PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF *Dacryodes edulis* LEAF EXTRACT AGAINST SOME CLINICAL BACTERIAL ISOLATES

BY

Enakeno Eru-Oghene MILLER (Miss)

LSC1605461

DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA IN PARTIAL FULFILLMENT FOR THE AWARD OF BACHELOR OF SCIENCE B.Sc. (Hons), MICROBIOLOGY

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CERTIFICATION

This is to certify that this project work was carried out by **Enakeno Eru-Oghene MILLER (Miss)**in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

_____ _____

Mr F.I. Ebiala **Date**

(Project Supervisor)

APPROVAL

This project work is accepted in partial fulfillment for the award of Bachelor of Science, B.Sc. (Hons.) in the Department of Microbiology, University of Benin, Benin City.

_____ _____

Prof. S.O. Omonigho **Date**

(Head of Department)

DEDICATION

To Almighty God for his infinite mercies, unmerited graces and love towards me and my parents Dr and Mrs.O. Miller for their support and show of love towards me.

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ABSTRACT

Plants have shown immense contribution to man's nutrition as they are also used for medicinal purposes. There is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases, microorganisms are rapidly developing resistance to the available once. Hence, the leaf of *Dacryodes edulis* (African pear) was analyzed for its phytochemical and anti-bacterial properties on clinical bacteria isolates *Klebsiella* sp., *Proteus* sp. and *Escherichia coli.* Samples of leaf were obtained from Okhoro community in- Benin metropolis, dried, blended into powder and macerated using sterile distilled water and methanol as solvent. Antibacterial assay was carried out via Kirby Bauer disc diffusion method. Findings from this research showed that *Dacryodes edulis* leaf aqueous extract was active against *Klebsiella* sp. with a mean zone of inhibition (ZOI) of 8.00±0.00mm across all concentrations (12.5, 25, 50 and 100 µg/ml). *Proteus sp.* and *E. coli* had mean ZOI of 8 ±0.70mm at 12.5µg/ml and at 100µg/ml the ZOI was 10 ±0.28mm and 12 ±0.14mm respectively. Methanol extract was only active to *Proteus* sp and *Klebsiella* sp with a mean ZOI of 8 ±0.00mm at 12.5µg/ml and 25µg/ml respectively. The minimum inhibitory concentration (MIC) of aqueous extract against all isolates is 12.5µg/ml and it was bactericidal to only *E.coli* at a concentration of 50µg/ml. Methanolic extract had a MIC of less than 12.5µg/ml for *Proteus* sp and 12.5µg/ml for *Klebsiella* sp. The MBC was 12.5 and 50 µg/ml for *Proteus* sp and *Klebsiella* sp respectively. All test organisms were resistant to all standard antibiotics used. They were susceptible to gentamycin which serves a control. Despite the fact that the extract was able to inhibit the growth of the organisms, the isolates are regarded as resistant to the extract because their ZOI were less than the standard value of resistance which is 14. Phytochemical screening reveals that phenol, flavonoid, saponin, tannin, alkaloid and steroids were present in aqueous and methanol extract of the leaves .More work should be done to test the presence of active metabolites to determine its antibacterial activity.

CHAPTER ONE

INTRODUCTION

The contribution of plants and their products to human nutrition cannot be overemphasized. In Africa, fruits are on high demand and this is because they are complemented with food to ensure balanced diet, and some serve as raw materials to industries. Fruits serve as sources of vitamins and minerals hence, they also become important when the functions of these vitamins and minerals, are being considered in the body (Majesty *et al*. 2012). Also, some of these fruits are used in folk medicine to salvage some diseases (Lawal *et al*. 2010). The ability of these fruits to remedy diseases could be as a result of bioactive constituents, which are generally present in plants (Okwu, 2004).

With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs (Preethi *et al*. 2010). Antibiotics undeniably are one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infection. However, only one third of the infectious diseases known, have been treated from these synthetic products (Sharma, 2011). This fact stems from the emergence of resistance pathogens that is beyond doubt the consequence of years of widespread indiscriminate use, incessant and mis-use of antibiotics (Enne *et al*. 2001; Westh *et al*. 2004). In addition to this problem, antibiotics are sometimes associated with side effects on the host including hypersensitivity, immune-suppression and allergic reaction. Antibiotics resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotics resistance inhibitors from plants (Kim *et al*., 2005; Alagesaboopathi, 2011).

Dacryodes edulis fruit (African pear), a Burseracea, is one of such fruits that could serve the dual purpose of being a source of minerals and vitamins to human nutrition and as a raw material for industries, if properly harnessed. The consumption of the fruit is wide spread in Nigeria especially in

the southeastern part of the country. In 2009, Ajayi and Adesanwo noted that the fruit pulp is eaten and the seeds usually thrown away. Akubugwo and Ugbogu also noted that *D.edulis* seed oil have potential of being used as domestic and industrial oil. *D.edulis* fruit is consumed traditionally in Nigeria, raw, roasted or boiled in hot water, and is eaten alone or used in garnishing cooked or roasted maize (Arisa and Lazarus, 2009). It could also be used as butter to eat bread. It has been reported that Africa pear (*D. edulis*) has many medicinal uses. The leaves, bark, stem and root of D. *edulis* tree, are used as local medicine against diseases (Ikhuoria and Maliki, 2007; Jirovet *et al*. 2003). Previous works on *D. edulis*, addressed the phytochemical parameters of the oil, mineral elements, fatty acid composition of the pulp and seeds and few on anti-nutrient composition (Okunomo *et al*. 2007).

This study provides an answer to the questions of whether pear leaves possess antimicrobial compounds against multi-drug resistant pathogens which could serve as a source of novel antibiotics and also elucidate the phytochemical present in pear leaf.

Worldwide, Infectious disease is the number one cause of death accounting for approximately half of all deaths in tropical countries (Cowan, 1999). Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States. As a result of multidrug resistance and emergence of new infectious diseases (e.g. mad cow infections. West Nile viruses) in harmony with the resurrection of previously eradicated infections (e.g. outbreak of E. *coli* 0157:H7, MRSA, Salmonella and Tuberculosis), considerable draw-backs in the use of existing antibiotics and in the developing countries, an inability of the average family to control infections using orthodox medicine. Therefore, there is the need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. In developing countries like Nigeria and particularly in the southern part, low income people like farmers, traders, and people in rural settlements use herbal

medicine for the treatment of common infections (Iwu *et al*. 1999). It is necessary to evaluate in a scientific base, the potential use of herbal medicine for the treatment of infectious disease produced by common pathogens. Literature reports and ethno-botanical records indicates a general consensus in the use of antimicrobial active medicinal plants to provide cheaper drugs that may complement existing supplies from orthodox medicine in the primary health care programme and/or provide novel or lead compound that may be employed in controlling infections in our community. The study was carried out to investigate the phytochemical and antibacterial composition of *Dacryodes edulis* leaf extract.

1.1 Aims and Objectives

This study is aimed at determining the antibacterial and phytochemical properties of *Dacryodes edulis* leaf extract on some selected clinical bacteria isolates.

Objectives are;

1. To determine the susceptibility pattern of test organisms at different concentration of the extract.
2. To determine the minimum inhibitory concentration (MIC) of the extract.
3. To determine the minimum bactericidal concentration (MBC) of the extract.
4. To determine the phytochemicals present in the extract.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Dacryodes edulis*

The generic name *Dacryodes* is derived from the Greek word Dakruon meaning tear, referring to resin droplets on the bark surface of its members, while *edulis* means edible, emphasizing the importance of the nutritious fruit in the plant's cultivation. The plant belongs to the family Burseraceae whose members are characterized by an ovary of 2 to 5 cells, prominent as in ducts in the bark, wood and intrastaminal disk (Chunduff, 1984).

The genus *Dacryodes* consists of about 40 species (Verheij, 2002). However, Rehm (1994) indicated 80 species to encompass subspecies, varieties, forms and cultivars. Two varieties are recognized: var. *edulis* and var. *paruicarpa* whose conical fruits are smaller with the pulp. Var. *edulis* exhibits verticillate or subverticillate branching, while the branching is slender and opposite or bifurcate in var. *paruicarpa* (Okafor, 1983; Kapseu and Tchiegang, 1996).H.J. Lam gives four synonyms of *D. edulis* (G.Don) viz., *Canarium edule* Hook. f., C. *saphu* Engl., *Pachylobus edulis* G. Don and *P. saphu* Engl (Burkill, 1985). *C. edule* (G. Don) Hook.f. and *P. edulis* G. Don have long been considered as the most unambiguous synonyms (Boutelje, 1980). The common names are (in English) African pear, African pear tree, Bush butter, Bush butter tree, Bush fruit tree, Eben tree, Native pear and, in French, Safoutier (Burkill, 1985).

2.1.1 Origin and Geographical distribution

It is an indigenous fruit tree in the Gulf of Guinea and Central African countries(Troupin, 1950), but to popularity of the nutritious fruit for consumption, the plant is widely cultivated, extending its area of distribution to Sierra Leone, Uganda, Angola, Zimbabwe and Nigeria. It rarely grows wild. Thus, the exact natural area of distribution is obscure (Verheij, 2002).

2.1.2 Botanical description

Dacryodes edulis is a dioecious shade loving species of non-flooded forests in the humid tropical zone. It is a medium-sized, evergreen tree reaching a height of 18-40 m in the forest but not more than 12 m in plantations (Hutchinson and Dalziel, 1958), comparable with the tree of *Co ula edulis* (Adebayo-Tayo and Ajibesin, 2008). It is generally low branching with a deep dense crown. The bole is 50-170 cm in diameter, short, shallow fluted and more or less sinuous. The bark is yellowish-grey to pale-grey, often rough with lenticels and horizontal folds exuding white aromatic resin. Leaves are compound, imparipinnate, with 5-8 pairs of leaflets. They are glossy and pubescent, the pubescence disappearing with age. They are oblong-lanceolate or ovate-lanceolate, up to 20 (-30)x6 (-8) cm, broadly cunaete to rounded and asymmetric at base. They are acuminate at apex and margin is entire and glabrescent. Flowers are unisexual, subtended, 3 lobed and conspicuous with caducous low bract (Verheij, 2002). However, Ikhuoria and Maliki (2007) reported bisexual state for the flower, emphasizing that the flowers gather in panicles which to fruit bunches. Flowers are fragrant, about 5 mm across and are trimerous except for the ovary. They are arranged in dense, ferruginous, stellate-tomentose inflorescence. Sepals and petals are 3, the former showing brown colour and the latter, cream colour. Stamens are 6 and are slightly shorter than petals. Disc is also 6 but lobed, surrounding the two-celled, glabrous ovary. Styles are very short and stigma is 2-4 lobed. Inflorescence axis is 10-42 cm long or may be longer and deeply grooved. Fruits are ellipsoid drupe and vary in size, 4-12x3-6 cm, resembling olives. The exocarp is thin and pink ripening to form blue-green, purple or brilliant black. The fruits are one-seeded, with pulpy pericarp, about 5 mm thick and thin, cartilaginous endocarp. Seeds are oblong-ellipsoid, up to 5.5 cm long. The cotyledons are very much thickened and deeply folded or conduplicate, appearing palmately lobed (Medenbach de Rooy, 1994),

In Nigeria, the fruit of *D. edulis* var. *edulis* is large, elongated and cylindrical and are usually more than 5x2.5 cm. The fruit pulp is thick, about 3.5-9 mm. The tree has whorled branching and the

branchlets are stout and ascending. Conversely, the fruit of *D. edulis* var. *paruicarpa* is small, rounded or more or less conical, usually less than 5x2.5 cm. The fruit pulp is thin, about 2-3.5 mm. Often, the tree has bifurcated branching with slender, drooping branchlets (Okafor, 1983).

Plate 2.1: Typical *Dacryodes edulis* plant

2.1.3 Ethno-medicinal uses

Dacryodes edulis is a versatile plant in African ethnomedicine, as its various parts are employed to treat several diseases. The bark of the plant has long been used to cicatrize wound in Gabon (Walker and Silans, 1961). In this case, the bark is pulped and then applied directly to the wound. In Democratic Republic of Congo, the plant is employed for the treatment of divers ailments. The decoction of the bark is taken orally to treat leprosy. It is also used as gargle and mouth-wash to treat

tonsillitis (Bouquet, 1969). The bark is comminuted with meleguetta pepper to cure dysentery, anaemia, spitting blood and as an emmenagogue; when mixed with palm oil, it is applied topically to relieve pains, debility, stiffness and skin diseases (Bouquet, 1969). The leaves are chewed with kolanut as an antiemetic. The leaf sap is used as ear drop to treat ear trouble, while a leaf decoction is prepared to produce vapour that treats fever and headache (Bouquet, 1969; Bouet, 1980), In Congo Brazzaville, the leaves are boiled with those of *Lanata camara, Cymbopogon citratus*and *Persea americana* in water to form a decoction for treating malaria. A steam bath can also be taken from the decoction to treat the same ailment (Diafouka, 1997). Boiling the leaves with those of *P. americana* alone can be used to treat headache, antalgic and cephalgy (Diafouka, 1997). Recently, Jiofack*et al.* (2010) reported that the leaves are made into plaster to treat snakebite in Southwest Cameroon. The bark resin is used in Nigeria to treat parasitic skin diseases and jiggers (Dalziel, 1937; Hutchinson *et al.* 1963). When applied in lotions and creams, the resin smoothens and protects the skin (Ekpa, 1993). The aroma of the resin when liberated through burning is believed to ward off evil spirit in Nigeria (Sofowora, 2008). The leaves are often crushed and the juice released to treat generalized skin diseases such as scabies, ringworm, rash and wound, while the stem or stem twigs are employed as chewing sticks for oral hygiene (Igoli *et al.* 2005; Ajibesin *et al.* 2008b).

2.1.4 Phytochemical property

A lot more work was carried out on the fruits and seeds of the plant for their chemical composition than on any other parts. Often, the fruit pulp and seeds were characterized for lipid, essential oil and proximate components.

Lipids and Terpenes

The fruit pulp of the plant is rich in lipid (Kinkela and Bezard, 1993), the oil content of which was determined on a dry basis to fall between 30 and 60%, depending on the origin and the ripening condition of the fruit (Bezard*et al.* 1991). However, Ikhuoria and Maliki (2007) reported a lower

value of 23.2% for oil content of the Nigerian fruit pulp when comparing is composition with that of Avocado pear. In rare cases, the oil content could go as high as 70% (Kinkela *et al.,* 2006). It has also been demonstrated that geographical orientation and fruit distribution of a tree by level to the ground can influence the percentage yield and relative composition of the oil (Kinkela *et al.,* 2006). The lipid yields fatty acids such as palmitic acid, oleic acid, linoleic acid and stearic acid, whose percentage composition vary markedly ranging from 30-62% (Palmitic acid), 15-24% (Linoleic acid), 18-60% (Oleic acid) and 1.3-5.5% (Stearic acid) (Mbofung*et al.* 2002; Kinkela *et al.* 2006; Ikhuoria and Maliki, 2007). In addition, arachidonic acid was identified as an important fatty acid in the pulp and seed oil (Ajayi andAdesanwo, 2009). Even at the regional level, relative variation in fatty acid content (49-58%) was observed for the fruits of Cameroon, Congo Brazzaville, Congo Kinshasha and Gabon, the constituent countries of Guinea Equatorial region (Mbofung*et al.* 2002). These values were found to be higher than those reported for Nigeria (32%) and Cote D'Ivoire (30%). The unsaponifiable fraction of the oil was reported to contain sterols, triterpene alcohols and traces of tocopherols-. Mineral elements such as phosphorus, calcium, magnesium, potassium, sodium, zinc and manganese are also found in the pulp and seed of the plant (Ajayi and Adesanwo, 2009).

Unlike other oily fruits, the seed oil possesses the same fatty acids as the fruit pulp (Silou, 1996) and the seed may contain up to 18-70% oil (Gunstone and Norris, 1982). In a recent study on the seed oil of the Nigerian fruits, Arisa and Lazarus (2008) reported oil content of 50 %. The good physicochemical properties shown by the seed oil suggests that the oil can be useful for consumption and industrial application. Soxhlet extraction method was reported to give the best yield of seed oil (Dzondo-Gadet *et al.,* 2004).

Essential oils have been isolated and analyzed from different parts of *D. edulis.* The fruit contains about 1.5% essential oil whose main constituents are a-pinene, a-terpineol, myrcene andgermacrene-D, while minor compounds include a-cadinol, 6- cadinol and 13-eudesmol (Onocha*et al.* 1999). In

another study on the essential oil of untreated, boiled and roasted fruits, many constituents were isolated among which a-pinene (47.1-60.5%), a-pinene (6.7-8.2%), myrcene (12.9-14.8%) and limonene (3.4-6.4%) were discovered to be the main compounds (Jirovetz *et al.* 2003). These and other compounds such as phellandrene, cadinol, sabinene, p-cymene, dimethyl sulfide and hexanal were found to be responsible for various odours of the plant (Jirovetz *et al.* 2003). The stem bark essential oil of the plant growing in Nigeria contains predominantly terpinen-4-ol, a-thujene and a-pinene, while a-phellandrene is the main constituent of root bark oil (Onocha*et al.* 1999). I3-caryophyllene, similarly found as the main sesquiterpene in the essential oil of the leaves of *Cinnamomum zeylanicum* growing in Nigeria also occurs as the main constituent of the leaf essential oil of *D. edulis* growing in Nigeria (Onocha*et al.* 1999). Furthermore, the ethanol extract of the stem bark gave oil that contained thirteen compounds including hydrocarbon (1-isopropyl-1-methyl-2nonylcyclopropane), carboxylic acid (octadecanoic acid), ketone (3-methylheptan-4-one) and an alcohol (6-methylheptan-1-ol) (Okwu and Ighodaro, 2009). The resin obtained from the tree has been reported to yield peppery essential oil rich in sabinene, 13-phellandrene and limonene and compounds such as crystalline canaric acid from non-volatile fraction and triterpene alcohols such as 3-epi- a-amyrin, 3-epi-lupeol and a-amyrin from neutral fraction (Ekong and Okogun, 1969).

Phenolics

Phenolics such as ethyl-gallate and quercitrin had been dentified in the plant leaves. Flavanols such as quercitrin, isoquercitrin, isorhamnetin and rhamnoside, as well as anthocyanins such as petunidin and cyanidin were also reported to be present in the fruit skin zone and pulp of *D. edulis* during ripening (Missang *et al.* 2003). The stem exudates of the plant were reported to contain tannin (0.47 mg/100 g) (Okwu and Nnamdi, 2008).

Other classes of compounds such as saponins (2.08 mg/100 g) and alkaloids (0.28 mg/100 g) were also detected from the stem exudates and quantified (Okwu and Nnamdi, 2008).

2.2 Biological activity

The presence of bioactive compounds such as saponins, tannins, alkaloids and flavonoids identified in the plant has been suggested to be responsible for the various uses of *D. edulis* in traditional medicine to cure ringworm, wound, scabies, skin diseases and inflammation (Okwu and Nnamdi, 2008). In addition, the potential health-related functions of dietary plants were found to include antibiosis, immunostimulation, nervous system action, detoxification, anti-inflammatory, antigout, antioxidant, glycemic and hypolipidemic properties (Johns, 2001).

2.3 Antimicrobial activity

The essential oils of the plant resin were investigated for antimicrobial and antioxidant activities. The essential oil showed more potent antibacterial effect against bacteria such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella enteric* and *Proteus mirabilis* than antifungal effect against Candida albicans and this effect was found to be due to the presence and high content of terpinen-4-ol (19.8%) and a-pinene (17.4) (Obame *et al.* 2008). In all the antibacterial cases, the Minimum Inhibitory Concentration (MIC) of 1-16 mm was equivalent to the Minimum Bactericidal Concentration (MBC). In another study, the antibacterial effect of the essential oil of the plant resin was confirmed to be due to the presence and high content of the same foregoing terpenes, but antifungal effect of the oil was reported to be lacking (Koudou *et al.* 2008). Since compounds such as alkaloids and saponins are known to be antimicrobial (Ajibesin*et al.* 2006), their presence has been suggested to account for the antimicrobial activity of the plant (Okwu and Nnamdi, 2008). In screening some Nigerian plants for antibacterial activity, the leaf extract demonstrated the best activity for which ethylgallate and quercitrin were identified as responsible (Ajibesin, 2005). Like many other Nigerian plants, the leaves showed better activity than the stem and root which lends credence to the wider application of leaves in Nigerian traditional medicine than the other plant parts (Ekpo *et al.,* 2008).

2.4 Antioxidant activity

In Cameroon, the extracts of 42 medicinal plants used for anaemia, diabetes, AIDS, malaria and obesity were investigated for phytochemical substances and antioxidant properties. The leaves of *Dacryodes edulis* elicited very high antioxidant effect when analyzed against three assay methods: Folin (Folin Ciocalteu Reagent), FRAP (Ferric Reducing Antioxidant Power) and DPPH (1, 1-diphenyl-2-picrylhydrazyl), ranking second behind Alchorneacordifolia (Agbor *et al.,* 2007). This antioxidant property was attributed to the presence of flavonoid in the plant. The essential oil of the plant resin also demonstrated good antioxidant activity. In a DPPH test system, the IC,, value of *D. edulis* oil was reported to be 68.5±2.29 µg mL^{-1}, while oxidation of linoleic acid was effectively inhibited by the plant (70%) in the 13-carotene-linoleic acid test (Obame *et al.,* 2008). However, this antioxidant capacity was ascribed to the mono and sesquiterpenes present in the plant essential oil. Employing similar antioxidant assay methods, Koudou *et al.* (2008) reported significant antioxidant effect of the resin oil, including DPPH radical scavenging activities and inhibition of lipid peroxidation and suggesting that *D. edulis* may help prevent oxidative damage in the human body such as lipid peroxidation associated with cancer, premature aging, atherosclerosis and diabetes. More recently (Nguefack, 2009) confirmed the significant antioxidant and free radical scavenging activities in the aqueous and ethanol extracts of *D. edulis.*

2.5 Cardiovascular activity

Dacryodes edulis oil was reported to decrease the HDL cholesterol level in serum of rats (Leudeu *et al.* 2006). Thus, potential health related functions of dietary plants such as *D. edulis* was reported to include immuno stimulation and nervous system action.

2.6 Antidrepanocytary activity (anti-sickle cell anemia)

The aqueous and ethanol extracts of *D. edulis* leaves were discovered to normalize the SS blood erythrocytes, following the deoxygenation of haemoglobin in anaerobic condition, thus validating their use in traditional medicine (Mpiana *et al.,* 2007).

2.7 Toxicity

No part of *D. edulis* is known to be toxic. During the survey of toxic plants of Akwa-Ibom State of Nigeria, *D. edulis* was not among the plants implicated for eventual toxicity evaluation (Ajibesin *et al.* 2002). Obasi and Okolie (1993) supported this position when they reported lack of toxic principles in the seed of the plant. However, the findings of Hanson (2009) opposed this report when he found the seed to contain anti nutrient factors such as oxalate, tannins, phytate and trypsin inhibitory activity. Thorough processing of the seed before use was therefore suggested. On the other hand, Dike (2010) reported lack of toxins in the fruit when he indicated values below toxic level for the antinutrient factors such as tannins and cyanide. Sometimes the fruit sold in the market may be contaminated with metal pollutants. Akinola and Adenuga (2008) reported the presence of heavy metals on the fruit wall, with lead showing a toxic concentration and others such as cadmium and zinc falling below the maximum permissible concentration. The source of the fruits before reaching the market and their exposure to atmospheric factors were advanced as possible reasons for the contamination. However, there is yet to be report on the toxicity study of the plant in experimental animals.

2.8 Economic properties

The tree wood is heavy, elastic and is found suitable to make axe-handles, mortals and can be for general carpentry (Walker and Silans, 1961). The dead branches of the tree are used as firewood (Ayuk *et al.* 1999), while the twigs serve as chewing sticks (Ajibesin, 2005). The resin is used as pitch on the inner surfaces of calabashes and for mending earthenware (Burkill, 1985). The resin can also serve as fuel (Ekong and Okogun, 1969). The fruit is the most important part to which the tree

owes its principal economic values and for which the tree is widely cultivated, domesticated and commercialized (Leakey *et al.* 2002; Anegbeh *et al.* 2005). The fruit consists of a seed (stone) surrounded by a pulpy butyraceous pericarp, which is the portion consumed either raw or cooked to form a sort of butter. The fruit is rich in lipids, proteins, minerals and vitamins which make it an excellent source of nutrition to consumers, stimulating its increase in production and commercialization for decades (Kenmegne *et al.* 1997). The fruit yields oil found suitable for cosmetics and food, while the flower nectar provides a good honey (Ayuk *et al.* 1999; Verheij, 2002). Safou fruit oil, when incorporated into foods can boost their nutritional values, thus making them more marketable. Mbofung *et al.,*(2002) while studying the economic values of safou fruit oil, observed that the protein content of a biscuit increased by 39% when the margarine in the biscuit recipe was substituted for safou pulp oil. The bark resin also finds application in the food and cosmetic industries as thickener, flavour, stabilizers and as and as an emulsifying agent (Ekpa, 1993).

So highly traded are the safou fruits that transactions now cut across local and international boundaries, so much so that the fruits are marketed in specialized markets in Europe (Awono *et al.* 2002). The farm-level value of fruit production may reach USD 161 a year per grower/collector (Ayuk *et al.* 1999). Whereas, the other medicinal parts of the plant such as leaves, stems and roots are sold in the herb section of domestic markets, the fruits are ubiquitous in every section of the market. The average price of the fruit in the markets in Nigeria where home consumption accounts for about 70% ranges from USD 300-700/ton of fruits. Between January and June 1995, almost 600 tons of fruits, valued at USD 244,000 were traded in the humid lowlands of Cameroon (Verheij, 2002). However, the marketed volume of safou fruits increased to 2,324 tons at a value of USD 1.5 million in 1999, when nine markets were surveyed in different parts of Cameroon (Awono *et al.* 2002). The trade is so active in Cameroon that it is extended to neighboring countries such as Gabon.

At international markets, African pear fruits imported into Europe are generally intended for nationals of the exporting countries, with the volume increasing since 1982 (Tabuna, 2000). The principal importing countries of safou fruits are Belgium, France and United Kingdom from African countries such as Cameroon, Nigeria, Congo Brazzaville, Democratic Republic of Congo and Central Africa Republic. The potential market in these importing countries in 2002 alone was well over 120,000 people (Awono *et al.* 2002), with the number increasing ever since. The major snag of safou international trade however is the perishability of the fruit. In spite of this, *D. edulis* has become the main source of food cash incomes, employment and enhanced livelihood for subsistence farmers and traders.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Sample

The leaves of *Dacryodes edulis* used in this experiment was collected from Okhoro community of Egor Local Government Area of Edo State, Nigeria and transported to the department of Microbiology Laboratory, University of Benin for analysis.

3.2 Identification and Authentication of leaf sample

The taxonomic identity of the plant was authenticated at the Department of Plant Biology and Biotechnology Herbarium, Faculty of Life Sciences, University of Benin, Nigeria.

3.3 Sources of Bacterial Isolates

The antibacterial screening of the extracts was assessed against clinical bacterial strains isolated from human urine, sputum, blood, ear swab and wounds collected from the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria.

The isolates of *Proteus* sp*, Klebsiella sp* and *Escherichia coli* were collected in agar slants and transported to the Department of Microbiology. These isolates were further identified in the laboratory based on their cultural, morphological and biochemical characteristics to ascertain their identity.

3.4 Preparation of Microorganisms Used

The test bacteria were sub-cultured on nutrient agar and incubated at 37^{o}C for 18-24hrs in an incubator. After incubation, the test organisms were sub-cultured on a fresh petri dish.

3.5 Preparation of Plant Extracts

The crude extract and solvent fractions were prepared as described by (Teke *et al*., 2007). The leaves were shade dried for 7-14 days after which it was blended into fine powder. A portion (500g) of the

ground leaves was macerated in 2.5 L of methanol (95%) for 72 hours in an air tight glass cylinder. The extract was filtered with muslin bag and concentrated in vacuum at 50°C using a rotary evaporator.

Same procedure was followed for aqueous extract, a portion (500g) of the ground leaves was macerated in 2.5 L of distilled water for 72 hours in an airtight glass cylinder. The extract was filtered with muslin bag and concentrated in vacuum at 50°C using a rotary evaporator.

The extract and solvent fractions were stored separately in well-labeled airtight dark bottles at 4°C until further use.

3.6 Phytochemical analysis

3.6.1 Qualitative test

Test for Alkaloids

A measure of 0.5 grams of the extract was extracted by warming with 20 ml 1% sulphuric acid in a 50 ml conical flask on a water bath, with intermittent shaking for 2 minutes. It was then centrifuged and the supernatant pipetted off into a small conical flask. One drop of Meyer's reagent was added to 0.1 ml supernatant in a semi-micro tube. Cream precipitate indicated presence of alkaloids (Trease and Evans, 2002).

Test for Saponins

Each seed powder sample takes 1 g separately and it separately boiled with 10ml of distilled water in a bottle bath for 10 minutes on a heater. Then the mixture was filtered while hot with Whatmann filter paper and it allows cooling. The following tests are then carried out. Test for frothing- 2.5 ml of seed extract sample are filter then it diluted with 10ml distilled water and shaken vigorously for 2 minutes, in solution frothing are present this indicate the presence of saponin in the filtrate sample (Trease and Evans, 2002).

Test for Phenols

Ferric chloride test was carried out where the extract was diluted to 5 ml with distilled water. To this, a few drops of neutral 5% Ferric chloride solution was added. A dark green or a blue-black color indicated the presence of phenolic compounds (Harbourne, 1998).

Test for flavonoids

A measure of 1 g of the leaf powdered sample were boiled separately in flask with 10 ml of distilled water for the time of 5 minutes and filtered it while extract solution are hot with whatmann filter paper. Few drops of 20% sodium hydroxide solution were added to 1 ml of the cooled seed filtrate sample. It shows change to colour like yellow colour to colourless when sodium hydroxide were added in seed extract. Theses colour change indicated that the presence of flavonoids (Harbourne, 1998).

Test for Tannins

Exactly 0.5g of the plant extract was stirred with 10ml sterile distilled water. This was filtered using a sterile filter paper and ferric chloride ($FeCl_2$) reagent was added to the filtrate. The presence of blue-black or green precipitate indicates the presence of tannin in the extract (Harbourne, 1998).

3.7 Antibacterial Assay

The sensitivity testing of the extract was determined using Kirby Bauer disc diffusion method. Freshly grown bacterial culture in nutrient broth for 18hrs was used for this experiment. For standardization the broth culture was diluted until the bacterial suspension matched with the turbidity of 0.5 McFarland turbidity standards. Exactly 0.1 ml of the standardized test isolates was evenly spread on agar medium using a sterile glass rod. Four concentrations (12.5, 25, 50 and 100µg/ml) of both methanolic and aqueous extractwere prepared in a plain sterile sample bottle and a paper disc of 6mm in diameter was added to each of the bottles and allowed to diffuse for 1hr. Standard Streptomycin and Ampicillin antibiotics were tested alongside as controls (Akinpelu, 1999).

3.8 Minimum inhibitory concentration (MIC) assay

The MIC values were studied for the microbial strains, being sensitive to the extracts in disc diffusion method. The minimum concentration with activity which does not kill the test organisms was the MIC. The extract in this study was tested in triplicate against each test organism (Akinpelu and Kolawole, 2004).

3.9 Minimum bactericidal concentration (MBC) assay

A loop full of the bacteria was added to a 10ml volume of various concentration (100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml) of the extract in McCartney bottles and incubated for 48hr. After which it was cultured on a petri dish to ascertain which of the concentration was able to kill the organism completely. The least concentration that kills the organisms completely was taken as the MBC. That is the concentration that had no growth on the media when inoculated from McCartney bottles (Olorundare *et al.,* 1992).

CHAPTER FOUR

RESULT

Table 4.1 shows the standard antibiotics susceptibility pattern of test organisms. The organisms were all resistant to ceftazidime, metronidazole, vancomycin, ertapenem and tetracycline. These organisms were all susceptible to gentamycin.

Table 4.1: Antibiotics sensitivity pattern of test organisms

Isolates	**Antibiotics used and class of antibiotics**							
	Fluoroquinolone	**Cephalosporin**	**Imidazole**	**Glycopeptide**	**Aminoglycosides**	**Carbapenems**	**Microlide**	**Tetracycline**
	CIP	**CAZ**	**MTZ**	**VA**	**CN**	**ETP**	**E**	**TE**
Escherichia coli	S	R	R	R	S	R	S	R
***Klebsiella* sp.**	R	R	R	R	S	R	S	R
***Proteus* sp.**	S	R	R	R	S	R	R	R

Key: ETP=Ertapenem, CN=Gentamycin, E=Erythromycin, MTZ=Metronidazole, VA=Vancomycin, CIP=Ciprofloxacin, TE=Tetracycline and CAZ=Ceftazidime,

S=Susceptible (>17)

R=Resistant(<14)

Table 4.2 reveals the mean zone of inhibition of *Dacryodes edulis* leaf aqueous extract against test organism. As seen in the table, the effect of the extract on *Klebsiella* sp. was not concentration dependent as it had a zone of inhibition of 8.0mm across all concentrations (12.5, 25, 50 and 100) µg/ml.

Table 4.2: Mean zone of inhibition of aqueous extract of *Dacryodes edulis* leaf extract in millimeter (mm).

Isolates	**Concentration**			
	12.5µg/ml	**25µg/ml**	**50µg/ml**	**100µg/ml**
***Proteus* sp.**	8.00±0.70	8.00±0.14	8.00±0.00	10.00±0.28
***Klebsiella* sp.**	8.00±0.00	8.00±0.00	8.00±0.42	8.00±0.00
E. coli	8.00±0.00	8.00±0.28	10.00±0.14	12.00±0.14

*Values are mean ± standard deviation

Table 4.3 shows the mean zone of inhibition of *Dacryodes edulis* leaf aqueous extract against the test organisms. There was no activity against *E. coli, Proteus* was inhibited by the extract with a zone of 8±0.00 mm *across* all concentrations hence not concentration dependent.

Table 4.3: Mean zone of inhibition of methanolic extract of *Dacryodes edulis* leaf extract in millimeter (mm).

Isolates	**Concentration**			
	12.5µg/ml	**25µg/ml**	**50µg/ml**	**100µg/ml**
***Proteus* sp.**	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00
***Klebsiella* sp.**	0.00±0.00	8.00±0.00	8.00±0.14	10.00±0.28
E. coli	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

*Values are mean ± standard deviation

Table 4.4 shows the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of both methanolic and aqueous extract against test organisms. The result revealed that the MIC for aqueous extract against all isolates is 12.5µg/ml and was bactericidal to only *E. coli* at a concentration of 50µg/ml.

Table 4.4: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous extract against test organisms

Organism	**Aqueous extract**		**Methanolic extract**	
	MIC(µg/ml)	**MBC (µg/ml)**	**MIC (µg/ml)**	**MBC (µg/ml)**
***Proteus* sp.**	12.5	NB	<12.5	12.5
***Klebsiella* sp.**	12.5	NB	25	50.0
E. coli	12.5	50.0	-	ND

Key:

NB= No bactericidal effect, ND= Not determined

Table 4.5 reveals result obtained for the qualitative phytochemical properties of *Dacryodesedulis*aqueous and methanol leaf extract. As shown in Table 4.5, phytochemical analysis reveals that phenol, flavonoid, saponin, tannin, alkaloid and steroids were present.

Table 4.5: Qualitative phytochemical properties *Dacryodes edulis* leaf extract

Phytochemicals	*Dacryodes edulis*	
	Aqueous extract	Methanol extract
Phenol	++	++
Flavoniod	+	++
Saponin	++	+
Alkaloid	+	++
Steriods	+	++
Tannins	++	++

Key: (++ high), (+ low)

CHAPTER FIVE

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* leaf had antimicrobial activity.

The results of preliminary phytochemical screening of the extract were presented in Table 4.5, which showed that the aqueous leaf extracts contained high concentrations of phenol, saponins and tannins while flavonoids, steroids and alkaloids were present but at low concentration, this is similar to Nwokonkwo, 2013. For the methanol extract, high concentrations of phenol, flavonoids, alkaloids, steroids and tannins were detected while low concentration of saponins was detected. Results reveals that aqueous was a better extracting solvent compared to methanol. These results are in good agreement with a similar study earlier conducted by Ajaiyeoba *et al*. (2003). The presence of the mentioned phytochemicals suggests that the extract and/or its metabolites may possess antibacterial potential against several human pathogens. This is because the different phytochemical compounds have been linked with various bioactivities (Evans *et al*. 1989; Sofowora *et al.*, 1969). The study of Olasunkanmi and Adeniyi (2017) on phytochemical analysis of *Dacryodes edulis* revealed the presence of tannins, flavonoids, saponins and alkaloids.

The antimicrobial results of the crude extracts (aqueous and methanol) of *Dacryodes edulis* leaf were shown in Table 4.2 and 4.3 respectively. It was observed that the aqueous extract was bacteriostatic and bactericidal to *E. coli* at various concentrations that is, at a concentration of 12.5 µg/ml, the extract was able to inhibit *E. coli* with a mean zone of inhibition of 8.0 ±0.00mm in diameter which is the minimum inhibitory concentration (MIC). At a concentration of

50µg/ml, *Dacryodes edulis* aqueous leaf extract was bactericidal against *E. coli* with a mean zone of inhibition of 10 ±0.14mm in diameter which is the minimum bactericidal concentration (MBC). The aqueous extract was bacteriostatic to both *Proteus* sp and *Klebsiella* sp with a minimum inhibitory concentration of 12.5µg/ml and a mean zone of inhibition of 8.0±0.00mm (Ejele and Nwokonkwo, 2013).

On the other hand, methanolic extract was inactive against *E. coli* while it was bacteriostatic and bactericidal to both *Proteus* sp and *Klebsiella* sp. The methanolic extract was active at different concentrations. The effect of methanolic extract against *Proteus* was not concentration dependent that is, at all concentrations (12.5, 25, 50 and 100µg/ml) of the extract had a mean zone of inhibition of 8.0±0.00mm in diameter however, the minimum inhibitory concentration was below 12.5µg/ml while the minimum bactericidal concentration was 12.5µg/ml as shown in table 4.5.

The methanolic extract was both inhibitory and bactericidal to *Klebsiella* sp with a minimum inhibitory concentration (MIC) 25µg/ml and a mean zone of inhibition of 8.0mm whiles the minimum bactericidal concentration of 50µg/ml. This is in agreement with earlier report by Ibekwe *et al*. (2001).Compared to the standard antibiotics, lower but significant zones of inhibition was obtained when both aqueous and methanol leaf extracts of *Dacryodes edulis* were tested against the bacteria. This revealed antibacterial capacities against potential pathogenic bacteria.

Similar case study carried out by Olasunkanmi and Adeniyi on antibacterial and antioxidant activities of *Dacryodes edulis* methanolic leaf extract revealed that the mean diameter of zones of inhibition exhibited by the extract ranged between 12.0±0.0 mm and 23.3±1.2 mm. Also the

conventional streptomycin and ampicillin diameter of zones of inhibition ranged from 13.0±0.0 mm – 31.0±1.0 mm and 18.3±0.6 mm - 29.3±1.5 mm respectively. The MIC exhibited by their extract against susceptible test organisms ranged between 0.27 mg/mL and 4.375 mg/mL while MBC ranged between 0.55 mg/mL and 17.5 mg/mL

In this study, standard antibiotics susceptibility pattern of test organisms revealed that *Klebsiella* sp was susceptible to gentamycin and erythromycin and resistant to ertapenem, metronidazole, vancomycin, ciprofloxacin, tetracycline and ceftazidime. *Proteus* sp was susceptible to ciprofloxacin and gentamycin while *Klebsiella* sp was susceptible to erythromycin and gentamycin. Bacterial isolates were all resistant to all extracts of *Dacryodes edulis*. Although the extract did not show significant level of inhibition of bacteria isolates, phytochemical analysis revealed that it contained components which have the potential for broad spectrum of activity at high concentrations and would be able to inhibit or kill the clinical isolates.

CONCLUSION

The results of the present study showed that the leaf extracts of *Dacryodes edulis* possess antibacterial activities but did not meet up with standard required measures of antibiotics used. All isolates were resistant to the extract. Further works should be done in the future to check if this plant can actively be used as an antibacterial agent and to check if there are metabolites in the extract.

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APPENDIX

Nutrient agar

Ingredient	**1 L**	**500 mL**
Yeast extract	2 g	1 g
Peptone	5 g	2.5 g
Sodium chloride (NaCl)	5 g	2.5 g
agar	15 g	7.5 g