IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 13, Issue 5 Ver. IV (Sep – Oct 2018), PP 08-17
www.iosrjournals.org

Comparative Assessment of Extracts and Fractions of *Dacryodesedulis* Leaves and Stem barks From Two Locations as Sources of Antimicrobial Ingredients forSkin Care Products

Enengedi I. S.[1], Ekpa O. D.[2]andAkpabio U. D.[3]
[1](Department of Chemistry,Faculty of Natural and Applied Science/ AkwaIbom State University, Nigeria)
[2](Department of pure and Applied Chemistry, Faculty of Science / University of Calabar, Nigeria)
[3](Department of Chemistry, Faculty of Science / University of Uyo, Nigeria)
Corresponding Author: Enengedi I. S

Abstract:*Plants are rich in a wide variety of phytochemicals with antimicrobial properties. Dacryodesedulis leaves and stem barks from two locations (Uyo and Ikom) were extracted with ethanol and water. The ethanol extracts were fractionated using solvent series of increasing polarity (dichloromethane, ethyl acetate, and n-butanol). Phytochemical screening of D. edulis leaves and stem barks extracts revealed the presence of phenols, flavonoids, tannins, saponins, cardiac glycosides, alkaloids, anthraquinones and terpenoids in some or all of the ethanol and aqueous extracts. The antimicrobial activity of the ethanol extracts, their respective fractions (dichloromethane, ethyl acetate, n-butanol and aqueous) and the aqueous extracts was determined using agar well diffusion technique. Ethanol extract of D. edulis leaves from Uyo inhibited all the micro-organisms (Staphylococcus aureus, Escherichia coli, Candida sp, Bacillus subtilis, Propionibacterium acnes and Pseudomonas aeroginosa) except Trichophyton sp., whereas ethyl acetate fraction of its counterpart from Ikom was active against all the pathogens. Also the ethyl acetate fractions of the stem barks from both locations inhibited more micro-organisms than the other fractions and extracts. The antimicrobial activity revealed by these extracts and fractions could be due to the secondary metabolites in the extract. This indicates the potential of D. edulis leaves and stem barks to serve as sources of antimicrobial ingredient for formulation of skin care products,to treat the underlying skin blemished caused by these micro-organisms. D. edulis leaves and stem barks from Ikom could be better antimicrobial ingredient for skin care products due to their lower minimum inhibitory concentration than its counterpart from Uyo.*

Keywords:*Dacryodesedulis, Phytochemicals, antimicrobial activity, skin care products*.

Date of Submission: 10-10-2018 Date of acceptance: 26-10-2018

I. Introduction

Plant parts such as leaves, stems, stem barks, roots and fruits have been used traditionally to treat skin diseases. They are used in form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations. Specific plants to be used and the methods of application for a particular ailment were passed down through oral history. Plants possess vast and complex arsenal of active ingredients (phytochemicals) not only able to calm or smooth the skin, but also actively restore, heal and protect the skin[1]. However, plants are very complex in their compositions and their therapeutic activity depends on their major active chemical constituents[2].

Dacryodesedulis, also known as African pear is an indigenous fruit tree in the humid low lands and plateau regions of West, Central African and Gulf of Guinea countries[3]. The stem bark yields a resin or exudates, which is also primitive oil. The stem (resin) exudates sometimes serve as glue, cosmetic components, or are burned for lighting[4]. *D. edulis* is used as a perennial cure for a variety of ailments, ranging from ear infection to fever and oral problems. The resin is medicinal and is applied to cure skin diseases such as ringworms, craw-craw and wounds[5]. Exudates from *D. edulis* have been used in cosmetics[6]. In Nigeria the resin is used for treating parasitic skin disease and jiggers, pulped bark is used to cicatrize wounds[7]. The leaves are chewed with kolanut as an antiemetic[8].

Investigation on the phytochemical constituents of *D. edulis* revealed the presence of phenols, carboxylic acids, tannins, saponins, steroids, flavonoids, alkaloids, terpenoids, anthraquinones, oxalate and cardiac glycosides[7,9,10].[11]Analysed essential oils of the leaves, resin and stem-barks of *D. edulis* growing in Cameroon. The resin essential oil contained p-cymene, α-thujene, α-phellandrene and β-phellandrene as the main components; the stem-barks essential oil had as abundant components p-cymene, trans-carveol, α-thujene, β-phellandrene and β-elemene. The leaves essential oil was distinct with elemol, caryophyllene oxide, trans-

carveol and spathulenol as major components. Caryophyllene oxide is well recognized as a stabilizer in foodstuffs, drugs and cosmetics and also shows growth inhibiting activity against dermatophytes[12].

Antimicrobial activity of methanolic leaf extract of *D. edulis* against *Bacillus subtilis*, *Bacillus stearothermophilus*, *Bacillus cereus*, *Enterococcus faecalis*, *Micrococcus luteus*, *Clostridium sporogenes*, *Staphylococcus aureus*, *Bacillus polymyxa*, *Trueperellapyogenes*, *Escherichia coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa*, *Citrobacterfreundii*and *Shigella*sphave been determined[13]. The extract was found to reveal appreciable antibacterial activity against the test micro-organisms except on *B. subtilis*, *B.cereus*and *C. sporogenes*.

This is the first time that*D. edulis*leaves and stem barksextracts and their respective fractions would be assessed on inhibitory effects on some pathogenic micro-organisms that are responsible for skin blemishes, for industrial application in skin care products.

II. Material And Methods

Sample Collection and Identification: *Dacryodesedulis* (leaves and stem barks) were collected from Uyo in AkwaIbom State and Ikom in Cross River State, Nigeria. The samples were transferred into polyethelene bags, labelled properly and taken to the laboratory for identification and preparation. The plant materials were identified and authenticated by a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, AkwaIbom State. Voucher specimen was deposited at the herbarium with the number, UUH 3541.

Extraction and Fractionation of Plant Extracts: The method of[14] was used for the extraction of plant materials. *D. edulis* leaves and stem barks were washed, chopped into pieces and dried at room temperature for about 14 days to a constant weight. The samples were coarsely powdered, each of the coarsely powdered samples were weighed, placed in a big glass jar and extracted by maceration with 80% ethanol for 72 hours (h) at room temperature. Also, wet samples were extracted by maceration with water for 8 h. The glass jar was covered with aluminum foil and the content stirred at interval using a glass rod. Then the extracts were filtered. The ethanol and aqueous extracts were concentrated in a rotary evaporator at a reduced pressure at 45^{o}C, and the solvent removed completely by evaporation in the water bath. The dried crude ethanol extracts were weighed to calculate the yield using Equation (1). Some amounts of the ethanol extracts were suspended in 200 - 300 ml of distilled water and subjected to sequential liquid-liquid extraction with a solvent series of increasing polarity: dichloromethane (DCM), ethyl acetate (EA) and n-butanol (BuOH). The fractionation was performed until the organic solvent became colourless in 1000 ml glass separatory funnels by mixing 200 ml of solvent with the aqueous phase and the content shaken. The separatory funnel was supported on a ring clamp, allowing the layers to separate. The pooled fractions: dichloromethane fraction, ethyl acetate fraction, n-butanol fraction and the remaining aqueous fraction were concentrated in a rotary evaporator, evaporated to dryness and weighed. Aqueous extracts, parts of the ethanol extract and fractions were stored in a functional refrigerator until used for the phytochemical screening of secondary metabolites and antimicrobial activity.

$$\text{Yield (\%)} = \frac{A1}{A2} \times 100 \qquad \text{Equation (1)}$$

Where A1 = weight of ethanol extract or fraction (g)

A2 = weight of the dry coarse powdered sample or ethanol extract (g)

Qualitative Determination of Phytochemicals: Phytochemical screening of the ethanol and aqueous extracts was carried out to detect flavonoids, phenols, saponins, tannins, alkaloids, terpenoids, anthraquinones and cardiac glycosides using standard phytochemical methods as described by[15,16,17,18,19,20].

Detection of Flavonoids: One milligram (1 mg) of each extract was dissolved in two millilitre (2 ml) of water in a test tube and filtered. 2 ml of dilute sodium hydroxide (NaOH) was added to the filtrate. A yellow solution that turns colourless on addition of 1 ml dilute hydrochloric acid (HCl) indicated the presence of flavonoids.

Detection of Phenols: The extract (5 mg) was dissolved in 2 ml of distilled water in a test tube. 3 ml of 10% lead acetic was added. A bulky white precipitate indicated the presence of phenols.

Detection of Saponins (Frothing Test): One half gram (0.5 g) of the crude extract in a test was dissolved in 3 ml of hot distilled water and then the mixture was shaken vigorously for one minute and persistent foaming observed indicated the presence of saponins.

Detection of Tannins: The extract (5 mg) was dissolved in 5 ml of distilled water in a test tube water and few drops of 5% ferric chloride solution was added. The formation of blue green colouration indicated the presence of tannins.

Detection of Alkaloids: The extracts (2 g) was dissolved in 5 ml dilute HCl in a test tube and filtered. The filtrate was treated with Dragendroff's reagent. Formation of red precipitate indicated the presence of alkaloids.

Detection of Terpenoids: The extract (0.5 g) was dissolved in 3 ml of chlorofoam in a test tube and filtered. 0.5 ml of acetic anhydride was added, the solution was cooled, and this was followed by addition of 2 ml concentrated tetraoxosulphate (VI) (H_2SO_4) acid by the side of the test tube. A red or pink colour at the interface indicated the presence of terpenoids.

Detection of Anthraquinones: The extract (2.5 g) and 5 ml of 10 % tetraoxosulphate (VI) acid in a test tube was boiled and filtered. The filtrate was shaken with 2.5 ml of benzene, then 1 ml of 10 % ammonia was added. A pink or red colouration in ammonia phase suggested the presence of anthraquinones.

Detection of Cardiac Glycosides: One gram (1 g) of extract was dissolved in 5 ml of distilled water in a test tube and filtered. 2 ml of glacial acetic acid and one drop of 5 % ferric chloride were added to the solution. This was underlayed with 1 ml of concentrated tetraoxosulphate(VI) acid. A brown ring at the interface indicated the presence of cardiac glycosides.

Determination of Antimicrobial Activities of Extracts and Fractions: Bacterial and fungal isolates were obtained from the University of Uyo Health Centre Laboratory, Nigeria. These isolates were inoculated on selective media for the isolation of *Staphylococcus aureus, Escherichia coli, Candida sp, Bacillus subtilis, Trichophytonsp, Propionibacterium acnes and Pseudomonas aeroginosa* respectively. Colonies that developed were sub-cultured on Nutrient agar and Saboraud dextrose agar for bacterial and fungal isolates respectively.The antimicrobial activities of crude extracts and fractions were determined using the agar well diffusion technique as described[21]. About 25 ml of Muller-Hinton agar was autoclaved at 121°C for 15mins. The medium was allowed to cool to about 40-47°C, poured into sterile dish and left to set. Minute inoculum size of the test organism was spread evenly on the surface of the Muller-Hinton agar and the dish was left on the laboratory bench to dry. Four holes were then bored on each plate using a sterile 5mm diametercork borer. Each hole was for a concentration of the crude extract or fraction. The extract or fraction (0.4 g) was dissolved in sterile water (4 ml) to obtain a stock solution of 100mg/ml. From this stock, dilutions were made with sterile water to obtain a concentration of 100 mg/ml, 75 mg/ml, 50 mg/l and 25 mg/ml. The holes were filled with their respective concentrations of the extract or fraction. The plates were then kept undisturbed for 15 minutes before incubation. Plates containing bacterial isolates were incubated for 24 h at 37^{o}C while plates containing fungi were incubated for 48 h at 37^{o}C. The measurements (in millimeters) of the zones of inhibitions of the extracts against the test organisms were measured. The extracts or fractions that were found effective, as antimicrobial agent, were later tested to determine the minimum inhibitory concentration for each micro-organism.

Minimum Inhibitory Concentration (MIC): The MIC of the extracts and fractions were determined using the tube dilution method as described[22]. Depending on the concentration of extracts or fractions that inhibited the different micro-organisms, concentrations of the extracts and fractions ranging from 10mg/ml to 95mg/ml were prepared and incorporated into a set of test tubes containing the culture media. 0.1 ml of the standard inoculums of the test organisms were added into each of the test tubes. The set of tubes containing a mixture of bacteria and the sample (extracts or fractions) were incubated at 37°C for 24 h while the tubes containing a mixture of fungi and sample (extract or fraction) were incubated at 37°C for 48 h. A positive control tube containing only the growth medium of each of the organisms was also set up. Turbidity indicated growth of the micro-organism and MIC was regarded as the lowest concentration of the extract or fraction that revealed no visible growth when compared with that of the control tubes.

III. Result

The yield and physical appearance of ethanol extracts and their respective fractions of *D. edulis* leaves and stem barks from Uyo and Ikomare presented in Table 1. The ethanol extracts of *D. edulis* leaves from Uyo and Ikom gave yields of 17.98% and 22.78% respectively with ethyl acetate fraction having the highest yield of 75.50 % from the *D. edulis*- Uyo ethanol leaf extract and n-butanol fraction having the highest yield of 37.26 % from the Ikom ethanol leaf extract. The ethanol extracts of *D. edulis* stem barks from Uyo and Ikom gave yields of 3.20% and 3.77% respectively with dichloromethane fraction having the highest yield of 47.60 % and 34.08 % respectively in both stem barks from the Uyo and Ikom ethanol extract.

Table 1: Yield and physical appearance of ethanol extracts of samples and fractions of the ethanol extracts.

Extract/fraction	Yield (% w/w)/physical appearance			
	D. edulis-Uyo		*D. edulis*-Ikom	
	Leaves	Stem barks	Leaves	Stem barks
Ethanol extract	17.98 Dark green	3.20 Sticky brown	22.78 Green	3.77 Sticky brown
DCM fraction	16.12 Dark green	47.60 Sticky brown	13.06 Brown	24.08 Sticky brown
EA fraction	75.50 Brown	8.44 Sticky brown	30.74 Sticky brown	22.83 Brown
BuOH fraction	1.50 Brown	15.48 Sticky brown	37.26 Sticky brown	15.80 Sticky brown
Aq fraction	6.84 Light brown	28.44 Brown	18.92 Brown	27.25 Brown

DCM = dichloromethane, EA = ethyl acetate, BuOH = n-buthanol and Aq = aqueous

The phytochemical screening of the ethanol and aqueous extracts of *D. edulis*-Uyo leaves, *D. edulis*-Uyo stem barks, *D. edulis*-Ikom leaves and *D. edulis*-Ikom stem barks are presented on Tables 2. Phenols, saponins, alkaloids and cardiac glycosides were present in all the ethanol and aqueous extracts. Tannins was present in all the extracts except in aqueous extracts of *D. edulis*-Uyo stem bark and *D. edulis*-Ikom leaves and stem barks. Flavonoids was present in ethanol and aqueous extracts of *D. edulis*-Uyo stem bark and ethanol extract of *D. edulis*-Ikom stem bark. Terpenoids was present in all the extracts except in ethanol extracts of *D. edulis*-leaves from both locations. Anthraquinones were present in all the extracts except inethanol extract of*D. edulis*stem barks from both locations and in aqueous extract of *D. edulis* stem bark from Uyo.

Table 2: Phytochemical screening of ethanol and aqueous extracts of *D. edulis*leaves and stem barks

Phytochemical	Ethanol				Aqueous			
	Uyo leaves	Uyo stem barks	Ikom leaves	Ikom stem barks	Uyo leaves	Uyo stem barks	Ikom leaves	Ikom stem barks
Flavonoids	-	++	-	+++	-	++	-	-
Phenols	+	+	+	+	+	+	+	+
Tannins	+	+++	+	+	+	-	-	-
Saponins	+++	+++	++	+++	+++	++	+++	+++
Alkaloids	+	+++	+	++	++	+	+++	+++
Cardiac glycosides	+++	+++	++	++	+	+	+++	+++
Anthraquinones	+	-	++	-	+	-	-	+++
Terpenoids	-	+++	-	+++	+	+++	++	+++

+ = present in low concentration, ++ = present in moderate concentration, +++ = present in high concentration, - = absent.

The results of the antimicrobial activities of the ethanol extract, dichloromethane fraction, ethyl acetate fraction, n-butanol fraction, aqueous fraction and aqueous extract of *D. edulis-Uyo* leavesagainst the test micro-organisms namely: *S. aureus, E. coli,B. subtilis, P. aeroginosa, Candida sp, Trichophytonsp* and*P. acnes* are shown in Table 3. The extracts and fractions revealed selective levels of activities against the micro-organisms. Ethanol extract inhibited *S. aureus*, *E coli*, *B. subtilis*, *P. aeroginosa*, *Candida sp* and *P. acnes*. Dichloromethane fraction inhibited *B. subtilis*, *P. acnes* and *S. aureus*. Ethyl acetate fractions inhibited *S. aureus*, *B. subtilis*, *P. aeroginosa* and *P. acnes*. None of the extracts and fractions inhibited *Trichophytonsp*, also aqueous fraction of *D. edulis*-Uyo leaves did not inhibit any of the micro-organisms.The MIC for the extracts and fractions of *D. edulis*-Uyo leaves that were found effective are shown in Table 4.

Table 3: Mean inhibitory zone diameter (mm) of different concentrations of ethanol extract, fractions and aqueous extracts of *D. edulis*-Uyo leaves against micro-organisms.

Micro-organism	Concentration (mg/ml) of extract/ fraction																			
	D. e (LU)				D. e (LU) DCM				D. e (LU) EA				D. e (LU) AqF				D. e (LU) AqE			
	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25
S.aureus	15	12	9	7	12	9	-	-	15	11	9	-	-	-	-	-	-	-	-	-
E. coli	10	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B.subtilis	20	17	12	10	15	12	10	7	20	17	14	10	-	-	-	-	15	10	8	7
P.aeroginosa	16	12	10	-	-	-	-	-	15	11	7	-	-	-	-	-	17	14	12	10
Candida sp	22	18	15	11	12	9	7	-	-	-	-	-	-	-	-	-	9	7	-	-
Trichophytonsp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. acnes	23	20	18	14	-	-	-	-	21	18	15	14	-	-	-	-	-	-	-	-

D. e (LU) = *D. edulis*-Uyo ethanol leaves extract, D. e (LU) DCM = dichloromethane fraction, D. e (LU) EA = ethyl acetate fraction, D. e (LU) AqF = aqueous fraction from the D. e (LU), D. e (LU)AqE = *D. edulis*-Uyo aqueous leaves extract and - = No activity.

Table 4: Minimum inhibitory concentration (MIC) for D. e (LU) extracts and fractions

Micro-organism	MIC (mg/ml)				
	D. e (LU)	D. e (LU) DCM	D. e (LU) EA	D. e (LU) AqF	D. e (LU) AqE

S.aureus	20	70	45	-	-
E. coli	65	-	-	-	-
B.subtilis	20	20	10	-	10
P.aeroginosa	45	-	45	-	20
Candida sp	10	30	-	-	65
Trichophytonsp	-	-	-	-	-
P. acnes	20	-	10	-	-

D. e (LU) = *D. edulis*-Uyo ethanol leaves extract, D. e (LU) DCM = dichloromethane fraction, D. e (LU) EA = ethyl acetate fraction,D. e (LU) AqF = aqueous fraction from the D. e (LU), D. e (LU)AqE = *D. edulis*-Uyo aqueous leaves extract and - = No activity.

The antimicrobial activities of the ethanol extract, dichloromethane fraction, ethyl acetate fraction, n-butanol fraction, aqueous fraction and aqueous extract of *D. edulis*-Uyo stem barks against the test organisms are shown in Table 5. Ethanol extract inhibited *S. aureus*, *Candida sp, P. acne* and *P. aeroginosa*. Ethyl acetate fraction inhibited *S. aureus*, *E. coli, P. aeroginosa*, *Candida sp,* and*P. acnes*. N-butanol fraction inhibited *P. aeroginosa* and *P. acne*s. Aqueous fraction inhibited *P. aeroginosa* and *Candida sp*., aqueous extract inhibited *P. aeroginosa* and *P. acne*. None of the extracts and fraction inhibited *B. subtilis*and *Trichophytonsp*, also dichloromethane fraction of *D. edulis*-Uyo stem barks did not inhibit any of the micro-organisms. The MIC for the extracts and fractions of *D. edulis*-Uyo stem barks that were found effective are shown in Table 6.

Table 5: Mean inhibitory zone diameter (mm) of different concentrations of ethanol extract, fractions and aqueous extracts of *D. edulis*-Uyo stem barks against micro-organisms.

Micro-organism	Concentration (mg/ml) of extract/ fraction																			
	D. e (SU)				D. e (SU)				D. e (SU) EA				D. e (SU)				D. e (SU) AqF				D. e (SU) AqE			
	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	100	75	5025	
S.aureus	12	10	7	6	-	-	-	-	17	14	10	6	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-	21	19	13	9	-	-	-	-	-	-	-	-	-	-	-	-
B.subtilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P.aeroginosa	8	-	-	-	-	-	-	-	23	20	17	16	20	18	15	12	12	8	7	-	16	12	10	8
Candida sp	11	9	8	-	-	-	-	-	12	9	7	-	-	-	-	-	10	7	-	-	-	-	-	-
Trichophytonsp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. acnes	16	14	12	-	-	-	-	-	12	9	7	6	20	17	15	12	-	-	-	-	12	10	-	-

D. e (SU) = *D. edulis*-Uyo ethanol stem barks extract, D. e (SU) DCM = dichloromethane fraction, D. e (SU) EA = ethyl acetate fraction, D. e (SU) BuOH = n-butanol fraction, D. e (SU) AqF = aqueous fraction from the D. e (SU), D. e (SU)AqE = *D. edulis*-Uyo aqueous stem barks extract and - = No activity.

Table6: Minimum inhibitory concentration (MIC) for D. e (SU) extracts and fractions

Micro-organism	MIC (mg/ml)					
	D. e (SU)	D. e (SU) DCM	D. e (SU) EA	D. e (SU) BuOH	D. e (SU) AqF	D. e (SU) AqE
S. aureus	10	-	10	-	-	-
E. coli	-	-	10	-	-	-
B.subtilis	-	-	-	-	-	-
P.aeroginosa	95	-	20	10	45	20
Candida sp	30	-	45	-	70	-
Trichophytonsp	-	-	-	-	-	-
P. acnes	45	-	10	10	-	65

D. e (SU) = *D. edulis*-Uyo ethanol stem barks extract, D. e (SU) DCM = dichloromethane fraction, D. e (SU) EA = ethyl acetate fraction, D. e (SU) BuOH = n-butanol fraction, D. e (SU) AqF = aqueous fraction from the D. e (SU), D. e (SU)AqE = *D. edulis*-Uyo aqueous stem barks extract and - = No activity.

The results of the antimicrobial activities of the ethanol extracts, dichloromethane fraction, ethyl acetate fraction, n-butanol fraction, aqueous fraction and aqueous extract of *D. edulis*-Ikom leaves against the test micro-organisms are shown in Table 7. Ethanol extract inhibited *S. aureus*, *P. aeroginosa* and *P. acnes*. Ethyl acetate fraction inhibited all the tested micro-organisms. N-butanol fraction also inhibited all the tested micro-organisms except *E. coli* and *Candida sp*.Aqueous extract and dichloromethane fraction inhibited *S. aureus* and *Candida sp* respectively.Aqueous fraction did not inhibit any of the micro-organisms. The MIC for the extracts and fractions of *D. edulis*-Ikom leaves that were found effective are shown in Table 8.

Table 7: Mean inhibitory zone diameter (mm) of different concentrations of ethanol extract, fractions and aqueous extracts of *D. edulis*-Ikom leaves against micro-organisms.

Micro-organism	Concentration (mg/ml) of extract/ fraction																			
	D. e (LI)				D. e (LI) DCM				D. e (LI) EA				D. e (LI) BuOH				D. e (LI) AqF				D. e (LI) AqE			
	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25
S.aureus	13	11	8	-	-	-	-	-	18	15	10	7	15	10	-	-	-	-	-	-	15	11	9	7
E. coli	-	-	-	-	-	-	-	-	20	17	15	12	-	-	-	-	-	-	-	-	-	-	-	-
B.subtilis	-	-	-	-15	-	-	-	-	20	18	13	10	20	15	12	10	-	-	-	-	-	-	-	-
P.aeroginosa	12	10	8		-	-	-	-	20	17	15	14	20	18	15	13	-	-	-	-	-	-	-	-
Candida sp	-	-	-	-	9	7	-	-	18	14	11	9	-	-	-	-	-	-	-	-	-	-	-	-
Trichophytonsp	-	-	-	-	-	-	-	-	15	11	9	7	20	16	12	9	-	-	-	-	-	-	-	-
P. acnes	14	12	-	-	-	-	-	-	30	26	22	18	24	21	18	16	-	-	-	-	-	-	-	-

D. e (LI) = *D. edulis*-Ikom ethanol leaves extract, D. e (LI) DCM = dichloromethane fraction, D. e (LI) EA = ethyl acetate fraction, D. e (LI) BuOH = n-butanol fraction, D. e (LI) AqF = aqueous fraction from the D. e (LI), D. e (LI)AqE = *D. edulis*-Ikom aqueous leaves extract and - = No activity.

Table 8: Minimum inhibitory concentration (MIC) for D. e (LI) extracts and fractions.

Micro-organism	MIC (mg/ml)					
	D. e (LI)	D. e (LI) DCM	D. e (LI) EA	D. e (LI) BuOH	D. e (LI) AqF	D. e (LI) AqE
S.aureus	30	-	10	65	-	10
E. coli	-	-	20	-	-	-
B.subtilis	-	-	10	10	-	-
P.aeroginosa	10	-	20	20	-	-
Candida sp	-	70	20	-	-	-
Trichophytonsp	-	-	20	20	-	-
P. acnes	70	-	20	20	-	-

D. e (LI) = *D. edulis*-Ikom ethanol leaves extract, D. e (LI) DCM = dichloromethane fraction, D. e (LI) EA = ethyl acetate fraction, D. e (LI) BuOH = n-butanol fraction, D. e (LI) AqF = aqueous fraction from the D. e (LI), D. e (LI)AqE = *D. edulis*-Ikom aqueous leaves extract and - = No activity.

The antimicrobial activities of the ethanol extracts, dichloromethane fraction, ethyl acetate fraction, n-butanol fraction, aqueous fraction and aqueous extract of *D. edulis*-Ikom stem barksagainst the test organisms are shown in Table 9. Ethyl acetate fraction inhibited all the micro-organisms except *E. coli*. Also n-butanol fraction inhibited all the micro-organisms except *E. coli* and *Trichophyton sp*. Ethanol extract inhibited *P. acnes*, *Candida sp* and *S. aureus*. Dichloromethane fraction inhibited *P. aeroginosa* and *Candida sp*.Aqueous fraction inhibited *B. subtilis*, *Candida sp*and*P. acnes*. Aqueous extract inhibited *S. aureus*,*B. subtilis* and *P. aeroginosa*. None of the extracts or fractions inhibited *E coli*. The MIC for the extracts and fractions of *D. edulis*-Ikom stem barksthat were found effective are shown in Table 10.

Table 9: Mean inhibitory zone diameter (mm) of different concentrations of ethanol extract, fractions and aqueous extracts of *D. edulis*-Ikom stem barks against micro-organisms.

Micro-organism	Concentration (mg/ml) of extract/ fraction																			
	D. e (SI)				D. e (SI) DCM				D. e (SI) EA				D. e (SI) BuOH				D. e (SI) AqF				D. e (SI) AqE			
	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25
S.aureus	7	-	-	-	-	-	-	-	15	12	10	8	15	11	9	-	-	-	-	-	12	9	7	-
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B.subtilis	-	-	-	-	-	-	-	-	25	20	17	15	22	18	14	9	13	10	8	6	12	10	7	6
P.aeroginosa	-	-	-	-	20	17	14	11	20	15	13	10	17	15	10	7	-	-	-	-	15	12	10	7
Candida sp	7	-	-	-	8	-	-	-	17	12	8	7	14	10	9	-	12	10	7	-	-	-	-	-
Trichophytonsp	-	-	-	-	-	-	-	-	20	15	12	10	-	-	-	-	-	-	-	-	-	-	-	-
P. acnes	22	20	15	13	-	-	-	-	20	17	14	12	15	12	-	-	20	15	12	11	-	-	-	-

D. e (SI) = *D. edulis*-Ikom ethanol stem barks extract, D. e (SI) DCM = dichloromethane fraction, D. e (SI) EA = ethyl acetate fraction, D. e (SI) BuOH = n-butanol fraction, D. e (SI) AqF = aqueous fraction from the D. e (SI), D. e (SI)AqE = *D. edulis*-Ikom aqueous stem barks and - = No activity.

Table 10: Minimum inhibitory concentration (MIC) for D. e (SI) extracts and fractions.

Micro-organism	MIC (mg/ml)					
	D. e (SI)	D. e (SI) DCM	D. e (SI) EA	D. e (SI) BuOH	D. e (SI) AqF	D. e (SI) AqE

Staphylococcus aureus	80	-	10	45	-	45
Escherichia coli	-	-	-	-	-	-
Bacillus subtilis	-	-	10	10	10	10
Pseudomonas aeroginosa	-	20	20	20	-	10
Candida sp	95	95	20	30	30	-
Trichophytonsp	-	-	10	-	-	-
Propionibacte-rium acne	20	-	10	70	10	-

D. e (SI) = *D. edulis*-Ikom ethanol stem barks extract, D. e (SI) DCM = dichloromethane fraction, D. e (SI) EA = ethyl acetate fraction, D. e (SI) BuOH = n-butanol fraction, D. e (SI) AqF = aqueous fraction from the D. e (SI), D. e (SI)AqE = *D. edulis*-Ikom aqueous stem barks extract and - = No activity.

IV. Discussion

Extraction is an important initial step for the recovery and isolation of bioactive compounds from plant samples. In Table 1, the percentage yield of the ethanol extracts of the samples differed. The percentage yield was in the decreasing order: *D. edulis*-Ikom leaves >*D. edulis*-Uyo leaves>*D. edulis*-Ikom stem barks >*D. edulis*-Uyo stem barks. This reveals that the percentage yield of the ethanol extracts of the samples depended on the chemical compositions of the samples. It was also observed that the percentage yield of the fractions depended on the polarity and non-polarity of the solvents.[23]Reported that, yield of extraction depends on solvent with varying polarity, pH, temperature, extraction time, and composition of the sample. Under the same extraction time and temperature, solvent and composition of sample are known as the most important parameters.

The physical appearance of all the leaves extracts were green while that of the stem and stem barks were brown. This indicates that the extracts tend to take the colour of the samples. The physical appearance of fractions were either green or brown or black. [24]In his report stated that plants' extracts are normally dark brown and greenish in colour.

Phytochemical screening is very useful in the evaluation of some active biological components of some vegetables and medicinal plants. Phenols, flavonoids, tannins, saponins, alkaloids, cardiac glycosides, anthraquinones and terpenoids were found to be present in all or some of the ethanol and aqueous extracts screened for phytochemicals (Tables 2).

Medicinal plants, since ancient time, have been noted for their diverse pharmacological and cosmeceutical properties which could be attributed to the presence of secondary plant metabolites such as phenols, flavonoids, tannins, saponins, alkaloids, cardiac glycosides, anthraquinones and terpenoids. Some of these phytochemicals in the form of plant extracts are incorporated in skin care product formulations, such as, soaps, creams, lotions, pomades and shampoos, because of their curative properties[25,26]. The rich phytochemical profile of the ethanol and aqueous extracts of *D. edulis* leaves and stem barks from Uyo and Ikom is an indication of their ability to serve as sources of therapeutic ingredients in skin care products.

Phytochemicals such as tannins, terpenoids, alkaloids, phenolics and flavonoids have been found *in vitro* to have antimicrobial properties[27]. Tannins are known to hasten the healing of wounds and inflamed mucous membranes. Tannins not only heal burns and stop bleeding, but they also stop infection while they continue to heal the wound internally. The ability of tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more[28]. Saponin is known for its foaming ability; it has been shown to be potent on several skin pathogenic microorganisms[29-31], which can be used as antimicrobial agents in skin care products for therapy of skin diseases. The astringent property of tannins and the foaming capacity of saponins make extracts effective against wounds[32]. Anthraquinones are also responsible for wound healing action[33]. The toxicity of alkaloids can be used in the treatment of skin infections.

The extracts and fractions of *D. edulis* leaves and stem barks revealed significant antimicrobial activity against the tested micro-organisms.Ethanol extract of *D. edulis*-Uyo leaves exhibited significant activity against all the tested micro-organisms except *Trichophyton sp*. The various fractions also inhibited some micro-organisms: dichloromethane fraction against *B. subtilis*and *Candida sp.*;ethyl acetate fraction against *S. aureus, B. subtilis, P. aeroginosa*and*P. acnes;* and the aqueous extract against *B. subtilis,* and*P. aeroginosa*(Table 3). These activities reveal that *D. edulis*-Uyo leaves is a potent antimicrobial agent. The extracts and fractions of the leaves did not show any activity against *Trichophyton sp.* The aqueous fraction did not inhibit any of the tested micro-organisms. This reveals that much of the components of the leaves responsible for antimicrobial activity had been extracted during the fractionation processes with dichloromethane, ethyl acetate and n-butanol.

The ethanol extract of *D. edulis*-Uyo stem barks inhibited *S. aureus, P. aeroginosa*, *Candida sp.* and*P. acne,* compared to ethyl acetate fraction, which was active against all the tested micro-organisms except B. *subtilis* and *Trichophyton sp.* Other fractions such as n-butanol and aqueous fractions exhibited inhibitory effect against *P. aeroginosa* and *P. acnes*; *P. aeroginosa* and *Candida sp.* respectively. The activity of aqueous extract against *P. aeroginosa* and *P. acnes* (Table 5) among others, reveal the potency of *D. edulis*-Uyo stem barks as antimicrobial agent.

From these observations, it can be inferred that the ethanol extract of *D. edulis*-Uyo leaves had better activity than the stem barks while the ethyl acetate fraction of *D. edulis*-Uyo stem barks had better activity than the leaves, against the tested micro-organisms. The aqueous fraction and the dichloromethane fraction of the leaves and the stem barks respectively did not inhibit any of the tested micro-organisms. None of the extracts and fractions of the leaves and stem barks inhibited *Trichophyton sp*.

The *D. edulis* samples from Ikom behaved similarly. *D. edulis*-Ikom leaves revealed significant antimicrobial activity against the tested micro-organisms (Table 7). The activity of ethyl acetate fraction against all the tested micro-organisms, n-butanol fraction against all the tested micro-organisms except *E. coli* and *Candida sp.*,ethanol extract against *S. aureus*and *P. aeroginosa*, and the aqueous extract against *S. aureus*indicate the potency of *D. edulis*-Ikom leaves as possible antimicrobial agents.

D. edulis-Ikom stem barks also revealed significant antimicrobial activity against the tested micro-organisms (Table 9). Apart from *E. coli,* ethyl acetate fraction was active against all the other tested micro-organisms, n-butanol fraction inhibited all the micro-organisms except *E. coli* and *Trichophyton sp*.Ethyl acetate and n-butanol fractions of *D. edulis*-Ikom leaves and stem barks were the most active against the micro-organisms. All the tested micro-organisms were resistant to aqueous fraction of *D. edulis*-Ikom leaves while *E. coli* was not inhibited by any extract or fraction of *D. edulis*-Ikom stem barks.

Comparatively, ethanol extract of *D. edulis*-Uyo leaves revealed the best activity inhibited all the micro-organisms except *Trichophyton sp.*, whereas, ethyl acetate fraction of *D. edulis*-Ikom leaves was the most active, inhibiting all the tested micro-organisms. Aqueous fractions of *D. edulis* leaves from both locations did not inhibit any of the tested micro-organisms. The ethyl acetate fractions of *D. edulis* stem barks from both locations were the most active against the micro-organisms. *B. subtilis and Trichophytonsp* were resistant to *D. edulis*-Uyo stem barks extracts and fractions, whereas, *E. coli* was resistant to *D. edulis*-Ikom stem barks extracts and fractions. All the tested micro-organisms were resistant to dichloromethane fraction of *D. edulis*-Uyo stem barks. Dichloromethane fraction of *D. edulis*-Ikom stem barks inhibited *P. aeroginosa*and *Candida sp*. The antimicrobial activity exhibited by *D. edulis*leaves and stem barks could be due to the presence of the phytochemicals in the extracts.It could also be that these extracts and fractions contain benzoic acid ester and caryophyllene oxide. Benzoic acid ester was isolated from *D. edulis* stem barks volatile oils[34]. Benzoic acid and its esters are employed externally as antiseptics, lotions, ointments, creams and mouth washes. It is used in the treatment of burns, frostbite, chaps, cracks, erythema, pruritus, ulcers, infected dermatitis and other minor wounds. [11]Analysed essential oils of the leaves of *D. edulis*, the leaves essential oil was distinct with caryophyllene oxide as one of the major components. Caryophyllene oxide is well recognised as a stabilizer in foodstuffs, drugs and cosmetics and also shows growth inhibiting activity against dermatophytes[12]. The disparity in result of antimicrobial activity of the leaves and stem barks of *D. edulis*from the two locations could be attributed to the variation in the differences in soil composition and probably also due to differences in the genetic variability in the plant between the two locations[35].

The activity of *D. edulis* extracts and fractions against the tested micro-organisms is an indication that *D. edulis* leaves and stem barks could be incorporated into skin care products as antimicrobial ingredients to relieve some of the skin diseases caused by these micro-organisms. Ethanol extract and some of the fractions of *D. edulis*-Uyo leaves could be used to treat boils, which is caused by *S. aureus*; toe web caused by *P. aeruginosa*; acne vulgaris in which *P. acnes* is considered as the major skin bacteria that cause its formation; soreness and itching around the anus and genitals in adults which is caused by *Candida sp.* and nosocomial infection caused by *B. subtilis*. This extract and some of its fractions might not be used for the treatment of ringworm, in which *Trichophyton sp.* is the causative agent. Ethyl acetate fraction and some of the extracts and fractions of *D. edulis*-Ikom leaves could also be used to treat the same skin diseases including ringworm. Aqueous fractions of *D. edulis*-Uyo leaves and dichloromethane fraction of *D. edulis*-Uyo stem barks might not be used for the treatment of any of the mentioned skin diseases. Also ethyl acetate fractions of *D. edulis* stem barks and some of the other fractions and extracts could be used to alleviate the mentioned skin diseases but not nosocomial infection and ringworm. *D. edulis*-Ikom leaves and stem barks could give better healing of the skin due to their lower minimum inhibition concentration (MIC) values (Tables 8 and 10), compared to its Uyo counterpart (Tables 4 and 6).

V. Conclusion

Personal care and pharmaceutical industries have an increasing interest in replacing synthetic antimicrobials in topical products. This study reveals that extracts and fractions of *D. edulis* leaves and stem barks could besuitable antimicrobial ingredients for skin care products formulations for the relieve of some of the skin diseases caused by these micro-organisms.*D. edulis*-Ikom leaves and stem barks could give better healing of the skin due to their lower minimum inhibition concentration (MIC) values, compared to its Uyo counterpart.

References

[1]. Patel, C. J., Tyagi, S., Kumar, U., Pinkesh, P., Bharat, C., Shreya, P. and Dhruv, M. Importance of different herbal plants in field of cosmetics: a recent review. Journal of Drug Discovery and Therapeutics. 2013;1(1): 19-27.

[2]. Pandey, S., Seth, A., Tiwari, R., Singh, S., Behl, H. M., and Singh, S. Development and evaluation of antimicrobial herbal cosmetic preparation. African Journal of Pharmacy and Pharmacology. 2014;8(20): 514-528.

[3]. Hez, N. U., Ngozika, C. O. and Chiaka, C. N. The chemical properties of African pear pulp at different stages of fruit development. International NGO Journal. 2009;4(9): 380-385.

[4]. Omonhinmin, A. C. (2012). Ethnobotany of *Dacryodesedulis* (G. Don) H.J. Lam in Southern Nigeria 1: Practices and applications among the Yoruba speaking people. *Ethnobotany Research and Applications*,10: 175-184.

[5]. Okwu, D. E. and Nnamdi, F. U. Evaluation of the chemical composition of *Dacryodesedulis* and *Raphiahookeri* Mann and Wendl exudates used in herbal medicine in South Eastern Nigeria. African Journal of Traditional, Complementary and Alternative Medicines. 2008;5(2): 194-200.

[6]. Ekpa, O. The use of exudates from *Raphiahookeri* and *Pachylobusedulis* in cosmetics formulation. Discovery and Innovation. 1993;5(4): 313-316.

[7]. Nwokonkwo, D. C. The phytochemical study and antibacterial activities of the seed extract of *Dacryodesedulis* (African native pear). American Journal of Scientific and Industrial Research. 2014;5(1): 7-12.

[8]. Ajibesin, K. K. *Dacryodesedulis* (G. Don) H. J. Lam: a review on its medicinal, phytochemical and economic properties. Research Journal of Medicinal Plants. 2011;5(1): 32-41.

[9]. Majesty, D., Chioma, A., Amadike, U., Adindu. E. and Benjamin, A. Phytochemical, vitamin and proximate composition of *Dacryodesedulis* fruit at different stages of maturation. Asian Journal of Plant Science and Research. 2012;2(4): 437-441.

[10]. Ogboru, R. O., Okolie, P. L. and Agboje, I. Phytochemical screening and medicinal potentials of the bark of *Dacryodesedulis* (G. Don) H. J. Lam. Journal of Environmental Analytical Chemistry. 2015;2(5): 1-3.

[11]. Riwom, S. H., Foe, F. M., Nyegue, M. A., Wanki, R. A., Olugu, S. V. and Etoa, F. Chemical composition and *in vitro* antibacterial activity of the essential oils of the leaves, resin and stem-barks of *Dacryodesedulis* (G. Don) H. J Lam growing in Cameroon on diarrhea associated strains. Journal of Applied Pharmaceutical Science. 2015;5(10): 6-11.

[12]. Yang, D., Michel, L., Chaumont, J. P. and Millet-Clerc, J. Use of caryophyllene oxide as an antifungal agent in an *in vitro* experimental model of onychomycosis. Mycopathologia. 1999;148(2): 79-82.

[13]. Oyetunji, O. O. and Opeyemi, A. P. Antibacterial and antioxidant activities of *Dacryodesedulis*methanolic leaf extract. Journal of Advances in Medical and Pharmaceutical Sciences. 2017;14(1): 1-11.

[14]. Apu, A. S., Al-Baizyd, A. H., Ara, F., Bhuyan, S. H., Matin, M. and Hossain, M. F. Phytochemical analysis and bioactivities of *Argemonemexicana* Linn leaves. Pharmacology Online. 2012;3: 16-23.

[15]. Poongothai, A., Sreena, K. P., Sreejith, K., Uthiralingam, M. and Annapoorant, S. Preliminary phytochemicals screening of *Ficusracemosa* Linn bark. International Journal of Pharma and Bio Sciences. 2011;2(2): 431-434.

[16]. Vijayalakshmi, R. and Ravindhran, R. Preliminary comparative phytochemical screening of root extracts *of Diospyrusferrea (Wild.)* Bakh and *Aervalanata*(L.) Juss. Ex Schultes. Asian Journal of Plant Science and Research. 2012;2(5): 581-587.

[17]. Ndam, L. M., Mih, A. M., Fongod, A. G. N., Tening, A. S., Tonjock, R. K., Enang, J. E. and Fujii, Y. Phytochemical screening of the bioactive compounds in twenty (20) Cameroonian medicinal plants. International. Journal of Current Microbiology and Applied Sciences. 2014;3(12): 768-778.

[18]. Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H. Phytochemical screening and extraction: areview. InternationalePharmaceuticaSciencia. 2011;1(1): 98-106.

[19]. Santosh, C. H., Attitalla, I. H. and Mohan, M. M. Phytochemical analysis, antimicrobial and antioxidant activity of ethanolic extract of *Vernoniaanthelmintica.*International Journal of Pharma and Bio Sciences. 2013;4(1): 960 – 966.

[20]. Arora, M. and Kaur, P. Phytochemical screening of orange peel and pulp. International Journal of Research in Engineering and Technology. 2013;2(12): 517-520.

[21]. Haruna, M. T., Anokwuru, C. P., Akeredolu, A. A., Akinsemolu, A. A. and Alabi, O. A. Antibacterial and Antifungal Activity of *Acalyphawilkesiana*. European Journal of Medicinal Plants. 2013;3(1): 52-64.

[22]. Jahan, F., Lawrence, R., Kumar, V. and Junaid, M. Evaluation of antimicrobial activity of plant extracts on antibiotic susceptible and resistant *Staphylococcus aureus*strains. Journal of Chemical and Pharmaceutical Research. 2011;3(4): 777-789.

[23]. Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S. and Ju, Y. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophilaaromatica*. Journal of Food and Drug Analysis. 2014;22: 296-302.

[24]. Chermahini, S. H. and Majid, F. A. A. Cosmeceutical values, antimicrobial activities and antioxidant properties of cashew leaves extract. African Journal of Biotechnology. 2011;10(65): 14573-14582.

[25]. Barbulova, A., Apone, F. and Colucci, G. Plant cell cultures as source of cosmetic active ingredients. Cosmetics. 2014;1: 94-104.

[26]. Igwe, O. U. Chemical constituents of the leaf essential oil of *Carica papaya* from South East Nigeria and its antimicrobial activity. International Journal of Research in pharmacy and Chemistry. 2015;5(1): 77-83.

[27]. Cowan, M. M. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999;12(4): 564-584.

[28]. Ashok, K. and Upadhyaya, K. Tannins are astringent. Journal of Pharmacognosy and Phytochemistry. 2012;1(3): 45-50.

[29]. Soetan, k. O., Oyekunle, M. A., Aiyelaagbe, O. O. and Fafunso, M. A. Evaluation of the antimicrobial activity of saponins extract of *Sorghum Bicolor* L. Moench. African Journal of Biotechnology. 2006;5(23): 2405-2407.

[30]. Maatalah, M. B., Bouzidi, N. K., Bellahouel, S., Merah, B., Fortas, Z., Soulimani, R., Saidi, S. and Derdour, A. Antimicrobial activity of the alkaloids and saponin extracts of *Anabasis articulate.* Journal of Biotechnology and Pharmaceutical Research. 2012;3(3): 54-57.

[31]. Ajibade, V. A. and Ibiyemi, M. F. The antibacterial potency of alkaloid and saponin extracts from *Solanummacrocapon*(Garden Egg). Journal of Advances in Microbiology. 2017;5(4): 1-5.

[32]. Amadi, B. A., Arukwe, U., Duru, M. K .C., Amadi, C. T., Adindu, E. A., Egejuru, L. and Odika, P. C. Phytonutrients and antinutrients screening of *D.edulis*fruits at different maturation stages. Journal of Natural Product and Plant Resources. 2012;2(4): 530-533.

[33]. Karodi, R., Jadhav, M., Rub, R. and Bafna, A.Evaluation of the wound healing activity of a crude extract of *Rubiacordifolia*L. (Indian madder) in mice. International Journal of Applied Research in Natural Products. 2009;2(2): 12-18.

[34]. Okwu, D. E. and Ighodaro, B. U. GC-MS evaluation of the bioactive compounds and antibacterial activity of the oil fraction from the stem barks of *Dacryodesedulis*. International Journal of Drug Development and Research. 2009;1(1): 117-125.

[35]. Yelwa, A. S., Mshelia, H. E., Cyril, O., Lawal, S. I., Bature, H. B., Shamsiya, A and Christiana, E. R. Phytochemical screening, acute toxicity study and evaluation of *in vitro* antimicrobial activities of the fractions of *Dacryodesedulis*against selected clinical bacterial isolates. Journal of Pharmacognosy and Phytochemistry. 2017;6(4): 1910-1915.

Enengedi I. S. "Comparative Assessment of Extracts and Fractions of Dacryodesedulis Leaves and Stem barks From Two Locations as Sources of Antimicrobial Ingredients for Skin Care Products" .IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.5 (2018): 08-17