



## ANTIBACTERIAL ACTIVITY OF *DACRYODES EDULIS* SEED EXTRACTS ON FOOD-BORNE PATHOGENS

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### ABSTRACT

**The antibacterial effect of aqueous and ethanolic seed extracts of *Dacryodes edulis* was studied using agar diffusion technique. Extracts tested at a final concentration 2000µg/ml produced *in vitro* antibacterial activities in assays against food-borne bacterial isolates of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus mirabilis*. The highest zone of inhibition was obtained from ethanol extract at 2000µg/ml against *Escherichia coli* with a diameter of 28mm. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the water extract ranged between 62.5-500µg/ml and 125-1000µg/ml respectively. The MIC and MBC of the ethanol extract ranged between 31.25-250µg/ml and 62.5-500µg/ml. The percentage inhibition of the isolates at 1000µg/ml was more in the ethanol extract (65.4-95.5%) compared to the water extract (30-60.2%). This study shows that the extracts of *Dacryodes edulis* have potential usage as a food preservative and pharmaceutical preparation.**

**Keywords:** *Dacryodes edulis*, Antibacterial activity, Aqueous, Ethanolic, Seed extract,

### INTRODUCTION

There has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents (Werner *et al.*, 1999; Evans *et al.*, 2002; Raghavendra *et al.*, 2006). Different extracts from traditional medicinal plants have been tested. Many reports have shown the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks of modern medicine to attain new principles (Evans *et al.*, 2002). The increasing interest on traditional ethnomedicine may lead to discovery of novel therapeutic agents. Thus medicinal plants are finding their way into pharmaceuticals, nutraceuticals, cosmetics, food supplements and food preservatives (Gupta *et al.*, 1996).

Furthermore, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of actions because of an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical practice. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. The drug resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in acquired immunodeficiency syndrome (AIDS) and cancer patient as reported by Harbarth and Samore (2005). Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens. Hence, more studies pertaining to the use of plants as therapeutic agents

should be emphasized especially those related to the control of antibiotic resistant microbes.

African pear (*Dacryodes edulis*) is a member of the Burseraceae which are mainly shrubs and trees that yield a resinous aromatic gum from their cut bark. The fruits are either eaten roasted, boiled or raw preferably with roasted or cooked maize (*Zea mays*). The seeds are thereafter discarded as waste (Omoti and Okiy, 1987). Although *Dacryodes edulis* has been used in folk medicine by traditional healers (in Nigeria and Democratic Republic of Congo) who employ it for the treatment of parasitic skin diseases, tonsillitis and as a mouth wash preparation, there is a dearth of information on the antimicrobial activity of the seed extract of this plant. The fact that plants represent a reservoir of effective chemotherapeutics and can provide valuable sources of natural preservatives led the researchers to screen *in vitro* for the antibacterial activity of the seed extract of *Dacryodes edulis* against both food spoilage and clinical microbial pathogens.

### MATERIALS AND METHODS

#### Collection of Plant Material

The fruits of *Dacryodes edulis* were obtained from New Benin market in Benin City, Nigeria.

#### Source of Test Microorganisms

The organisms *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella aerogenes* were isolated from spoiled food samples in Lahor Public Health and Research centre, Ikpoba Hill, Benin City, Nigeria. Pure cultures of *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus mirabilis* were obtained from the Department of Medical Microbiology, University of Benin Teaching Hospital (UBTH), Benin City, Nigeria.

All microbial isolates were properly identified by gram staining, morphological and biochemical characterization (Cowan and Steel, 1965; Cheesbrough, 2000). They were thereafter plated on nutrient agar and stored in slants in the refrigerator (4°C) prior to use.

#### Preparation of Extracts

Aqueous and ethanolic extracts of *Dacryodes edulis* were carried out by the procedure of Babu *et al.* (2007) with modifications. The fruit of *Dacryodes edulis* were defleshed to obtain the seeds which were cut into tiny bits. They were macerated using a warring electric blender (Binatone, England). Forty gram (40g) of the ground seeds were separately soaked in 200ml of water and ethanol in separate conical flasks and allowed to stand for 24h. The fluid was filtered through double layered muslin cloth. The filtrate was thereafter concentrated using a rotary evaporator. The concentrate was reduced to dryness by placing in an oven maintained at 40°C. The solid extract obtained was preserved aseptically in brown air-tight bottles stored at 4°C until needed for use. For re-constitution 0.004g of the extract was dissolved in 20% DMSO and diluted with sterile distilled water to obtain a stock concentration of 4000µg/ml.

#### Preparation of Inoculum

A loopful of the test bacteria was taken from four or five well isolated colonies from an overnight agar plate and suspended directly in 5ml of saline. The resulting bacterial suspension was diluted until the turbidity matched that of the McFarland standard prepared by adding 0.5ml of 0.048 BaCl<sub>2</sub> (1.75% [wt/vol] BaCl<sub>2</sub>.2H<sub>2</sub>O) to 99.5ml of 0.36N H<sub>2</sub>SO<sub>4</sub> (1%, vol/vol). This turbidity is half the density of a McFarland No 1 standard and is often referred to as McFarland 0.5 standard. The turbidity standard was agitated on a vortex mixer immediately before use. This turbidity is estimated to be equivalent to approximately 5×10<sup>7</sup> CFU/ml. Inoculation of media for susceptibility test was made within 30min of adjusting the inoculums (Barry and Thornsberry, 1985).

#### Antibacterial Activity Assay

The modified agar well diffusion method of Barry and Thornsberry (1985) was employed to determine the antibacterial activities of both aqueous and ethanolic extracts of *Dacryodes edulis* seed. Cups were made in nutrient agar plate using sterile cork borer (5mm) and inoculum containing 5×10<sup>7</sup>cfu/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50µl of each double fold serial dilution blanks of the stock of

$$\% \text{ inhibition} = \frac{AS - AU}{AS} \times \frac{100}{1}$$

Where AS = Absorbance of control

AU = Absorbance of the test sample

#### RESULTS

Table 1 shows the results of the antibacterial activity of both ethanol and aqueous extract of the seed of *Dacryodes edulis*. The diameter of the zones of inhibition was concentration dependent in both extracts.

As the concentration increased, the diameters increased and vice versa. The highest zone of

all aqueous and ethanol extracts were placed in the cups made in inoculated plates. The treatments also included 50µl of sterile distilled water and ethanol separately which served as control. The plates were incubated for 24h at 37°C and zone of inhibition if any around the wells were measured in millimeter (mm).

#### Determination of Minimal Inhibitory Concentration

A double fold serial dilution was made using nutrient broth according to the methods of Ibekwe *et al.* (2001) and Idu *et al.* (2007). Double strength nutrient broth of 5ml was pipetted into universal bottles (9 bottles for each of the extracts) and they were labeled N, 2, 4, 8, 16, 32, 64, 128 and 256. Using a sterile graduated pipette, 5ml of the extract was pipetted into the bottle labelled 2 from N. The solution was thoroughly mixed and 5ml was pipetted from this bottle into the next labeled 4. This procedure was repeated until the last bottle labeled 256, 5ml was then pipetted and discarded. In another bottle, broth only was put, without the extract to serve as control. The following concentrations were obtained in order: N-4000µg/ml, 2-2000µg/ml, 4-1000µg/ml, 8-500µg/ml, 16-250µg/ml, 32-125µg/ml, 64-62.5µg/ml, 128-31.25µg/ml and 256-15.63µg/ml. Having obtained the different dilutions and concentrations, 100µl of an over-night broth culture of the test organisms were inoculated into the dilutions and incubated at 37°C for 24h. The lowest concentration of the extracts which inhibited microbial growth was recorded as the minimal inhibitory concentration (MIC).

#### Determination of Minimal Bactericidal Concentration

Tubes showing no visible growth from the MIC test were sub-cultured into nutrient agar plates and incubated at 37°C for 24h. The lowest concentration of the extract yielding no growth was recorded as the minimal bactericidal concentration (MBC).

#### Determination of Percentage Inhibition

Standard solution of the extracts was prepared from the stock (4000µg/ml) by double fold serial dilution to obtain a concentration of 1000µg/ml. The tubes were inoculated with over-night broth cultures of the test organisms and incubated at 37°C for 24h. The absorbance was measured using a spectrophotometer. A control was also prepared (having been inoculated but without the extracts) and the absorbance determined.

The percentage inhibition was calculated using the relationship:

inhibition was obtained from ethanol extract against *Escherichia coli* with a diameter of 33mm. The lowest zone of inhibition in ethanol extract was observed with *Klebsiella aerogenes* with a diameter of 7.0mm. The zones of inhibition produced by the aqueous extract was generally lower than that of ethanol.

The highest zone of inhibition in aqueous extract was recorded for *Escherichia coli* (27mm) at 2000µg/ml. This was followed by *Pseudomonas aeruginosa* with a zone diameter of 25mm. The least zone of inhibition produced by aqueous extract was 8.0mm against *Klebsiella aerogenes*.

Tables 2 and 3 presents the minimal inhibitory and minimal bactericidal concentrations of aqueous and ethanol extracts of the seed of *Dacryodes edulis*. Both aqueous and ethanol extracts had activities against the seven test microorganisms used. In the aqueous extracts of *Dacryodes edulis* seed, the highest activity was against *Escherichia coli* and *Klebsiella aerogenes* with a minimal inhibitory concentration (MIC) of 62.5µg/ml. This was followed by *Salmonella typhi* and *Staphylococcus aureus* with an MIC of 250µg/ml. The least antimicrobial activity of the aqueous extract of *Dacryodes edulis* was against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Proteus mirabilis* with the same MIC of 500µg/ml. *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Salmonella typhi* had the same minimal bactericidal concentration of 1000µg/ml (Table 2).

The minimal inhibitory and bactericidal concentrations of the ethanol extract of *Dacryodes edulis* were considerably lower compared to the aqueous extract. Three organisms: *Bacillus subtilis*, *Escherichia coli* and *Klebsiella aerogenes* had similar low MIC of 31.25µg/ml respectively. The highest MIC of the ethanol extract was against *Proteus mirabilis* (Table 3). The highest activity of the ethanol extract was against *Escherichia coli*, *Klebsiella aerogenes*, *Bacillus subtilis* and *Staphylococcus aureus*. The least MBC of 62.5µg/ml was recorded for *Bacillus subtilis* and *Klebsiella aerogenes* while the highest MBC of 500µg/ml was against *Pseudomonas aeruginosa*.

In general, ethanol extract of *Dacryodes edulis* seed demonstrated a higher percentage inhibition compared to aqueous extract (Figure 1). At a concentration of 1000µg/ml, the highest percentage inhibition of the aqueous extract was 60.2% against *Klebsiella aerogenes* while the least was 30.2% against *Salmonella typhi*. With ethanol extract, the highest percentage inhibition was 95.5% against *Klebsiella aerogenes* while the least was 65.4% against *Pseudomonas aeruginosa* (Figure 1).

**Table 1: antibacterial activity of *dacryodes edulis* seed extract zone of inhibition(mm)**

CONCENTRATION (µg/ml)	ETHANOL EXTRACT							AQUEOUS EXTRACT						
	BS	SA	EC	KA	PA	ST	PM	BS	SA	EC	KA	PA	ST	PM
2000	23	25	33	27	29	24	28	17	19	27	23	25	18	24
1000	20	23	29	24	25	21	23	13	15	19	20	21	13	19
500	18	20	20	21	15	15	20	10	12	13	16	15	10	17
250	16	17	18	19	11	12	15	0	09	11	13	0	07	12
125	13	14	18	15	08	10	0	0	0	11	10	0	0	0
62.5	11	10	15	11	0	0	0	0	0	09	08	0	0	0
32.25	09	0	11	07	0	0	0	0	0	0	0	0	0	0
15.63	0	0	0	0	0	0	0	0	0	0	0	0	0	0

**Key:** BS = *Bacillus subtilis*, SA = *Staphylococcus aureus*, EC = *Escherichia coli*, KA = *Klebsiella aerogenes*, PA = *Pseudomonas aeruginosa*, ST = *Salmonella typhi*, PM = *Proteus mirabilis*

**TABLE 2: MINIMAL INHIBITORY/BACTERICIDAL CONCENTRATION OF AQUEOUS EXTRACTS**

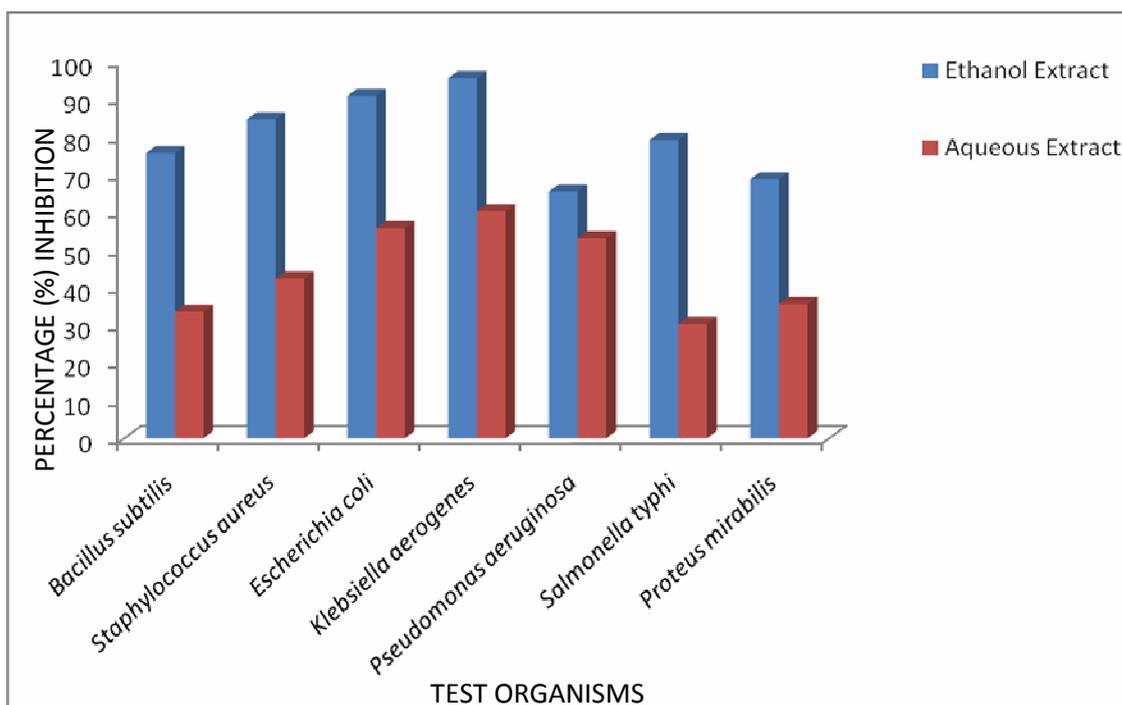
Test Organisms	BS	SA	EC	KA	PA	ST	PM
MIC (µg/ml)	500	250	62.5	62.5	500	250	500
MBC (µg/ml)	1000	500	500	125	1000	1000	500

**Key:** BS = *Bacillus subtilis*, SA = *Staphylococcus aureus*, EC = *Escherichia coli*, KA = *Klebsiella aerogenes*, PA = *Pseudomonas aeruginosa*, ST = *Salmonella typhi*, PM = *Proteus mirabilis*

**TABLE 3: MINIMAL INHIBITORY/BACTERICIDAL CONCENTRATION OF ETHANOL EXTRACTS**

Test Organisms	BS	SA	EC	KA	PA	ST	PM
MIC (µg/ml)	31.25	62.5	31.25	31.25	125	125	250
MBC (µg/ml)	62.5	125	125	62.5	500	125	250

**Key:** BS = *Bacillus subtilis*, SA = *Staphylococcus aureus*, EC = *Escherichia coli*, KA = *Klebsiella aerogenes*, PA = *Pseudomonas aeruginosa*, ST = *Salmonella typhi*, PM = *Proteus mirabilis*



**Figure 1: PERCENTAGE (%) INHIBITION OF THE TEST ORGANISMS BY 1000µg/ml OF *Dacryodes edulis* EXTRACTS.**

**DISCUSSION**

Several researchers have reported that plants contain bioactive substances (Bylka *et al.*, 2004; Kilani, 2006; Babu *et al.*, 2007). The results of the present study corroborate the reports of previous workers. The results demonstrates that both aqueous and ethanol extracts of *Dacryodes edulis* seed had significant *in-vitro* antimicrobial activity on both gram positive and gram negative bacteria. However the degree of inhibition by the extracts varied with ethanol extract being more effective than the aqueous extract. This is possibly due to the better solubility of the bioactive components in this organic solvent compared to water. This is in agreement with earlier report by Ibekwe *et al.*(2001).

The fact that the extracts were active against both gram positive and gram negative bacteria tested indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing food preservative or therapeutic substances that will be active against food spoilage and multidrug resistant organisms. The gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* were more susceptible to the activity of *Dacryodes edulis* seed extract compared with some of the gram negative bacteria tested such as *Salmonella typhi*, *Pseudomonas aeruginosa* and *Proteus mirabilis* (Table 3).

Thus, the low MICs of these gram positive isolates is a good indication of high efficacy against

these bacteria. Furtherance to this a food preservative developed from the ethanol extract will rapidly control the spoilage caused by these bacteria in foods. The high MICs of some gram negative organisms obtained in this study which ranged from 125µg/ml – 250µg/ml may be due to the inherent resistance of some gram negative organisms to a large number of antimicrobial agents. This is an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds in *Dacryodes edulis* seed extract.

On the whole the percentage killing or inhibition (Figure 1) of *Dacryodes edulis* seed extract on the microbial isolates tested suggests that ethanol extracts of *Dacryodes edulis* seed have potential antibacterial effects which can be adequately explored further in food preservative and pharmaceutical preparation of antimicrobial agents against infections possibly caused by these microbes.

**Conclusion and Recommendation**

In conclusion, the results of the present study showed that the seed extracts of *Dacryodes edulis* possess potential antibacterial activity against some food borne and spoilage organisms. It is believed that these findings reported for the first time on the seed of *Dacryodes edulis* will be helpful to many researchers in the field of antibacterial activities in plant seeds.

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