/mL

Key: Column fractions from AQU1cf and AQU2cf=Aqueous, DCMcf =Dichloromethane, LIO=Locally Isolated Organisms, NCIB=National Collection for Industrial Bacterial

There is a significant difference between the variable of AQU1cf when compared to AQU2cf ($P<0.05$, $P=0.001$) There is no significant difference between the variable of AQU1cf when compared to DCMcf ($P>0.05$, $P=0.994$) There is a significant difference between the variable of AQU2cf when compared to DCMcf ($P<0.05$, $P=0.001$). Shown in Figure 2 are the antibacterial activities of column fractions (AQU1cf, AQU2cf and DCMcf) at 35 mg/mL indicating that both AQU1cf and DCMcf had greater zones of inhibitions and therefore more active compared to AQU2cf ($P < 0.05$).

Table 6: Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of Aqueous (AQU1pf, AQU2cf) and Dichloromethane (DCMcf) fractions

Test Organisms	AQU1cf		AQU2cf		DCMcf		MIC Index
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	
S. aureus (LIO)	0.78	1.56	3.12	6.25	0.39	0.78	2.0
S. pneumoniae (LIO)	3.12	6.25	3.12	6.25	1.56	3.12	2.0
Micrococcus luteus (NCIB 196)	0.39	0.78	0.78	1.56	0.39	0.78	2.0
E. coli (LIO)	1.56	3.12	1.56	3.12	1.56	3.12	2.0
K. pneumonia (NCIB 418)	1.56	3.12	1.56	3.12	0.78	1.56	2.0
P. aeruginosa (NCIB 950)	3.12	6.25	3.12	6.25	0.78	1.56	2.0
P. fluorescens (NCIB 3756)	0.78	1.56	1.56	3.12	0.39	0.78	2.0
C. pyogenes (LIO)	1.56	3.13	1.56	3.12	1.56	3.12	2.0
B. subtilis (NCIB 3610)	1.56	3.12	0.78	1.56	0.78	1.56	2.0
P. vulgaris (LIO)	0.39	0.78	1.56	3.12	0.39	0.78	2.0

AQU1cf = Aqueous column fraction 1; AQU2cf=Aqueous column fraction 2; DCMcf = Dichloromethane column fraction. LIO=Locally Isolated Organisms, NCIB= National Collection for Industrial Bacteria, MIC index =Ratio of MBC to MIC.

The MIC of AQU1cf ranged from 0.39 - 3.12 mg/mL, AQU2cf from 0.78 - 3.12 mg/mL and DCMcf from 0.39 - 1.56 mg/mL, while the MBC for AQU1cf ranged from 0.78 – 6.25 mg/mL, AQU2cf from 1.56-6.25 mg/mL and DCMcf from 0.78 – 3.12 mg/mL. The

MIC_{index} is 2 for all column fractions indicating bactericidal activity.

Discussion

Plants and herbs are the current points of focus as alternative sources of safer or more effective therapeutics than synthetically produced antimicrobial agents. Over time, pathogenic bacteria have developed resistance to antibiotics through mutations or acquisition of genetic materials conferring resistance against broad-spectrum drugs. Antimicrobial susceptibility testing (AST) is a necessary tool to determine micro-organisms' sensitivity or resistance during the management of infectious diseases. It is also a way to detect multi-drug resistance organisms as well as synergistic activities of antimicrobial agents. The antibacterial activity of acetone crude extract (35 mg/mL) of *D. edulis* leaf in this work showed higher activity than what Idu *et al,2013* obtained with ethanol and chloroform extracts (100 mg mL) with *S. aureus, P. aeruginosa* and *B. subtilis* but a lower activity than the partition and column fractions. The results in Table 1 agree with those of Agbo, 2016; Olasunkanmi and Adeniyi, 2017 who extracted different parts of *D. edulis* plant with other solvents and also recorded significant zones of inhibition against the same bacterial organisms. The MIC of the crude extract of the plant against the test organisms ranged from 1.56 to 6.25 mg/mL which is much lower than 3.125 to 100 mg/mL recorded by Idu, *et al 2013* and the MIC index is 2. MIC index of plant extract which is equal or less than 2 mg/mL is considered bactericidal while those above 2 mg/mL but less than 16 mg/mL are bacteriostatic (Biradar and Rachetti, 2013). These outcomes of antibacterial activities agree with the works of Obame, *et al, 2008*; Fonkeng *et al* 2015 who extracted different parts of *D. edulis* plant with other solvents and also recorded significant MIC and MBC. The DCMpf had the highest inhibitory activity against *P. aeruginosa* and *P. fluorescens* (with a zone diameter of inhibition 28 mm) and higher activity than ampicillin (the standard control antibiotic) indicating a very promising source of antibiotics against these highly resistant bacteria. For *K. pneumoniae, P. pyogenes, P. aeruginosa, S. aureus, S. pneumoniae* and *B. subtilis,* the fractions were also more effective than the standard control antibiotics. This disparity between the activities of the extract and the standard antibiotics may be due to the mixtures of bioactive compounds present in the fractions compared to the pure compound contained in the standard antibiotics. The different structures of microorganisms and their susceptibility potentials influence the size of zones of inhibition produced by the plant extract and fractions. The minimum inhibitory concentrations (MIC) of antimicrobial agents are usually an adequate guide for the treatment of most infections (Olorundare, 1992). Acetone crude extract in this study showed higher activity against *S. aureus* with a MIC value of 3.13 mg/mL as compared to 18.75 mg/mL reported by Riwon, *et al*. 2015 for the same microorganism. The highest activity as determined by MIC was observed against *E. coli, B. subtilis* and *P. vulgaris* at MIC of 1.56 mg/mL, which was far lower than the values obtained by Riwon, *et al 2015*. While AQUpf fractions showed higher activity against *P. fluorescens, P. vulgaris*, DCMpf had higher activity against *S. aureus, M. luteus, P. fluorescens and B. subtilis* at 0.78 mg/mL. AQU2cf had the highest activity at 0.78 mg/mL against *M. luteus* and *B. subtilis* while AQU1cf had the highest activity at 0.39 mg/mL against *M. luteus*, *P. vulgaris* and at 0.78 mg/mL against *S. aureus* and *P. fluorescens*. DCMcf had overall best activity at 0.39 mg/mL against more organisms-: *S. aureus, M. luteus, P. fluorescens, P. vulgaris* and at 0.78 mg/mL against *K. pneumoniae, P. aeruginosa,* and *B. subtilis*. This suggests that *D. edulis* leaf can be utilized as alternative antimicrobial agent as low MICs indicate that only a small quantity of the extract will be required to impair microbial growth. It is also noteworthy that the MIC of DCM fraction against resistant bacteria such as -: *P. aeruginosa*, and *S. aureus* is 0.78 mg/mL and it a justification of the high efficiency of this plant being used for wound treatment as earlier reported by Okunomo and Egho 2010. Different Rf values of the compounds were recorded and these values provide an idea about their polarity that may also help in selecting a particular solvent system for further isolation of any compound from the plant extracts using chromatographic and spectroscopic techniques (Trease K. and Evans W. C. 1996). Compounds showing high Rf value in less polar

solvent systems have low polarity while those with a low Rf value have high polarity(Ukwuani-Kwaja,2016). In the present study, the TLC profiling of the leaf extracts again revealed the presence of different active fractions: AQU1cf, AQU2cf and DCMcf. The column fractions with lower Rf values that produced one pure spot were AQU1cf=Rf 0.308; AQU2cf=Rf 0.80; DCMcf=Rf 0.243 and they were tested for antibacterial activities as observed in Figure 2.

The selection of an appropriate solvent system for a particular plant extract can only be achieved by analyzing the Rf values of compounds in different solvent systems (Sidjui 2016). In the present study, the TLC profiling of the leaf extracts again revealed the presence of different active ingredients. The high degree of antimicrobial activity of the fractions and the observed MICs confirms the traditional therapeutic claims of *D. edulis* plant. The antimicrobial activity of the leaf extract and fractions were compared with a standard antibiotic (ampicillin) under the same laboratory conditions. The activities of the fractions were greater than that of the standard antibiotics. The results indicate that fractions of *D. edulis* leaf extract possess antibacterial activity and this is justified by the values of inhibition zones and MIC indices obtained from all the fractions. Our finding agrees with other researchers who have also reported antibacterial activities of partition and column fractions obtained from medicinal plants (Ambarwati et al 2017, John, 2009). The broad-spectrum activity of the fractions against pathogenic bacteria like *S. aureus*, *P. aeruginosa, E. coli, K. pneumoniae, S. pneumonia* and *P. vulgaris* allows possible discovery of new, effective components for downstream antibiotics development. This present study the biocidal efficacies of AQUpf, DCMpf, AQU1cf, and DCMcf fractions and hence an alternative antimicrobial agent

Conclusion

Broad-spectrum antibacterial activity is observed with crude acetone extract, the partitioned and column fractions though the column factions had highest activity. Also indicated is the higher activity of the fractions than the standard antibiotic especially with pathogenic bacteria. The result of this study could be harnessed and employed by pharmaceutical industries for the production of new antimicrobial drugs to address urgent global therapeutic needs.. Further research work is ongoing to isolate and identify active compounds present in the fractions which could be drug templates for pharmaceutical use.

Competing Interests

The authors hereby declare that there are no competing interests in this work.

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