CHAPTER ONE
INTRODUCTION

1.1 Background

The World Health Organization defined a therapeutic plant as any plant which, in one or more of its organs (leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds), has substances which could be used for curative purposes, or that are precursors for chemo-pharmaceutical semi synthesis. The medicinal plant will have its organ useful in controlling or treating a disease state and thus, has chemical constituents that are therapeutically potent (Kar, 2007).

Therapeutic plants are prospective sources of novel drugs as well as preliminary materials for manufacturing drugs and alternative remedies for different health problems. Moreover, a way of preventing antibiotic resistance of pathogenic microorganisms is by using novel compounds which are different from current artificial antimicrobial products. Therefore, the curiosity in researching curative plants as a source of active pharmacological compounds has globally increased (Firas and Hassan, 2008).

1.1.1 *Dacryodes edulis* and *Anacardium occidentale*

Dacryodes edulis (G. Don) H. J. Lam, known as African plum or African pear, belongs to the family Burseraceae. The fruit is an ellipsoidal drupe that varies in length. The skin of the fruit is dark blue or violet, whereas the flesh is pale to light green. The bark, leaf, fruit and resin of *D. edulis* are used for treating acute malaria, wounds, elephantiasis, skin diseases; as anthelmintics, astringent, and for clearing pregnancy stretch marks (Orwa *et al*., 2009; Omonhinmin, 2014).

Anacardium occidentale Linn. (Cashew tree) belongs to the family Anacardiaceae. The fruit of the cashew tree is an accessory fruit (pseudocarp or false fruit). The true fruit of the cashew tree is a kidney or boxing-glove shaped drupe that grows at the end of the cashew apple. Within the true fruit, is a single seed; the cashew nut. The bark, leaf and fruit of *A. occidentale* are used for treating scurvy, caries, malaria, typhoid, warts, ringworms, elephantiasis, leprosy, for managing diabetes, and as anthelmintics (Orwa *et al*., 2009; Aderiye *et al*., 2015).

1.1.2 Skin and its infections

The skin, which is the largest organ of the body, takes about fifteen percent of the total adult weight. It protects the body against intruders. It prevents excess water loss from the body, thus functioning in thermoregulation. It is composed of three layers: the epidermis, the dermis, and subcutaneous tissue (Kanitakis, 2002). Skin diseases may be infectious, congenital, degenerative, inflammatory and cancerous, and they may affect all ages from cradle to grave, but a disproportionate burden falls on the elderly and young children (Abel-Rahman *et al*., 1997; Asiedu and Etuaful, 1998).

Staphylococcus aureus is the universal etiological agent of boils, carbuncles, breast abscess, and different skin infections, including infantile-impetigo and folliculitis. It is difficult to get rid of these infections, particularly in the deeper skin layers, sweat gland, sebaceous gland, and the hair-follicles, through daily washing and scrubbing, even with antiseptic soaps. Besides *S. aureus*, *Pseudomonas aeruginosa* also causes folliculitis (Ratnam *et al*., 2017).

Mycoses (fungal infections) with highest incidence are candidiasis and dermatophytosis. Tinea pedis (athlete's feet), tinea cruris (groin), tinea unguium (onychomycosis: nails), tinea barbae (beard) and tinea incognito (skin) are caused by *Trichophyton rubrum*, *T. mentagrophytes* and *Epidermophyton floccosum*, while tinea faciei (face) and capitis (scalp) are also caused by *T. rubrum* and *T. mentagrophytes* (Sharma *et al*., 2015).

The World Health Organization estimated about twenty percent global occurrence of dermatomycosis (Sharma *et al*., 2015). The infections are generally widespread in developing countries, because of poor hygienic environment, poor socio-economy, closeness to animals, as well as favourable climatic conditions for the growth of dermatophytes (Sharma *et al*., 2015).

1.2 Statement of the problem

Skin infections are of global concern, most especially in developing countries. Illness that directly affects the skin is the fourth most frequent cause of all human diseases, affecting almost one third of the world's population (Abel-Rahman *et al*., 1997). Also, some modern chemical based creams predispose the skin to irritations and other contraindications. Unfortunately, some indigenous medicinal plants that are traditionally used in combating skin infections, are not scientifically proven, documented or formulated into herbal skin products.

1.3 Justification for the study

The World Health Organization estimated that eighty percent of the global population still relies on herbs for their major healthcare (Himal *et al*., 2010). In developing countries, many people prefer herbal medicine to modern drugs because of current resistance of microbes, allergic reactions, adulteration, relatively high cost, scarcity especially in rural areas, and also people's socio-cultural beliefs.

Plants have been used in solving various health problems, since the beginning of human existence, thus many researchers are now interested in investigating them. Moreover, there are increasing global researches on incorporation of medicinal plants into conventional pharmaceutical dosage forms, for health and safety reasons.

It is essential to scientifically evaluate, authenticate and also guarantee the safety of acclaimed ethnomedicinal potentials of *Dacryodes edulis* (G. Don) H. J. Lam and *Anacardium occidentale* Linn. leaves, especially in treating some skin infections; and if these readily available plants in our locality are antimicrobially active, they could be formulated into standard dosage forms for use.

1.4 Aim of the study

This research investigated the effectiveness of *Dacryodes edulis* and *Anacardium occidentale* leaves and creams formulated against selected microorganisms causing skin infections.

1.4.1 Specific objectives

The following are the specific objectives of this research:

i. Ethnobotanical survey of *D. edulis* and *A. occidentale* in Ibadan.
ii. Antioxidant evaluation of *D. edulis* and *A. occidentale* leaf extracts.
iii. Quantitative phytochemical evaluation of *D. edulis* and *A. occidentale* leaf extracts.
iv. Antimicrobial screening of *D. edulis* and *A. occidentale* leaf extracts.
v. Functional group determination and acute toxicity test of *D. edulis* and *A. occidentale* leaf extracts.
vi. Formulation and evaluation of creams containing *D. edulis* and *A. occidentale* leaf extracts.

CHAPTER TWO
LITERATURE REVIEW

2.1 The human skin

The human skin is the largest organ and outer covering of the body. It is similar to most of the other mammal's skin, especially to pig's skin (Herron, 2009; Liu *et al*., 2009). It has three layers: the epidermis, the dermis, and the hypodermis. The epidermis is divided into five layers (strata): stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum (prickle cell layer), and stratum basale (stratum germinativum). The dermis consists of two layers, the papillary and reticular layers. The dermis contains the sweat glands, hair, hair follicles, muscles, sensory neurons, and blood vessels. The hypodermis (subcutaneous tissue), which is the deepest layer of the skin, contains adipose lobules, hair follicles, sensory neurons, and blood vessels (Yousef *et al*., 2019).

2.1.1 Importance of the human skin

The skin serves as the body's first barrier against pathogens, UV light, chemicals, and mechanical injury (Yousef *et al*., 2019). According to the British Association of Dermatologists (2015), it functions include sensation, heat regulation, water conservation, and immunological surveillance. Also, it is the only most significant determinant of human appearance and identity, and the interface for much of our physical and social contact with our environment.

2.2 Microorganisms and skin infections

2.2.1 Microorganisms

A microorganism is a single celled or multicellular microscopic living organism. They have many varieties which include all the bacteria and archaea, nearly all the protozoa, numerous fungi, algae, and some animals like rotifers. Microorganisms are broadly categorized as prokaryotes or eukaryotes. Prokaryotes are bacteria and blue-green algae, while eukaryotes are slime moulds, protozoa, fungi, and other algae (Challis and Hopwood, 2003; Madigan and Martinko, 2006).

Microorganisms are well utilized in food, beverage, biotechnology, and genetic engineering. However, a minute percentage of microorganisms are pathogenic (often referred to as microbes), causing diseases and even death in plants and animals (WHO, 2002a). For example, a gram-negative bacillus bacterium called *Pseudomonas aeruginosa*, which causes broad range clinical infections such as pneumonia or bacteraemia, is a primary source of nosocomial infections that are associated with increase in death rate and are often difficult to treat (Wahab and Rahman, 2013). Therefore, the Public Health Agency of Canada (2004), because of the problems of some microbes, emphasized on the decontamination of every waste that has come in contact with any contagious microorganism by autoclaving, gamma irradiation, incineration, or chemical disinfection.

Pseudomonas aeruginosa, *Staphylococcus aureus*, *Streptococcus pyogenes* and many coliform bacilli are the main frequent pathogenic bacteria isolated from burn injuries. However, in some community health and hospital practices, *S. aureus* is commonly isolated, and it is also a prominent cause of nosocomial infection from burn wounds. *Escherichia coli* is the principal cause of urinary tract infections; yet, *Staphylococcus* spp., *Klebsiella* spp., as well as *P. aeruginosa* are usually isolated from urinary tract infections. In addition, *Staphylococcus* and *Streptococcus* species cause skin infections, pneumonia, meningitis and devastating sepsis (Fish, 2002).

2.2.2 Bacterial skin infections

Kumar *et al*. (2007) explained some skin infections caused by bacteria as:

Impetigo: This is mostly caused by *Staphylococcus aureus*, and at times by *Streptococcus pyogenes*. It is contagious and mainly frequent among toddlers.

Erysipelas: This is a deep and acute epidermal infection caused by *Streptococcus* species.

Cellulitis: This is a diffuse and severe inflammation of dermal and subcutaneous layers of the skin. It could be caused by exogenous bacteria (normal skin flora), where there are insect bites, burns, blisters, sites of intravenous drug injection or catheter insertion, and surgical wounds. It commonly occurs on the face or lower legs, although it can affect any part of the body.

Bacterial skin infections (or pyoderma) cause irritation and some discomfort. In some cases, the infections penetrate deep down through the epidermis, causing a necrotic ulcer, a condition known as ecthyma (Hay *et al*., 2006).

2.2.3 Fungal skin infections

The infection of the skin, hair and nail by colonization of the keratinized layers of the body is termed dermatomycoses. Infections are caused by the microorganisms in the genera – *Microsporum*, *Trichophyton* and *Epidermophyton*, which belong to anamorphic (asexual or imperfect fungi) with about forty species. Moreover, the genus *Candida*, and non-dermatophytic moulds in the genera – *Scopulariopsis*, *Fusarium* and *Aspergillus*, may also cause infections. *Trichophyton* and *Microsporum* are anthropophilic and zoophilic pathogens, while *Epidermophyton* (*E. floccosum*) is only an anthropophilic pathogen (Farahmand *et al*., 2016).

In many parts of Africa, tinea capitis is a commom condition affecting more than thirty percent of children in primary schools. Also, *Trichophyton tonsurans*, the form of tinea capitis endemic in the United States and in parts of Europe, such as France and the United Kingdom, is extremely resistant to treatment (Hay *et al*., 2006).

2.2.4 Associated consequences of skin infections

Skin diseases were associated with mortality rates of 20,000 people in Sub-Saharan African in 2001 (Mathers *et al*., 2006). Also, in 2010, dermatophytes affected about one billion people globally (Vos, 2012). Intolerable itching and disfigurement have severe consequences on the skin. Inflammation of the skin can lead to inflammation of other tissues and organs, and eventually certain diseases. Moreover, the impairment of joints and internal organs may result to mental illness causing severe depression, social isolation, and even suicide (British Association of Dermatologists, 2015).

2.3 *Dacryodes edulis*

Dacryodes: *Dakruon* (Greek) means tear, while *edulis* means edible.

Common names: African pear tree, African plum tree, African palm, bush butter tree, butter fruit tree, native pear (English), eben tree (U.S.A.), safoutier, prunier, atanga (French).

Local names: Ube (Igbo), elemi/eleme (Yoruba), bir (Hausa).

Some local names in Yoruba land: Ibeni (Igbodu), akor (Ijebu Ijesha), orunmwun (Ijagba), oro (Okeisa), mawe (Iperindo).

Synonyms: *Pachylobus saphu* (Engl.), *Pachylobus edulis* G. Don.

Origin: Africa

Habit: Tree

(Orwa *et al*., 2009; Omonhinmin, 2012)

2.3.1 Scientific classification of *Dacryodes edulis*

Kingdom: Plantae

Sub-kingdom: Tracheobionta

Super-division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Sapindales

Family: Burseraceae

Genus: *Dacryodes*

Species: *Dacryodes edulis*

2.3.2 Taxonomic description of *Dacryodes edulis*

Dacryodes edulis is a medium-sized, evergreen tree that normally branched from lower part, having a deep and crowded top. Its short bole (50 - 170 cm in diameter) is slightly fluted and roughly sinuous; the rough, pale grey, scented bark produces a whitish resin. The tree lacks buttress roots and can reach a height of 12 m in plantations, but 18 - 40 m in the forest (Orwa *et al*., 2009).

2.3.2.1 Leaf, flower, fruit

Its leaf is compound, imparipinnate, having 5 - 8 pairs of leaflets; adaxial is glossy, and its pubescence disappears with maturity. The fragrant three-lobed flowers are subtended, conspicuous with caducous brown bracts approximately 5 mm across, trimerous (excluding the ovary), densely arranged, ferruginous, stellate-tomentose inflorescence; three brown sepals; three cream-yellow petals; six white stamens; six lobed disc, surrounding the two-celled, glabrous ovary; inflorescence axis (10 - 42 cm) is intensely grooved (Orwa *et al*., 2009).

The fruit is an ellipsoidal drupe, varying in dimension (4 - 12 x 3 - 6 cm), and resembles olive. At maturity, its pink, thin exocarp becomes dark blue to violet; the pulp is thin and firm. The

leathery, shelled stone is bound by a mild turpentine smelling pulpy, butyraceous pericarp (approximately 5 mm thick) (Orwa *et al*., 2009).

The two varieties of *D. edulis* in Nigeria are differentiated by their fruits. *Dacryodes edulis var. parvicarpa* is small, round and roughly conical, generally below 5 x 2.5 cm; the thin fruit pulp is approximately 2 - 3.5 mm; its tree frequently has bifurcated branching with slender and drooping branchlets. *Dacryodes edulis var. edulis* is big, elongated and cylindrical, frequently above 5 x 2.5 cm; the thick fruit pulp is approximately 3.5 - 9 mm; its tree usually has whorled branching with plump and rising branchlets. Moreover, *D. e. var. edulis* has lower proximate water content, more oil content and more stable than *D. e. var. parvicarpa*. However, the fruit variety among the Igbos showed that *D. edulis* possesses a greater degree of distinct accessions (up to seven fruit types) than the two varieties (Orwa *et al*., 2009; Omonhinmin, 2012).

2.3.3 Ecology of *Dacryodes edulis*

2.3.3.1 Origin, distribution and habitat requirements

Dacryodes edulis is believed to have originated from Southern Nigeria and was introduced to the Western Nigeria (Omonhinmin, 2014). It is planted in Democratic Republic of Congo, Cameroon and Southern Nigeria, because of its nourishing fruit that is rich in oil. Moreover, it is now commonly spread in Central and West Africa. It is a shade-loving plant of non-flooded forests in moist tropical regions; that is, it is only established in gallery forests and on marshy areas, where there is a well-marked season. It adapts well to fluctuations in temperature, rainfall, humidity, soil type, day length and altitude; but does not perform well in the very wet southern and very dry northern areas of Central and West Africa (Orwa *et al*., 2009; Omonhinmin, 2012; Omonhinmin, 2014).

2.3.3.2 Planting and reproduction

In orchards, the recommended spacing for its planting is 10 x 10 m and application of fertilizer or manure is only during planting; pruning as well as crop protection are not practised. The tree is either dioecious or hermaphroditic, that is, male trees can produce a restricted amount of female flowers, and therefore produce a few fruits. Latitude and genotype determine the flowering time and duration. Flowering occurs between January and April while main and minor fruiting seasons are between May and October, and between November and March respectively.

However, some trees flower before time while some trees flower belatedly and may blossom continually for many months. Bees are its common pollinators; the fruit is dispersed by man and animals (Orwa *et al*., 2009; Omonhinmin, 2012). Moreover, the physiological maturity stage of the fruits for optimum utilization is 17 - 21 weeks after fruit set.

2.3.3.3 Infestation of diseases and pests

The fruits of *Dacryodes edulis* are delicate, thus almost half of them are usually lost when gathered, owing to softening and spoiling. Fruit post harvest rotting is usually caused by *Botryodiplodia theobromae*, *Rhizopus stolonifer*, *Aspergillus niger*, and *Erwinia* bacterium: the first two accounting for eighty percent of the loss. Polyphagous fungi causing dieback of branches and leaf, dead spots with galls on leaves, together with fruit drop have been documented in Gabon (Noumi *et al.*, 2006).

Sylepta baltoata (caterpillar of pyralid moth) which causes burnt form of *D. edulis* leaves is the main significant pest in Congo. However in Cameroon, *Carpophilus* sp. (larva of nitidulid beetle) consumes the seed while the adult bores out of the fruit causing secondary infections that frequently leads to rot. Generally, most of the fruits on the tree are destroyed by birds (Orwa *et al*., 2009).

2.3.4 Food uses of *Dacryodes edulis*

The main value of the tree of *Dacryodes edulis* is its fruit, which is either eaten raw, boiled for about three minutes, fried or roasted over charcoal or in hot ashes. The fruits are frequently eaten with corn, yam, rice, cocoyam or bread, because they are very oily (contains approximately 48 percent edible oil), and also contains many amino acids and vitamins. The fruits of *D. edulis* are for household uses and also sold in neighborhoods, and occasionally in transnational trade centres (Orwa *et al*., 2009; Ajibesin *et al*., 2011a; Omonhinmin, 2012). The fruit pulp of *D. edulis* is locally used as spice in vegetable soup; also the pulverized seeds are added to soups as a condiment. Its fruits produce cooking oil that can go along as common domestic cooking oil (Omonhinmin, 2012; Omonhinmin, 2014).

Orwa *et al*. (2009) reported that *D. edulis* wood contains oil that is composed of fatty acids and their esters when extracted with petrol ether solvent, a tree can turn out 7 - 8 t/ha of oil. The yield and the richness of the oil (amino acids and triglycerides) from its fruit could augment available

oil for man (Omonhinmin, 2012). The oily seeds of *D. edulis* have dietary value which can be exploited as supplement feed for domestic animals. The kernel (approximaly 3.3 % proteins) is usually given to household livestock like goats and sheep (Orwa *et al*., 2009).

Powder from *D. edulis* fruit could be utilized directly as a partial alternative for butter in producing biscuits even without undergoing the expensive rigorous process of oil extraction, while the oil from the fruit displayed excellent physicochemical proprieties, showing that it could be a beneficial raw substance for lipid industry (Ondo-Azi *et al*., 2013).

2.3.5 Medicinal uses of *Dacryodes edulis*

The leaves, resin, fruits and bark of *Dacryodes edulis* are employed in the treatment of headache, fever and malaria. The leaf or bark decoction is also applicable for treating earache, toothache, gum problem, tonsillitis; as gargle or mouthwash (Ajibesin *et al*., 2008).

Okwu and Nnamdi (2008) reported that the leaves and bark of *D. edulis* are used as antihelmintics and astringent; also for treating skin diseases, wounds, diarrhea, dysentery, leprosy, elephantiasis, and for clearing pregnancy stretch mark. Moreover, its pulp oil, avocado pulp, palm kernel oil and spices are used as lotion for skin smoothening, while the pulped bark is for cicatrizing wounds and treating chiggers (Orwa *et al*., 2009).

The resin exudate of *D. edulis* is used alone or together with other oils (such as palm oil) and applied on the skin for treating ecto-parasitic infection (from ticks and chiggers), skin diseases (such as ringworm) and disorders (such as craw-craw); moreso, the leaf extract is efficacious when applied on bruises, cuts and wounds (Okwu and Nnamdi, 2008; Omonhinmin, 2012; Omonhinmin, 2014; Adeniji *et al*., 2018).

Omonhinmin (2012) reported that hot water infusion (tea) from dried, powdered seed of *D. edulis* is taken to combat stress. As reported, a decoction of boiled leaves is used for treating internal body heat, high fever and for muscles relaxation (Omonhinmin, 2014). Fruit oil, pulverized bark, or stem exudates of *D. edulis* in oil, are used for treating muscle pain or stiffness (such as cramps); additionally, its resin exudates is used separately or together with local oils for treating arthritis and stiffness (Omonhinmin, 2012).

Boiled roots or leaves of *D. edulis* singly or together with *Cymbopogon citratus* or *Parkia biglobosa* (bark or leaves), are used for treating hypertension or peptic ulcer. Boiled leaves or bark of *D. edulis* with *Pennisetum purpureum*, are used for treating malaria. Bark or leaves of *D. edulis* are boiled in pap water for treating epilepsy and retarded growth in children. Bark decoction of *D. edulis* is for treating anaemia or dysentery. Root or root bark extracts of *D. edulis* are used for treating leprosy (Omonhinmin, 2012; Omonhinmin, 2014).

Decoction from the entire fruit, pulp oil, and leaves of *D. edulis* are used for treating stomach disorder, constipation, digestive tract discomfort, heartburn, aches, and indigestion. The fruit is a mild antacid that aids digestion, but causes purging when consumed in excess. The root boiled with other herbs is taken orally for treating beriberi and rickets, while dried pulverized leaves, plant oils and some substances are mixed together to ease labor pains for safe delivery (Omonhinmin, 2012).

Orwa *et al*. (2009) explained that the bark decoction of *D. edulis* is utilized as gargle, mouthwash and in the treatment of tonsillitis in the Democratic Republic of Congo. Also, the bark decoction with *Aframomum melegueta* is for treating anaemia, blood spitting, and dysentery or as emmenagogue. The bark decoction is added to palm oil and topically used in relieving stiffness, general pains as well as treating cutaneous conditions. The root bark decoction is for treating leprosy, while the leaves are consumed raw with kola nut for anti-emetic action. Leaf decoction as a vapour bath is for combating fever with headache, and leaf sap is used for treating ear problems (Orwa *et al*., 2009). *Dacryodes edulis* is employed as a medication for tonsillitis and dermatitis in Gabon; the leaf decoction is utilized in treating malaria in Cameroon; while the wood charcoal is used for treating eye disorder in South-western Cameroon (Zofou *et al*., 2011).

2.3.6 Other uses of *Dacryodes edulis*

The tree is planted for shade in Nigeria and frequently seen as an ornamental garden tree in rural communities. Its anthesis gives attractive flowers, while the ovary produces sweet nectar that attracts bees and many insects, thus useful as a melliferous tree. It is intercropped with shade tolerant species (such as *Colocosia esculenta*, *Xanthosoma saggittifolium*) and also for perennial crops like coffee and cocoa in Nigeria (Orwa *et al*., 2009).

The tree symbolizes fruitfulness and peace to some people, thus its parts are used for religious and socio-cultural activities, such as marriage and naming ceremonies, settlement and communal festivals, worship of gods, and in expelling demons (Omonhinmin, 2012; Omonhinmin, 2014).

The wood of *D. edulis* is resilient, greyish white to pinkish; the sapwood and heartwood are not easy to demarcate, yet useful for tool handles, especially for axes; sometimes for mortars, and also fitting for woodwork, road and shelter construction – animal shelters, crop storage shelters (such as yam barns), huts and building shades (Orwa *et al*., 2009; Ajibesin *et al*., 2011a; Omonhinmin, 2012).

The dried trunk, branches and twigs of *D. edulis* serve as good firewood; its stem twigs are utilised as chewing sticks, while its leaves are used as wrapping materials for storage (Omonhinmin, 2012; Omonhinmin, 2014).

The leaves of *D. edulis* have a colorant; the leaves and the fruit remnants have been found to considerably improve soil fertility and thus, used by peasant farmers in south Cameroon for soil fertility (Orwa *et al*., 2009; Ajibesin, 2011; Omonhinmin, 2012).

The aromatic resin produced from bark of *D. edulis* when injured, is utilized as pitch on calabashes for patching up ceramic; serves as glue, cosmetic component, and as an ancient lamp oil or bush candle (Orwa *et al*., 2009; Omonhinmin, 2012). In addition, the small relative density of its fruit oil, signifies excellent ignition and burning quality (Tee *et al*., 2014).

2.4 *Anacardium occidentale*

Anacardium (Greek: *ana* means without; *cardium* means heart) means the seed is outside the center of the fruit; *occidentale* means of or from the West.

Common names: Cashew nut tree, cashew (English), Anacardier, cajou (French).

Local names in Nigeria: Kaju (Yoruba), sashu (Igbo), kanju (Hausa).

Synonyms: *Acajuba occidentalis* (L.) Gaertn., *Anacardium microcarpum* Ducke, *Cassuvium pomiferum* Lam.

Origin: Brazil

Habit: Tree

(Orwa *et al*., 2009)

2.4.1 Scientific classification of *Anacardium occidentale*

Kingdom: Plantae

Sub-kingdom: Tracheobionta

Super-division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Sapindales

Family: Anacardiaceae

Genus: *Anacardium*

Species: *Anacardium occidentale*

2.4.2 Taxonomical description of *Anacardium occidentale*

Anacardium occidentale is a spreading, low-branched, evergreen (perennial), medium-sized tree that is majorly cultivated for its nut and pseudo fruit. It grows to a height of 6 - 12 m; the crown has a globose shape, ranging 6 - 15 m large in diameter based on soil conditions; the tree is deeply tap rooted and develops many lateral roots that allows it to survive during dry periods; its wood is moderately hard with 500 kg/cm as density (Orwa *et al*., 2009).

2.4.2.1 Leaf, flower and fruit

Anacardium occidentale has simple leathery leaves with obvious pinnate venation, smooth margins, oval-obovate in shape (10 - 20 x 5 - 10 cm), clustered at terminals, alternately arranged, pale green or reddish when young, and dark green at maturity. Its inflorescences (15 - 25 cm) are terminal panicles of male, female and bisexual flowers. Flowers are pentamerous (1 cm diameter at most); yellowish pink, highly scented and inviting to bees, hence it provides an excellent source of honey. After flowering, the fruits mature in three months (Orwa *et al*., 2009).

The kidney-shaped nut (true fruit) of *A. occidentale* has a double-walled shell (pericarp), consisting of a hard epicarp, a toxic mesocarp, a slim endocarp and an edible kernel enclosed with a thin testa. The nut never cracks at maturity; when fully grown, but not ripe. Its receptacle enlarges and develops into a very conspicuous plump, succulent, apple or pear-shaped edible cashew apple with a yellow, red, or red plus yellow colour (Orwa *et al*., 2009).

2.4.3 Ecology of *Anacardium occidentale*

2.4.3.1 Origin, distribution and habitat requirements

It is originally indigenous to northeastern Brazil. Some Portuguese took it to Goa, India, around 1560 to 1565 and then scattered all over Southeast Asia and finally Africa. *Anacardium occidentale* tree is now widely cultivated in Vietnam, Nigeria, India, Brazil, and Indonesia as major production countries; the main importers of cashews are the United States, Canada, Hong Kong, Singapore, Japan, Australia and some Middle Eastern nations. The largest cashew tree (Pirangi Cashew Tree in Brazil) covers a whole park across 8,500 m^2; main stem has five branches, approximated as the size of eighty common *A. occidentale* trees and turns out about eighty thousand fruits in a season (FAO, 2013).

Some Portuguese traders introduced *A. occidentale* into Nigeria around 16^{th} century, and was first planted in Agege, Lagos State, where it started spreading to different areas of the country, through seed transfer; only the apple was majorly utilized without attaching commercial value to the nut and this lasted for over four hundred years. However, cashew now presents continual prospect for investment and huge prospective for economic growth (Adeigbe *et al*., 2015).

The tree of this plant has acclimatized to different climatic areas around the globe, between the latitudes of 25 ^{o}N and 25 ^{o}S and requires high temperatures (mean annual temperature: 17 - 38 ^{o}C) while frost is deleterious. *Anacardium occidentale* has a preference for deep, fertile, loamy or sandy soils (pH of 4.5 - 6.5), although can grow up well on nearly all soils, except on impermeable clays or poorly drained soils that is subjected to episodic flooding. Moreover, it requires altitude 0 - 1000 m and mean annual rainfall 500 - 3,500 mm (Orwa *et al*., 2009).

2.4.3.2 Dispersal and reproduction

The seeds of *A. occidentale* are spread by bats, wild birds, and some animals that eat the cashew apples. Bats, bees, flies and ants and wind perform pollination. However, bees cause greater pollination, since they are attracted to its scented flowers and sticky pollen grains. The trees reproduce sexually with seeds, and also asexually through vegetative propagation (side, softwood and wedge graftings; ground and air layerings, top-working, chip-budding, and cutting) (Orwa *et al*., 2009).

The tree of *A. occidentale* usually takes like three years before it starts producing, but it begins economic harvests after eight years. However, some new breeds like the dwarf cashew trees (about 6 m), begin production the second year, and economic yields by the fourth year. Cashew is frequently intercropped with quick-growing crops, such as vegetables, legumes, chilies, peanuts, cotton, or tobacco within the first three years. A single tree may produce 200 - 300 fruits per annum, and keep on producing for twenty years or more; and it may survive for 50 - 60 years (Azam-Ali and Judge, 2006).

2.4.3.3 Diseases and pests

Orwa *et al*. (2009) reported that *A. occidentale* is vulnerable to more than sixty identified species of insect pests throughout the phases of its development. Few of its numerous insect pests in Nigeria are: *Analeptes trifasciata* (stem girdler), *Selenothrips rubrocinctus* (red-banded thrips), *Plocaecderus ferrugineus* L. (stem and root borer), and *Pachnoda cordata* (fruit scrapper).

2.4.4 Food, beverage and cosmetic uses of *Anacardium occidentale*

The immature leaves and shoots are consumed fresh or cooked. The nuts are popular snacks, food sources, while the apple is processed as jellies, juices, syrups, and as intoxicating beverages like wine, brandy, and gin. Moreover, cashew kernels and apples are commonly employed in candy in China, Thailand, and India. The edible oil (similar to olive oil) produced from the kernels is used in cooking. The apples are utilized in body care products like lotions, shampoos and anti-aging creams (Marc *et al*., 2011).

2.4.5 Medicinal uses of *Anacardium occidentale*

Anacardium occidentale are used in treating cough, bronchitis, intestinal colic, diarrhea, diabetes, and as common tonic by the Northern American healers. The leaves are utilized in treating gum challenges and toothaches, while the buds and immature leaves are used for treating skin diseases in African herbal medicine and Ayurveda (Orwa *et al*., 2009).

The leaf of *A. occidentale* is used for treating diarrhea, while the bark as a vaginal douche in Peru. Its immature leaves are used for treating hemorrhoids, diarrhea, and dysentery, while mature leaves are used as hot poultices for burns and skin disorders in the Philippines. The leaves and bark of *A. occidentale* have amebicidal, antioxidant, antivenom, and astringent

properties, and hence employed in the treatment of inflammation, gingivitis, syphilitic ulcers, and diarrhea (Orwa *et al*., 2009).

In Ayurveda, the bark of *A. occidentale* is used as a laxative to purge intestinal parasites, for treating fevers, diabetes, and detoxifing snake bite. The bark is for treating toothache as well as gum inflammations, while the leaves are used for treating toothaches, sore throat, and as a febrifuge in Brazil and Nigeria. The bark, stem and leaf are extensively used in treating dysentery, diarrhea, and colonic pain. The leaf is also used in treating malaria, while the seeds are used for treating diabetes in Malaysia (Mahendran *et al*., 2014; Adeniji *et al*., 2018).

The leaf decoction of *A. occidentale*, is utilized in treating diarrhea and diabetes in Venezuela, but is known for treating amenorrhea and dysentery in Haiti. Its pulverized bark is used for treating diabetes in Colombia, and its resin is used for cold treatments in Cuba. Its fruit juice is utilized as a diuretic, a therapy for vomiting, diarrhea, and sore throat, while the gum is applied to fungal infections, corns, and leprosy in Brazil. Moreso, the leaves are used in Brazil for treating eczema, psoriasis, dyspepsia, scrofula, cough, bronchitis, intestinal colic, leishmaniasis, syphilis-related skin disorders, impotence, genital and venereal diseases (Rajesh *et al*., 2015).

The caustic shell oil and fruit of *A. occidentale* are utilized as cauterizing agents and skin stimulants. Its oil is carefully utilized in eradicating warts, corns, ringworm, psoriasis, ulcers, elephantitis, and leprosy; and also for treating gastrointestinal disorder in Brazil and Nigeria.

The seeds are used as antidote for snakebites; the nut oil is applied to cracked heels; the fruit, bark and leaf are used as an anti-pyretic, anti-fungal, anti-diarrheal agent, and in treating rashes and sores. Additionally, the resin from its seeds is used for treating palpitations, rheumatism, and mental derangement. It was used to restore memory loss caused by smallpox (Orwa *et al*., 2009).

2.4.6 Fodder uses of *Anacardium occidentale*

Cashew nut testa was mixed with "dusa" (leftover from sorghum in alcohol production) in Nigeria to replace groundnut meal for pigs. Moreover, the seed coats are utilized as poultry feed; the kernels (about 30 - 40 %) are discarded during the process of roasting, and the residues of cashew apple after juice extraction (peels, fibrous pulp) are fed to livestock, particularly monogastric animals like pigs (Orwa *et al*., 2009).

2.4.7 Other uses of *Anacardium occidentale*

The bark of *A. occidentale* has an acerbic sap (3 - 5 % tannin) of deep brown resin (which changes to black when exposed to air), which is utilized as permanent ink in cottons and linens, as a varnish, an additive for fishnets, and a flux for solder metals. Also, the stem produces an amber-coloured gum (partially dissolved in water and swells into a jelly-like mass), which is useful as an adhesive (for book binding, plywood, woodwork panels), since it partly possesses insecticidal property (Orwa *et al*., 2009).

2.5 Assays and bioassays of medicinal plants

An assay is a chemical test carried out to determine the composition of a substance or the concentration of its components, while bioassay is a technique used to determine the concentration or effectiveness of a material such as a drug, by measuring its consequence on an organism. Assay and bioassay of medicinal plants in search for novel drugs, for improved and effective treatment of a number of diseases, are critical to human and animal survival. However, only infinitesimal percentages out of two hundred and fifty thousand species of higher plants in the world have been effectively studied for their drug potentials (Dubey *et al*., 2004).

2.5.1 Free radicals and oxidative stress

Atoms with an unpaired (odd) number of electrons are called free radicals. Any chemical reaction that generates free radicals, producing chain reactions that can impair cells is called oxidation. Oxidative stress arises when free radicals overpower the body's capability to control them. Equilibrium between free radicals and antioxidants is necessary for normal physiological function (Lobo *et al*., 2010).

2.5.1.1 Antioxidants

Halliwell (1995) defined antioxidant as a molecule that is sufficiently stable to donate an electron to a free radical and neutralize it, consequently reducing its damaging ability. It delays or inhibits cellular injury mostly by its free radical scavenging capability.

Antioxidants could exert their effect on biological systems through diverse means, such as electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation. Generally, free radical scavengers either prevent the reactive species from being produced, or eliminate them before they harm very important components of the cell (Sies, 1997).

Antioxidants are mainly grouped into two, based on their solublility in water (that is, hydrophilic) or in lipids (that is, lipophilic). Generally, hydrophilic antioxidants (such as ascorbic acid, lipoic acid, uric acid, glutathione) react with oxidants in the cell cytosol and the blood plasma, whereas lipophilic antioxidants (such as carotenes, α-tocopherol, ubiquinol) prevent cell membranes from lipid peroxidation (Sies, 1997).

2.5.2 Phytochemicals

Phytochemicals (plant chemicals) are biologically active, naturally occurring chemical compounds in plants which are not straightforwardly linked with plant's natural growth, development or reproduction. They play a main function in a plant's defense coordination and its environmental adaptation. They are useful to humans as medicines, and flavorings (Ogboru *et al*., 2015).

Phytochemicals (secondary metabolites) are principally grouped into three: phenolics, alkaloids and terpenes. However, they can be categorized by their chemical configuration (such as those with rings, or sugar), composition (such as those with or without nitrogen), solubility in different solvents, or the pathway by which they are produced (such as phenyl propanoid synthesizes tannins). Moreover, by their biosynthetic sources, they can be grouped mainly into three: (i) terpenoids, (ii) alkaloids with nitrogen and compounds with sulphur, (iii) flavonoids, related phenolics and polyphenolics (Tee *et al*., 2014).

2.6 Topical medication

Topical drug delivery system is a system of medication, in which drug substances are administered to the skin for local action, and the active components remain on the skin surface, or infiltrate the epidermal layers, and may get to the dermis, but not into the blood circulatory system. Contrarily, transdermal drug delivery system (transdermal patches) is systemic, because the drug traverses through the different layers of the skin to deliver the active ingredient(s) into the general blood circulation by different mechanisms, based on the composition and method of fabrication (Murthy and Shivakumar, 2010).

Topical drugs are applied to a specific site on the outer surface of the body to treat local dermatological conditions. The absorption takes place through sweat glands, hair follicles, sebaceous gland, and the stratum corneum (Gidwani *et al*., 2010).

Kranthi *et al*. (2011) highlighted some of the factors affecting topical permeation based on the physicochemical properties of drug materials as: particle size, partition coefficient, pH, drug solubility, polymorphism, concentration, and molecular weight.

2.6.1 Advantages of topical medication

Some of the advantages of topical medication over other methods of drug administration as reported by Srivastava (2006), Moody (2010), and Chen (2014) are:

1. It has minimal systemic side effects when applied to the affected tissues, because the drug does not reach the systemic circulation.
2. It avoids drug metabolism before reaching the target site of action (the skin).
3. It is easy to apply and suitable for self-medication, therefore increases patient's compliance.
4. It does not need complicated application procedures, thus circumvents the risks and inconveniences of intravenous drug delivery.
5. It provides a relatively large area of application (since the skin is the largest surface area on the human body), and has the capability to release drug more selectively to a particular area.
6. It can retain a high local concentration of drug in the adjoining tissues above an increased time, thus reducing dosing frequency.

2.6.2 Disadvantages of topical medication

Some of the disadvantages of topical medication as reported by Moody (2010), and Chen (2014) are:

1. There could be skin irritation (allergic reactions), because of drugs or excipients.
2. The challenge of poor permeability through the skin for some medications.
3. They are not suitable for drugs with large particle size.
4. There could be denaturation of drugs, due to the enzymes found in the epidermis.

2.6.3 Categories of topical medication

There are three categories of topical dosage forms. They are: liquid topical dosages, which are solutions, suspensions, and lotions; semi-solid topical dosages, which are foams, collodions, pastes, gels, ointments, and creams; solid topical dosages, which are patches, powders, gauzes, sticks and tapes (Murthy and Shivakumar, 2010; Chen, 2014).

2.6.4 Differences between a lotion, a cream, an ointment and a gel

A lotion is defined as a low viscous topical preparation purposed for application to an intact skin, while gels and creams contain higher viscosities. Creams are non-greasy viscous system with dense form, while ointments are more viscous and translucent. Also, creams have more than forty percent water plus volatiles, while ointments have less than twenty percent water plus volatile materials. Moreover, creams are suited for discharging lesions, while ointments are appropriate for chronic, dry wounds and contraindicated in oozing injuries (Reuter *et al*., 2010).

Every topical dosage type, based on the area of application and curative require, offers special features. However, creams are frequently chosen instead of other topical preparations, because they are more appealing and easier in application. Moreover, creams are suitable for people with tender or dry skins that need a less sensitive, damp formulation (Kranthi *et al*., 2011).

2.6.5 Pharmaceutical creams

Pharmaceutical creams comprise medicaments dissolved or suspended in water or emollient bases. Combination of unmixable compounds is achievable by mechanical shaking or heating, thus creams are classified as water-in-oil (W/O) or oil-in-water (O/W). Lately, creams have been limited to formulations having oil-in-water emulsions or aqueous microcrystalline dispersions of long chain fatty acids or alcohols that are water removable and more cosmetically and aesthetically satisfactory (Kranthi *et al*., 2011; Ajala *et al*., 2016).

2.6.5.1 Types of creams

2.6.5.2 Oil-in-water creams

Oil-in-water creams are small droplets of oil dispersed in a continuous aqueous phase. An example of oil-in-water cream is cold cream, which has four main ingredients: water, oil, emulsifier, and thickening agent. They have small amount of oil, and are easily removed by water (Kranthi *et al*., 2011).

2.6.5.3 Water-in-oil creams

Water-in-oil creams contain small droplets of water dispersed in a continuous oily phase. An example of water-in-oil cream is fluocinolone acetonide cream. They are not easy to deal with, although several drugs that are integrated into creams are hydrophobic, and they will be readily released from a water-in-oil cream than an oil-in-water cream. They are also more moisturising,

as they give a greasy barrier that decreases water evaporation from the skin epidermal stratum (Kranthi *et al*., 2011). Some generally accessible creams on the basis of their purpose are classified as vanishing and foundation cream, cleansing and cold cream (lotion), hand and body cream, night and massage cream, moisturizing cream, and all purpose cream (Kranthi *et al*., 2011).

2.6.5.4 Uses of creams

Cream penetrates well the outmost layer of skin (stratum corneum), and is useful in providing a barrier (physical or chemical barrier as with sunscreens) in protecting skin. They help in retaining moisture (particularly, w/o creams) in skin, sanitizing skin, soothing skin, treating sun burns, and also act as a medium for drug materials, such as local anaethetics, antibiotics, anti-inflammatories, and hormones (Kranthi *et al*., 2011).

2.6.6 Herbal topical preparations

Herbal medications for skin care with antimicrobial activities have been prepared from a diversity of flora components (fruits, leaves, stems, roots, barks), and administered topically as solvent extracts, lotions, ointments, creams, gels, or soaps (Ajala *et al*., 2016). Herbal cosmetics are products that possess pleasing physiological actions, such as smoothing and enhancing looks, and healing features. The incorporation of medicinal plant materials into the modern pharmaceutical dosage forms is gaining much importance. The usage of two or more herbs in formulations is known as polyherbal formulation (Rajasree *et al*., 2012).

The demand for herbal topical formulations is increasing rapidly. This could be that plant parts used for topical preparations contain mixture of attributes such as; emollient, antioxidant, antiseptic, anti-inflammatory, anti-seborrhatic, and anti-kerolytic activities among others. Moreso, herbal topical preparations are advantageous for being effective, non-toxic in nature or having reduced allergic responses (Yadav and Yadav, 2015).

2.6.6.1 Some formulated herbal creams

Herbal creams, which incorporate medicinal plants into cream formulations for particular desirable physiological activities, have been attempted by many researchers. Soisuwan *et al*. (2010) created an anti-wrinkle cream from ethanol extracts of *Caesalpinia pulcherrima* orange petals, and its quality evaluation was assessed by using heating–cooling cycle's method. The

results showed unaffected physical features; no irritation was seen on the twenty-one volunteer's skins, thus concluded that the product has the efficacy of enhancing the elasticity of the skin.

Vinod *et al*. (2011) extracted piperine from black pepper with ethanol. The piperine cream was formulated for treating Vitiligo using bees wax as base. The Thin Layer Chromatography (TLC), Scanning Electron Microscopic studies (SEM), irritancy test, organoleptic properties among others were studied. The alkaloid showed violet colour under UV radiation, the Rf value was 0.26 for TLC, globule size range was 4.72 - 52.72 μm, moisture absorption was insignificant, pH was 6.5; no redness, edema nor irritation on rabbits. It has a smooth texture, consistent, light yellow colour, peppermint odour and was physically stable throughout the shelf life.

Kale *et al*. (2011) formed sunscreen cream from lutein ester extracted from *Tagetes erecta* flowers by petroleum ether, and investigated the Sun Protection Factor (SPF) by COLIPA method. The cream showed less activity (SPF = 1.08 ± 0.02) with Boot Star Rating 4, which is close to suitable sunscreen action. It was concluded that the cream possessed fine sunscreen activity, and could be integrated into other sunscreen preparations for activity enhancement.

Verma *et al*. (2011) formulated polyherbal depilatory cream from *Prosopis cineraria* leaf, *Zingiber officinale* extract, *Citrus limon* juice, *Allium sativum* extract and *Azadirachta indica* leaf. They evaluated the physical properties such as particle size, viscosity, pH, adhesion force, and depilatory activity of the cream. The result showed that the cream had acceptable physicochemical properties. In addition, the weight ratio of depilated hair of mice was 200 % of depilatory cream when compared with the untreated mice. The cream also reduced the amount of depilated hair, and impaired the quality of undesired hair, thus suggested that the depilatory cream has depilatory effects.

Nair *et al*. (2012b) produced herbal cream from *Curcuma longa*, after testing the antioxidant property of the extract, and evaluated the stability on many factors such as visual form, pH, nature, fragrance, viscosity for two months; and also examined for the existence of pathogenic microbes on it. The two formulations (F1 and F2) showed good spreadability 12.78 g cm/sec and 10.67 g cm/sec with pH of 5.8 and 6.2 respectively, good consistency without phase separation, no microbial growth, and then concluded that it could be developed for skin protection.

Manimaran *et al*. (2014) formulated an antimicrobial and wound healing topical herbal creams from aqueous extracts of *Cissus quadrangularis*, *Eupatorium glandulosum*, and assorted extracts of the two botanicals. The creams were screened for some physicochemical properties, as well as for antifungal and antibacterial activities, using Amphotericin B and Gentamycin as control drugs respectively. Also, the wound healing experiment on rats was conducted with Nitrofurazone (0.2 % w/w) cream as a control drug. It was reported that the formulated topical herbal cream gave significant antimicrobial activity. Moreover, *E. glandulosum* cream (10 %) was significantly higher than the control in wound healing experiment on rats.

Pavitha and Poornima (2014) created herbal repellent (incense log and cream) from *Tagetes erecta* flowers, and *Callistemon brachyandrus* leaves. They reported that the repellent activities investigated for the cream gave 87.5, 89.87 and 90 % safety, while smoke toxicity experiment for incense log gave 70, 66.25 and 67.5 % safety against *Culex infulus*, *Anopheles stephensi*, *and Aedes aegypti*, respectively.

Matangi *et al*. (2014) formed an anti-aging polyherbal cream from *Punica granatum* leaves, neem oil, jamul powder, and carrot powder with different concentrations (F1 - F4); and tested the pH, viscosity, homogeneity, texture, removal, irritancy test, appearance, microbial test, spreadability and stability. It was reported that F3 and F4 were especially safe and stable for about one year.

Laxmi and Pranita (2014) produced four multipurpose herbal creams A – F (varying the proportion of all the ingredients of herbal origin) from *Aloe vera*, *Carica papaya*, *Phyllantus emblica*, *Azadiractha indica*, *Ocimum santum*, and *Curcuma longa*. Their physicochemical parameters such as appearance, type of emulsion, stability of colour, and odour, extrudability, pH, texture, feeling upon application, particulate contamination, and spreadability were evaluated. They all have cooling effect after application and good extrudability. However, cream C (orange in colour, stable after two weeks, pH of 6.4, smooth texture, and very good spreadability) was the best with regards to its use and demonstration of better product stability. Likewise, Alobaidi *et al*. (2014) formulated the alcohol extract of *Glycyrrhiza glabra* root. The formulation and base were stored at different accelerated environment (8 ^{o}C, 25 ^{o}C, 30 ^{o}C, 40 ^{o}C and 40 ^{o}C + 75 % RH) for a month to foresee the cream stability. They concluded that the cream

was physically and chemically stable over the experimented storage conditions, and there was no contact dermatitis.

Trailokya *et al.* (2014) created and evaluated herbal cream from *Calendula officinalis*, *Arnica montana*, *Aloe vera*, *Clerodendrum indicum*, *Panax ginseng*, and *Rosa canina* for wound healing activity. The stability was ascertained by storing the cream at different temperatures (4 ^{o}C, 27 ^{o}C and 37 ^{o}C) for a particular time. The pH was 6.6, spreadability was 16.17 gcm/sec, viscosity was 17,650 cP with 25 rpm, and there was no skin irritation, erythema or edema on the tested rats. Moreover, the standard cream (Povidone-Iodine/Betadine), and formulated cream significantly ($p < 0.01$) reduced the injury regions when compared with the untreated on 18th day of application, thus showed that the formulation has wound healing ability.

Majekodunmi and Nubani (2014) formed creams for treating microbial skin infections from *Acalypha wilkesiana* ethanol leaf extract. Initially, the extract was tested against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Candida albicans*, using Streptomycin and Nystatin as standard drugs for the bacteria and fungus, respectively. The extract was incorporated into cetomacrogol cream, zinc cream, and emulsifying wax ointment, and each was tested on the microbes. The cetomacrogol cream showed better antimicrobial activity than the zinc cream, and emulsifying wax ointment. It was concluded that the cream might be effective in treating some skin infections.

Yadav and Yadav (2015) formulated and evaluated a polyherbal facial powder from *Rubia cordifolia*, *Santalum album*, *Symplocos racemosa*, and Calcium bentonite. The particle size of the powder was 20 - 25 μm, angle of repose ($16^{o} \pm 1.05^{o}$), bulk density (0.436 g/cc) and tapped density (0.413 g/cc). It was concluded that the herbal face pack has good properties as a cosmetic product.

Ajala *et al*. (2016) produced herbal cream and ointment from *Phyllanthus amarus*, and evaluated their physicochemical, safety, and antimicrobial properties. The pH of the formulations was acidic (3.61 - 6.04), while the viscosities for ointments and creams were 1250 - 4950 and 570 - 1233 cP, respectively. The irritation index on rabbits ranged from 0.06 - 0.13 for creams and ointments, while there were no visible lesions from the toxicity experiment after three weeks. The antimicrobial activity for the formulations was significantly higher ($p < 0.001$) than *P.*

amarus extract, but the cream antimicrobial activity was higher than the ointment. It was concluded that the formulations might be helpful in treating some skin infections.

Soje *et al*. (2016) formulated the methanol extract of *Allium cepa* bulb into an herbal cream, using fusion method with hard paraffin as a base. The extract and the creams were tested under different storage conditions of 5 ^{o}C, 25 ^{o}C and 45 ^{o}C, in order to determine the reliability of the formulation process. Physical appearance, enumeration of microbial count, odour, texture, pH, spreadability, and nature of the cream were evaluated. The creams stored at room temperature (25 ^{o}C) were within acceptable range for quality, while those stored at extreme temperatures (5 ^{o}C and 45 ^{o}C) had values outside the normal range (in terms of change in physical appearance, poor spreadability, and altered nature). The study concluded that fusion method was suitable for the formulation.

Zibaee *et al*. (2016) produced creams from essential oils of *Matricaria chamomilla* and *Rosmarinus officinalis*, and tested their repellency against *Paederus fuscipes*, *Anopheles stephensi*, and *Culex pipiens*. This was compared with N, N-diethyl-meta-toluamide (a chemical repellent). Cream IV gave repellency (91 %) against *P. fuscipes*, which was close to the value for the synthetic repellent. The formulation could therefore be utilized in making a useful natural repellent, and as a substitute to the chemical ones.

Kumari *et al*. (2016) formed herbal vanishing creams containing seeds and peel of *Punica granatum* fruit with olive oil and almond oil. They evaluated the colour, odour, pH, homogeneity, viscosity, type of smear, texture, dye test, spreadability, patch test, stability, and skin irritation on rat and human skins. The pH was 5.6 - 6.8, while viscosity was 12.01 - 13.12 cP. There were no erythema and hypersensitivity on the rats' skins; and also, no redness or edema was observed on the human skin. The study concluded that the creams had acceptable safety profile.

Mirela *et al*. (2016) formulated and evaluated an anti-acne cream from alcohol extracts of *Saponaria officinalis*, *Rosmarinus officinalis*, *Rosa centifolia*, and *Thymus vulgaris*. The antimicrobial activity of the extracts was tested on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The activity of sebaceous glands on the mid foreheads, cheeks, and chins was assessed on human volunteers. The results were statistically significant and revealed the efficacy

of the tested cream, which reduced the seborrhoea after four weeks of cream application. It was concluded that it has excellent cosmetic properties, and very good efficacy in decreasing the sebum secretion levels, and could be helpful for the treatment of acne.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

The city of Ibadan, covering a total area of 3,080 Km^2, the Capital of Oyo State in south-western Nigeria, is situated within Latitude 7°23'47" N and Longitude 3°55'0" E. It is an ancient city rich in Yoruba culture. It is the largest city in Nigeria and West Africa. It is third most populous city (over 3 million people) in Nigeria and second in African continent. It is totally within the tropical forest zone with mean total rainfall of 1420.06 mm, mean maximum and minimum temperatures of 26.46 °C and 21.42 °C respectively, and relative humidity of 74.55 % (Demographia, 2015). Figure 3.1 shows the location of the study area.

3.2 Ethnobotanical survey of *Dacryodes edulis* and *Anacardium occidentale* in Ibadan

Ethnomedicinal survey, following the approach of Oladunmoye and Kehinde (2011) was conducted, using two hundred structured questionnaires that were randomly administered to people living in Ibadan, so as to know about their medicinal knowledge of *D. edulis* and *A. occidentale*. Moreoso, in view of the low educational level of herbsellers at Bode market in Ibadan, the questionnaire was used as an interview guide and to really know about the preparations of the plants for various treatments. The branch containing leaves and fruits of *D. edulis* were shown to the herb sellers for proper recognition, and the prices of the two plants were also enquired. The sample of the questionnaire, pictures of the plant parts, apparatus used and activities during the research are in the appendixes I and II.

3.3 Collection and preparation of plant samples

Dacryodes edulis leaves were collected in February, 2015 behind house 13, Abadina quarters, University of Ibadan, while *Anacardium occidentale* leaves were collected beside Faculty of Veterinary Medicine, University of Ibadan. They were authenticated with voucher specimen numbers: **UIH 22488** and **UIH 22489** respectively in the University of Ibadan Herbarium (UIH), Ibadan. The leaves were thoroughly washed with clean water and then spread to air dry at the Department of Botany, University of Ibadan. Two weeks after, the air dried samples were ground

with a milling machine (Crusher, British Jeffrey-Diamond Ltd, Sternnard Works Wakefield, England) at the Department of Chemistry of the same institution.

3.3.1 Methanol extraction of the plant

The pulverized sample (4 kg) was divided into eight, each sample was put inside a 5 L round bottom flask, and 1.5 L of analytical grade methanol (BDH, England) was added to each flask, corked and left for 48 hours. The liquid extract was decanted and filtered with Whatman filter paper No. 1; the filtrate was kept in a refrigerator for a week at the Department of Chemistry, University of Ibadan. It was concentrated with rotary evaporator (Heidolph Laborota 4000 efficient, 517-01002-00-2, Germany) at the Department of Pharmaceutical Chemistry, University of Ibadan; dried to solid extract with freeze drying machine (Gallenkamp, UK) at the Department of Pharmacognosy, University of Ibadan.

3.3.2 Fractionation of each plant

The methanol extract was successively partitioned into fractions using analytical grade solvents: n-hexane, ethyl acetate, butanol and distilled water. Each fraction was concentrated with rotary evaporator and dried to solid extract using freeze drying machine.

3.4 Antioxidant tests

3.4.1 1, 1 diphenyl-2-picrylhydrazyl radical scavenging method

One milliliter of 0.3 mM 1, 1 diphenyl-2-picrylhydrazyl (DPPH) in methanol was added to 1 mL of different concentrations (0.5, 0.25, 0.125, 0.0625, 0.03125 mg/mL) of each plant extract and a sample ascorbic acid (standard) in test tubes; mixed together, incubated in the dark for 30 min, and absorbance was spectrophotometrically measured at 517 nm. The DPPH in methanol (1 mL) served as a control. All the tests were carried out in triplicates and the percentage inhibitions (% I) were calculated from the means of the absorbance values (Brand *et al*., 1995).

$\% I = [(A_{control} - A_{sample}) / A_{control}] \times 100$

$A_{control}$ is the absorbance of the control.

A_{sample} is the absorbance of the plant extract or ascorbic acid.

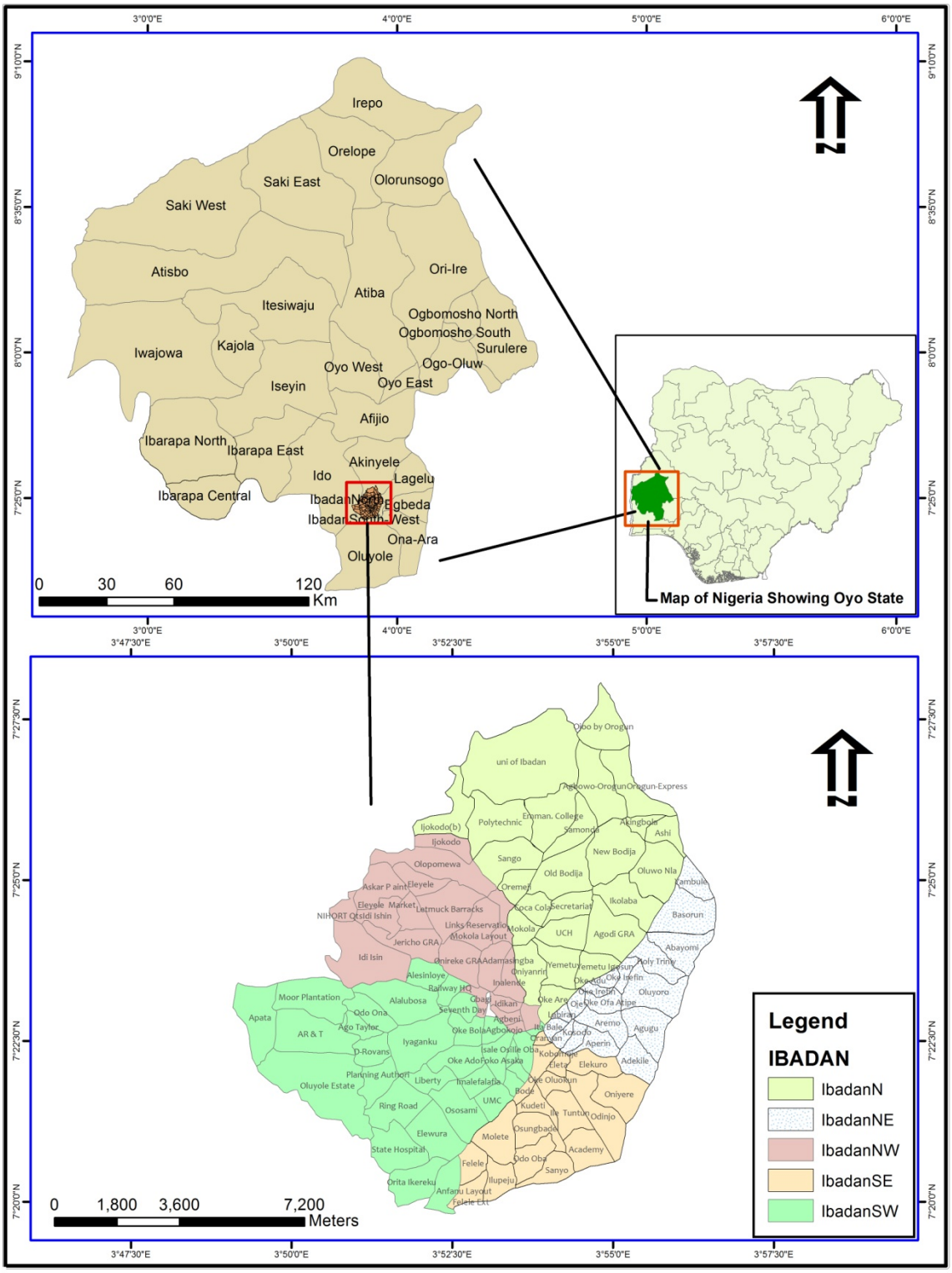


Figure 3.1: Map of Nigeria showing the ethnobotanical study area

3.4.2 Ferrous ion-chelating method

Solutions of 2 mM $FeCl_2 \cdot 4H_2O$ and 5 mM ferrozine were diluted twenty times. An aliquot (1 mL) of varied concentrations (0.5, 0.25, 0.125, 0.0625, 0.03125 mg/mL) of the plant extract was mixed with 1 mL $FeCl_2 \cdot 4H_2O$; incubated for 5 min, 1 mL ferrozine was added, shaken vigorously and incubated for 10 min and the absorbance was spectrophotometrically measured at 562 nm. Ethylene diamine tetra-acetate (EDTA) was used as a standard. All the tests were carried out in triplicates and the mean results were obtained. The percentage inhibition of ferrozine – Fe^{+2} complex formations was calculated. This was done in line with the modified method described by Singh and Rajini (2004).

Chelating effect (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

$A_{control}$ is the absorbance of control ($FeCl_2$ and ferrozine complex formation molecules).

A_{sample} is the absorbance of the tested plant or EDTA.

3.5 Phytochemical tests

The phytochemical assays were done on the methanol extracts, hexane, ethyl acetate, butanol and aqueous fractions of *D. edulis* and *A. occidentale*, and also on their pulverized leaves. The samples were quantitatively screened for saponins, tannins, alkaloids, phenolics, anthraquinones, flavonoids, steroids, terpenoids, cardiac glycosides and carotenoids. This was done in October, 2015 at Kappa Biotechnology Laboratories, Bodija, Ibadan.

3.5.1 Saponins

One gram of the plant sample was added to 5 mL of 20 % ethanol and placed in a water bath at 55 ^{o}C for 4 hours. The residue was filtered and washed with 20 % ethanol twice. The extract was reduced to 5 mL in the oven. Five millilitres of petroleum ether was added to the concentrated extract inside a separating funnel. The petroleum ether layer was discarded and 3 mL of butanol was added to it. Five millilitres of 5 % sodium chloride was used to wash it. The butanol layer was poured into a weighed Petri dish, evaporated to dryness and the weight of the residue was taken (Harborne, 1998; Mayuri, 2012).

3.5.2 Tannins

One gram of the plant sample was extracted with 25 mL of the solvent mixture of 80:20 acetone: 10 % glacial acetic acid for 5 hours. It was filtered and the absorbance was measured at 500 nm.

The absorbance of the reagent blank was also measured. The concentration of tannin was read off against a standard graph (10, 20, 30, 40, 50 mg/100g of tannic acid) and the dilution factor was taken into consideration (Harborne, 1998; Mayuri, 2012).

3.5.3 Alkaloids

One gram of the plant sample (W) was added to 20 mL of 10 % acetic acid in ethanol. It was mixed, allowed to stand for 4 hours, and then filtered. The filtrate was evaporated to a quarter of its original volume. One drop of concentrated ammonia was added. The precipitate formed was filtered through a weighed filter paper (W_1). The filter paper with the residue was left to dry in the oven at 60 ^{o}C. The filter paper was weighed after drying to a constant weight (W_2) (Harborne, 1998; Mayuri, 2012).

$$\% \text{ Alkaloids} = \frac{W_2 - W_1}{W} \times 100$$

3.5.4 Phenolics

Two milligrams of the plant extract was mixed with 0.5 mL of Folin-Ciocalteau reagent, 1.5 mL sodium carbonate (20 %), and left for 30 minutes at 40 ^{o}C to develop colour. The absorbance was measured 765 nm and expressed as Gallic Acid Equivalent (GAE/g) (Harborne, 1998; Mayuri, 2012).

3.5.5 Anthraquinones

One gram of the plant sample was added to 25 mL water and boiled with 10 mL of sulphuric acid. It was filtered while hot; the filtrate was mixed with 5 mL of chloroform and shook for a minute. The chloroform layer was pipetted into another test tube and 1 mL of diluted ammonia was added. The absorbance of the resulting solution was measured at 530 nm (Harborne, 1998; Mayuri, 2012).

3.5.6 Flavonoids

One gram of the plant sample was extracted with 10 mL of 80 % methanol. It was allowed to stand for 2 hours, after which it was filtered into a weighed Petri dish, and it was dried at 40 ^{o}C in the oven. The Petril dish was weighed when the sample has dried to a constant weight (Harborne, 1998; Mayuri, 2012).

3.5.7 Steroids

One hundred milliliters of water were added to 5 g of the plant sample. 0.1 M ammonium hydroxide was added to adjust the pH to 9.1, then 2 mL petroleum ether, 3 mL acetic anhydride and conc. H_2S0_4 were added one after the other. The absorbance was read at 420 nm (Harborne, 1998; Mayuri, 2012).

3.5.8 Terpenoids

One gram of the plant sample was dissolved in 10 mL petroleum ether. It was allowed to extract for 15 min, after which it was filtered and the absorbance was read at a wavelength of 420 nm in a spectrophotometer (Harborne, 1998; Mayuri, 2012).

3.5.9 Cardiac glycosides

One gram of the plant sample was extracted with 40 mL water and placed in the oven 100 ^{o}C for 15 min. One millilitre from the extract and 5 mL water was added to 2 mL glacial acetic acid. A drop of $FeCl_3$ and 1 mL conc. H_2S0_4 were added. The absorbance of the resulting solution was measured at 410 nm (Harborne, 1998; Mayuri, 2012).

3.5.10 Carotenoids

One gram of the plant sample was mixed with 20 mL acetone. It was left for an hour and then filtered. Ten millilitres water was added to the filtrate. The filtrate was poured into a separating funnel and 5 mL petroleum ether was added to it. The funnel allowed it to flow into it by the side of the funnel and left for some minutes to separate. The lower layer was discarded; the absorbance was measured at 440 nm and read off a standard graph (Harborne, 1998; Mayuri, 2012).

3.6 Antimicrobial assays

3.6.1 Collection of test organisms

Two typed strains of Gram positive bacteria: *Staphylococcus aureus* (NCIB 8588), *Bacillus cereus* (NCIB 6349); four typed strains of Gram negative bacteria: *Serratia marcescens* (NCIB 1377), *Klebsiella pneumoniae* (NCIB 418), *Pseudomonas aeruginosa* (NCIB 950), *Proteus vulgaris* (NCIB 67); and six fungal isolates: *Penicillium camemberti*, *Trichophyton mentagrophytes*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Trichoderma* sp., and *Cladosporium herbarum* were obtained from the Department of Microbiology, Obafemi Awolowo University,

Ile-Ife, Osun State, Nigeria. They were used for the preliminary antimicrobial screening of the methanol extracts and fractions of *Dacryodes edulis* and *Anacardium occidentale* leaves in September and October, 2015.

3.6.2 Preparation of culture media

Dissolution and autoclaving of the nutrient media were according to the manufacturers' instructions: 38 g of Muller Hinton Agar (Sigma Aldrich), 48 g of Malt Extract Agar (Sigma Aldrich), 62 g of Sabouraud Dextrose Agar (SDA, Biolife Lab., Italy) were separately dissolved in 1 L of distilled water, while 2.7 g of Mannitol Salt Agar (MSA, Biowark Lab., Pune, India) was dissolved in 25 mL of distilled water. Each was heated to boil; 20 mL was dispensed into each MacCartney bottle, autoclaved at 121 ^{o}C, 15 psi (1 kg/cm^{3}) pressure for 15 minutes and kept in a molten state.

3.6.3 Serial dilution

One gram of each plant extract was measured and re-dissolved in 10 mL of each solvent (methanol, nhexane, ethyl acetate, and water). Five millimetres from the extract was aseptically transferred into a Bijou bottle containing 5 mL of the solvent and successively diluted five times and the resulting concentrations (mg/mL) were: 100, 50, 25, 12.5, 6.25 and 3.125.

3.6.4 Antibacterial tests

Agar well diffusion technique was used; the broth culture of each test organism (0.1 mL) was added to the 20 mL sterile molten Muller Hinton Agar which had been cooled to 44 ^{o}C. The bottle was gently rotated to mix the microbe with the medium, poured into a properly labeled sterilized glass Petri dish (120 x 20 mm) and allowed to set. Seven holes (wells) were bored on the seeded medium using a sterile cork borer (8 mm). The holes were about 0.5 mm to the edge of the plate and labeled respectively. Each hole was filled with the appropriate plant extract (3 - 6 µL) using sterile Pasteur pipette. The culture plates were incubated at 35 ^{o}C for 24 hours and care was taken not to stock-pile the culture plates. Susceptibility of each test organism to the plant extract was examined, measured with a pair of divider and a ruler, recorded in millimeters, and 8 mm of the cork borer was deducted from the zone of inhibition (Arekemase *et al*., 2011).

3.6.5 Antifungal tests

Ditch plate method was used; the prepared Malt Extract Agar was poured into a plate (120 x 20 mm) and allowed to solidify, then a trough (5 x 9 mm) was cut out of the agar. A loopful of each test organism (0.1 mL) was streaked outwards from the ditch on the agar surface and each plant extract or fraction (15 - 20 µL) was carefully run into the ditch, about three-quarters full. Each plate accommodated three test organisms but one concentration of the plant extract. The plate that served as the control had its ditch filled with the solvent used to extract the plant. The width of inhibition zone was examined, measured in millimeters, recorded at 72 hours of incubation at 30 ^{o}C and 5 mm of the trough was deducted from the zone of inhibition (Sodha *et al*., 2015).

3.7 Functional group determination

A portion (0.2 g) of the ethyl acetate fraction of each plant was put on the disc and then inserted into the sample chamber of Fourier Transform Infrared (FTIR) machine (Perkin Elmer Spectrum BX, Llantrisant, UK) at the Multidisciplinary Central Research Laboratory (MCRL), Univeristy of Ibadan, to generate the spectrum. The peaks of the spectrum were interpreted with IR Pal 2.0 Tabledriven Infrared Application.

3.8 Ethical approval and animal grouping for toxicity experiment

The ethical approval for animal use was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (**UI-ACUREC/App/2016/033**). One hundred and thirty male *Rattus norvegicus domestica* (15 - 80 g) were purchased from the Central Animal House, University of Ibadan, and were randomly grouped into cages (5 per group), while two groups served as the controls. They were given food and water *ad libitum* for three days to acclimatize them prior and throughout the experimentation. Permanent colour markers were used on the rats for identification.

3.8.1 Acute toxicity test

Different doses (10, 100, 1000, 1,600, 2,900 and 5,000 mg/kg) were prepared from the aqueous and ethyl acetate fractions of *Dacryodes edulis* and *Anacardium occidentale* leaves using 20 % Dimethyl sulphoxide (DMSO) as a dissolvent. For each plant's fraction, the rats were picked, weighed and orally administered different doses using syringes with cannulas. Only 20 % DMSO was orally given to five rats (control I) while five rats were not dosed (control II). The rats were

fasted and observed for 24 hours to monitor their behaviour and also for any mortality. All the rats were sacrificed by quick cervical dislocation on the third day and their carcasses, spleens, kidneys, hearts and livers were harvested and weighed. The kidneys and livers were preserved in 10 % formalin. Serial sectioning (5 μm thickness), paraffin embedding, block making, staining with heamatoxylin and eosin, and microscopic examination of the kidneys and livers were done (Lorke, 1983).

3.9 Cream formulation

Chlorocresol (0.1 % $^{w}/_{w}$) dissolved in warm distilled water (69.9 % $^{w}/_{w}$) was mixed with hot wax (emulsifying ointment: 30 % $^{w}/_{w}$) and was continuously stirred until it was cold. The aqueous cream BP formed (Table 3.1) was the base for the creams (BPC, 1979). Humectant (propylene glycol: 10 % $^{w}/_{w}$) was mixed with each ethyl acetate fraction of the plant (0.75, 1.5 and 3.0 g) and then incorporated into each of the base (26.25, 25.5 and 24.0 g, respectively) by continuous stirring, until desired products were formed.

The cream formulations were done at Pharmaceutics Dispensing Laboratory, University of Ibadan, the organoleptic properties (colour by visual outlook, texture by feeling between fingers, odour by smelling) were recorded and each product was dispensed into plain tubes for different analyses: density, extrudability, spreadability, pH, diffusion rate, viscosity, globule size, *in vitro* and *in vivo* antimicrobial tests, stability studies, dermal irritation and toxicity tests. The statistics from the creams are in the appendix V. Tables 3.1, 3.2 and 3.3 respectively show the ingredients for preparing the emulsifying ointment, the ingredients for preparing the aqueous cream, and the composition of the formulated creams.

3.9.1 Density

The weight of a 2 mL syringe was firstly measured; 2 mL of a cream was syringed and then re-weighed. The density was calculated thus: Density (g/cm^3) = $(W_2 – W_1) / V$

Where W_1 is the weight of the syringe only, W_2 is the weight of the syringe and cream, V is the volume of the cream.

3.9.2 Extruding time

The time taken for extruding the cream from the 2 mL syringe was also recorded and the mean calculated.

Table 3.1: Ingredients for preparing the emulsifying ointment BP

Ingredients	Quantity (g)	Percentage ($^{w}/_{w}$)
Emulsifying wax	90	30
White soft paraffin	150	50
Liquid paraffin	60	20
Total	300	100

Table 3.2: Ingredients for preparing the aqueous cream BP

Ingredients	Quantity (g)	Percentage ($^{w}/_{w}$)
Emulsifying ointment	90	30
Chlorocresol	0.3	0.1
Purified water	209.7	69.9
Total	300	100

Table 3.3: Composition of the formulated creams

FC	Base (% $^{w}/_{w}$)	PG (% $^{w}/_{w}$)	LEF (% $^{w}/_{w}$)	Total (%)
FDe1	87.5	10	2.5	100
FDe2	85.0	10	5.0	100
FDe3	80.0	10	10.0	100
FAo1	87.5	10	2.5	100
FAo2	85.0	10	5.0	100
FAo3	80.0	10	10.0	100

FC = Formulation code, Base = Aqueous cream, PG = Propylene glycol, LEF = Leaf ethyl acetate fraction, FDe = *Dacryodes edulis* cream, FAo = *Anacardium occidentale* cream.

3.9.3 Spreading length

A glass slide (20 x 5 cm) and a die were separately weighed and then together. The die on the glass slide was filled with a cream and re-weighed; the cream was pushed out with a punch on the glass slide while another glass slide and a known weight (142 g) were placed on it for 5 minutes. The lengths of the spread cream were measured with a pair of divider and a ruler and the mean was calculated.

3.9.4 The pH

The pH of the formulated creams was determined by a calibrated pH meter (Jenway, UK) at 25 ± 2 ^{o}C; the electrode was dipped into each cream sample. The average values from five measurements were calculated.

3.9.5 Diffusion rate

Melted nutrient agar (20 mL) was poured and allowed to set in a glass Petri dish. This was flooded with 5 $\%^{w}/_{v}$ ferric chloride solution (Iron III chloride hexahydrate) and the excess was drained off and dried. Three holes were bored into the agar by a 6 mm cup borer, and the holes were filled with a cream sample, incubated at 37 ^{o}C. Diameters of the diffused cream were measured at varying times between 1 - 40 hours, while the mean diameters were calculated and plotted on a graph. The gradient difference of the graph (that is, the diffusion rate) was determined.

3.9.6 Viscosity

The viscosity of the formulated creams was determined with Brookefield viscometer (VT 181, Karlsrule, Germany) at 28 ± 2 ^{o}C using spindle number 7. The spindle was lowered perpendicularly into the cream without touching the bottom of the plain tube and readings were recorded after they became constant. The preliminary viscosity studies of each cream at rotational speed 2.5, 4, 5, 10, 20, 50 and 100 revolutions per minute (rpm) were done. The graphs of viscosity against rotational speed were plotted. The subsequent viscosity studies of the formulated creams with spindle number 7 were done at 50 rpm (Ajala *et al*., 2016).

3.9.7 Globule size

The formulated creams were stained with Gentian violet to create contrast between hydrophilic and hydrophobic components and thinly smeared on glass slides. Microscopical pictures were

taken at x400 magnification with a digital microscope, VJ-2005 DN model Bio-microscope®. The globule diameters (morphometrical analysis) of one hundred particles were randomly determined using TS View CX Image® Software, version 6.2.4.3 and Motic Image 2000 (China). This was done at the Department of Veterinary Anatomy, University of Ibadan.

3.10 Antimicrobial tests of the creams

Two typed isolates: *Staphylococcus aureus* (ATCC 2785) and *Pseudomonas aeruginosa* (ATCC 29213); two clinical isolates: *S. aureus* and *P. aeruginosa*; three clinical fungi: *Trichophyton rubrum*, *Epidermophyton* sp. and *Candida albicans* were obtained from Medical Microbiology Department, College of Medicine, University College Hospital (UCH), Ibadan; the tests were done at the Department of Pharmaceutical Microbiology Laboratory, University of Ibadan.

3.10.1 *In vitro* antimicrobial test

Agar well diffusion technique was used for the obtained microorganisms and the concentrations prepared for the ethyl acetate fractions of *Dacryodes edulis* and *Anacardium occidentale* were 200, 100, 50, 25, and 12.5 mg/mL. The cork borer used was 6 mm for the ethyl acetate fractions and their formulated creams. A portion (0.5 g) of the cream was filled into each well, allowed to stand and diffuse for 30 minutes, incubated at 37 ^{o}C for 24 hours for bacteria while at 25 ^{o}C for fungi for 48 hours. Ethyl acetate, Amoxillin tablet (Pacmentin-625, Medicef Pharma, India) and Ketoconazole tablet (Mycozoral, Ciron Drugs, India) were used as the controls for the antimicrobial test of the ethyl acetate fractions; while the aqueous cream (base), Tydineal cream (Adams Pharmaceutical (Anhui) Co., Ltd., China) and Mycozoral cream (Ciron Drugs, India) were used as controls for the cream antimicrobial experiments. The zones of inhibition were measured (mm) and 6 mm of the cork borer was deducted from them (Ajala, *et al*., 2016).

3.10.2 *In vivo* antibacterial test

Forty male *Mus musculus* (15 - 20 g) were purchased, given food and water *ad libitum* for three days, in order to acclimatize them to the environment (Animal House of Veterinary Anatomy, University of Ibadan). They were grouped into eight (5 per group), two groups served as the controls. A circular area (2 cm diameter) was marked out with a permanent colour marker, clipped free of fur and 0.5 mL of *Staphylococcus aureus* (a log-phase culture of dilution 10^{-8} CFU) was intradermally injected into each mouse. Administration of creams commenced after 24 hours of inoculation; 200 mg of the six formulated creams and Tydineal cream (reference

cream) were topically applied once per day on each group of mice for three days. The untreated group (3 mice injected with the *S. aureus* but not treated; 2 mice not injected nor treated with the *S. aureus*) was the second control.

The mice were sacrificed on the fourth day by quick cervical dislocation; the treated site of each mouse skin was excised, half was put in 1 mL peptone water and kept in a refrigerator for 38 hours, while the other half was put inside 10 % formalin for histopathological studies. Serial dilution (10^{-2} CFU) was done by adding 0.1 mL of the peptone water to 9.9 mL sterile peptone water; 0.2 mL was cultured on mannitol salt agar at 40 - 45 ^{o}C and incubated for 24 hours. Viable count of *S. aureus* was determined with a colony counter (Stuart Scientific Ltd, Great Britain) and the percentage bacteria killed was calculated (Gisby and Bryant, 2002).

Colony forming unit (CFU/mL) = a x b x c

Where a = viable count, b = reciprocal of the volume taken, c = reciprocal of the dilution factor.

3.11 Stability studies of the creams

The stability studies of the formulated creams for 120 days were determined by two analyses: the viscosity and the organoleptic properties of the creams (that is, colour, odour, and texture) when stored under different temperatures of 29 ± 4 ^{o}C (room), 0 ^{o}C (frozen) and 46 ^{o}C (high temperature).

3.12 Dermal irritation and sub-acute toxicity tests of the creams

A four week old, thirty male *Mus musculus* (7 - 12 g) were purchased, acclimatized, grouped into six (5 per group), given food (Top feed super starter) and water *ad libitum* prior and throughout the experimentation at the Animal House of Veterinary Anatomy, University of Ibadan. The side (2 cm diameters) of each mouse was clipped free of fur and a cream (200 mg) was topically applied. The untreated group (2 mice not injected with the *S. aureus* nor treated) from the *in vivo* antibacterial test also served as the control.

The behaviours of the mice were observed and the sites of application were visually assessed after 24 hours for any redness or oedema on their skins. For the toxicity tests, the creams were applied daily to the same sites for 21 days. The mice were humanely sacrificed by quick cervical dislocation; the sites of the skins were harvested and kept inside 10 % formalin, from which

serial sections of 5 µm thickness were made after paraffin embedding, block making, staining with heamatoxylin and eosin, and microscopically examined for histopathological aberrations (Ajala *et al*., 2016).

3.13 Statistical analyses

ArchMap GIS software version 10.6.1 (2018) was used to draw the map of the study area. Microsoft Office Excel software (2010) was used to plot the graph of viscosity at different rotational speed, and to analyse the antimicrobial data: results were presented as mean ± standard deviation (mean ± SD). Statistical System of Analysis software (SAS) version 9.1 (2003) was used to analyse the phytochemical data: results were presented as mean ± standard error of mean (mean ± SE), at the level of significance $p < 0.05$. Prism Software (Graphpad) package 5.00 was used to analyse the data of acute toxicity, viscosity and globule size. The data were analysed ($p < 0.05$) using unpaired student's t test, analysis of variance (ANOVA) and Turkey Kramer's multiple comparison tests: results were presented as mean ± standard deviation (mean ± SD).

CHAPTER FOUR
RESULTS

4.1 Findings of the ethnobotanical survey of *Dacryodes edulis* and *Anacardium occidentale* in Ibadan

The respondents were mostly men (consisting 62.5 %) while others were women (37.5 %). The age ranges and educational statuses of the respondents are shown in Figures 4.1 and 4.2, respectively. The occupations of the respondents; 89 % for civil servants, artisans and students, 5 % for herb sellers, 5 % for farmers while 1 % for herbalists. Looking at religions of the respondent, Christianity had 60.5 %, Islam had 28.5 %, and 6.5 % for those practicing Christianity and Islam, 2.5 % for traditional religion, 1.5 % observe the three religions while 0.5 % practices no religion. The respondent sources of knowledge on medicinal plants (Fig. 4.3) showed that parents (that is, parents, grandparents, in-laws) and apprenticeship had the highest and least values (61.5 % and 1 %, respectively), while there was no value for others (supernatural means – dreams, trances etc). All the herb sellers interviewed at Bode market belonged to the market association of herb sellers. Generally, 94 % of the respondents do not belong to any tradomedicinal association outside the market, while only 6 % of the respondents are members of tradomedicinal association outside the market.

Majority of the respondents (65 %) do not mix medicinal herbs and orthodox drugs together; 27.5 % indicated that they sometimes combine medicinal herbs with orthodox drugs, while only 7.5 % specified they always combine medicinal herbs with orthodox drugs. Also, eighty percent of the respondents are familiar with the medicinal uses of *Anacardium occidentale*, while only 10 % with *Dacryodes edulis*. Information gathered from the respondents in Ibadan on the medicinal preparations and utilization of *D. edulis* and *A. occidentale* is shown in Table 4.1.

The abundance of *A. occidentale*, based on the scale of 80 - 100 percentages, is ten times than *D. edulis* in Ibadan. Only 7.5 % of the respondents have earlier planted *A. occidentale* and 2 % for *D. edulis*. Thirty-five percent indicated that *A. occidentale* is highly effective medicinally and 7.5 % for *D. edulis*. From the data gathered, 2.5 % indicated side effects which are skin itching and rashes from raw nuts of *A. occidentale* and none for *D. edulis* (Fig. 4.4). Meanwhile, 40 % of the

respondents procured *A*. *occidentale* (leaves, bark, fruits) from markets and 25 % indicated that it is easy and cheap. A pack of *A*. *occidentale* leaves (150 g) and barks (450 g) are sold for fifty naira and one hundred naira respectively at Bode and Oja Oba markets; however, leaves and barks of *D*. *edulis* are not available in the markets in Ibadan. Consequently, *A*. *occidentale* is better known locally for treating skin challenges in Ibadan than *D. edulis*.

4.2 Antioxidant activity of *Dacryodes edulis* and *Anacardium occidentale* leaf extracts using 1, 1 diphenyl-2-picrylhydrazyl method

Butanol fraction of *D*. *edulis* had the highest inhibition of free radical (86.29 %) at 0.25 mg/mL while hexane fraction of *D*. *edulis* had the least (68.37 %) at 0.25 mg/mL; there was difference of 13.33 % between ascorbic acid (control) and hexane fraction. The ethyl acetate fraction of *D*. *edulis* had the highest inhibitions: 80.28 and 58.63 % at 0.125 and 0.0625 mg/mL, respectively; while ascorbic acid had the least inhibitions: 54.09 and 29.74 % at 0.125 and 0.0625 mg/mL, respectively. There was a difference of 16.36 % between ethyl acetate fraction and methanol extract at 0.0625 mg/mL, while a difference of 10.12 % was observed between hexane fraction and ascorbic acid. The methanol extract of *D*. *edulis* had the highest inhibition (34.89 %) at 0.03125 mg/mL, while ascorbic acid (22.88 %) was the least at 0.03125 mg/mL (Fig. 4.5).

The ascorbic acid (control) at every concentration (0.25, 0.125, 0.0625 and 0.03125 mg/mL) was higher in inhibition of free radical than *A*. *occidentale*. The hexane fraction of *A*. *occidentale* had the least inhibitions (16.88, 13.24, 8.09 and 2.27 %) for all the concentrations (0.25, 0.125, 0.0625 and 0.03125 mg/mL, respectively). There was higher difference of 39.24 % between ascorbic acid and methanol extract at 0.25 mg/mL, and lower difference of 12.11 % between ethyl acetate and butanol fractions at 0.25 mg/mL. There was difference of 31.21 % between ascorbic acid and methanol extract of *A*. *occidentale* at 0.125 mg/mL. There was difference of 15.34 % between ascorbic acid and aqueous fraction of *A*. *occidentale* at 0.0625 mg/mL (Fig. 4.6).

Comparatively, in Figures 4.5 and 4.6, *D. edulis* had an antioxidant potential for showing higher inhibition of free radical than ascorbic acid (control), except for hexane fraction at 0.25 mg/mL. However, *A*. *occidentale* had inhibition of free radical below ascorbic acid at every concentration (0.25, 0.125, 0.0625 and 0.03125 mg/mL).

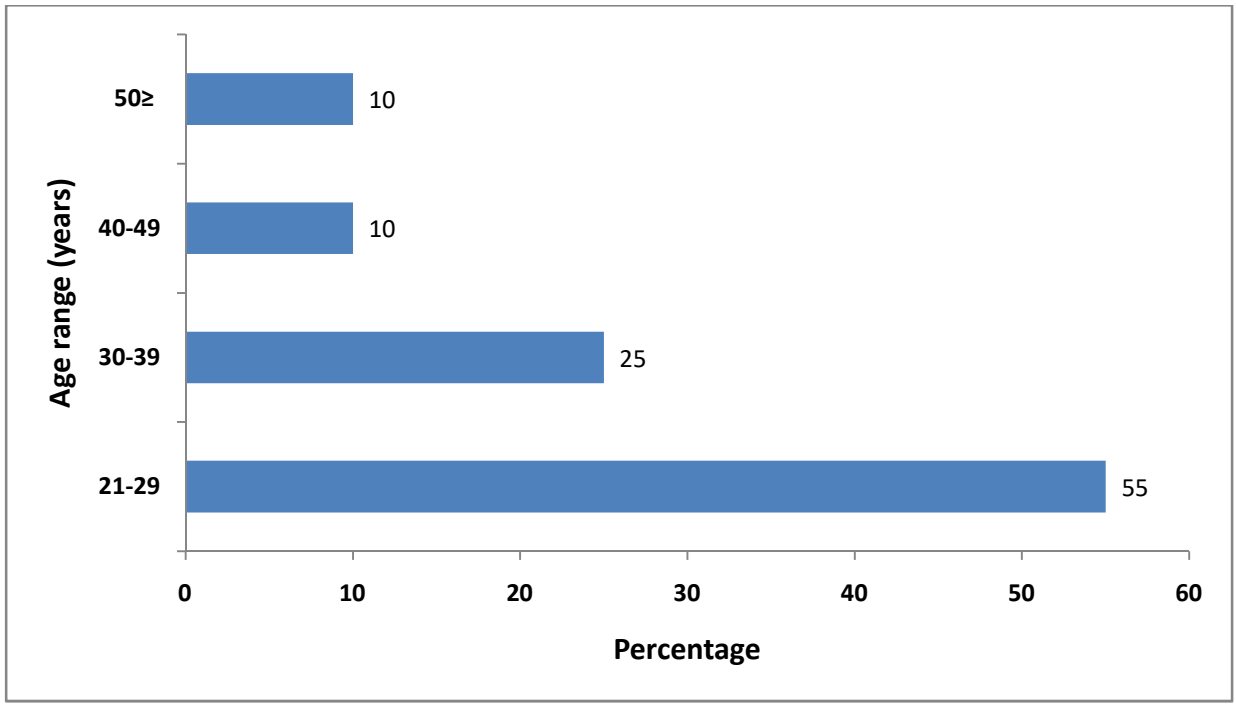


Figure 4.1: Age ranges of respondents from survey of *Dacryodes edulis* and *Anacardium occidentale* in Ibadan

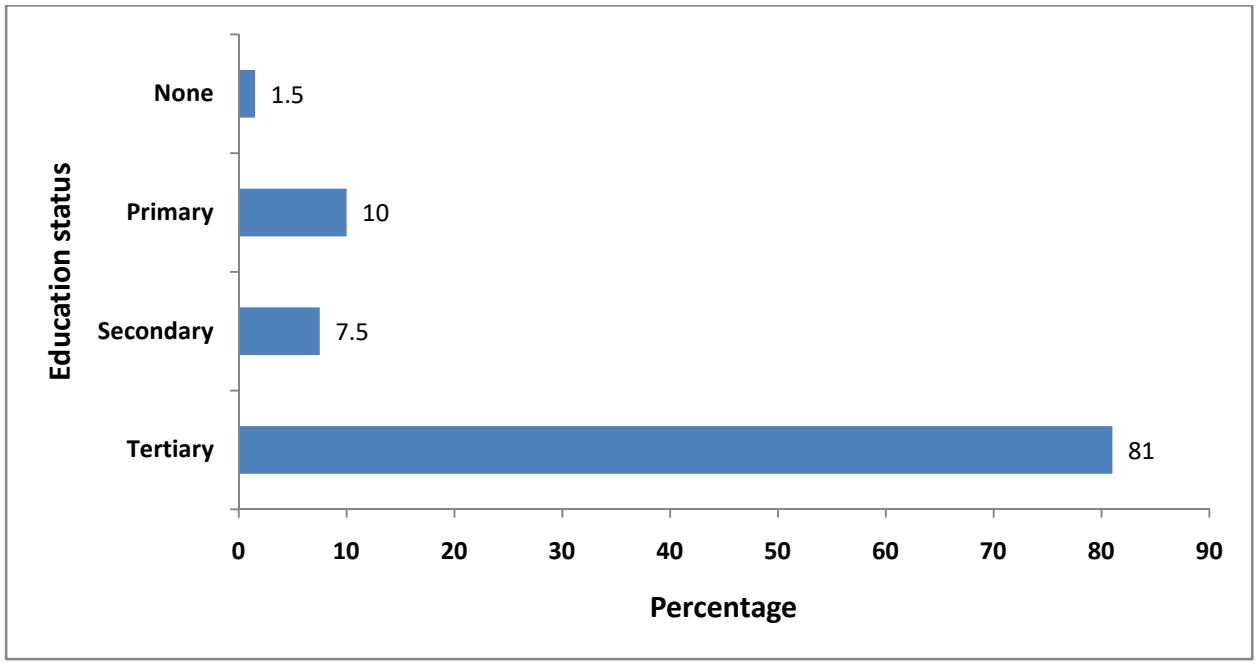


Figure 4.2: Educational statuses of respondents from survey of *Dacryodes edulis* and *Anacardium occidentale* in Ibadan

Table 4.1: Medicinal uses of *Dacryodes edulis* and *Anacardium occidentale* in Ibadan

Plants	Parts used	Preparations	Treatment
Dacryodes edulis	Leaf	Decoction with lime juice for drinking and/or bathing. Decoction with some materials.	Skin itching, measles, body ache, joint pains (*àwóká*), child fever, high blood pressure, boosting blood volume, combating insomnia, managing diabetes, and for skin freshness
	Fruit	Dried fruit added to cream	Skin smoothness
Anacardium occidentale	Leaf, bark	Decoction with some herbs in water for drinking.	Malaria
	Leaf, bark	Soak in maize pap water (*omi ògi*) and add some materials for drinking	Typhoid
	Leaf	N	Blood parasites (*kòkòrò inú èjè*)
	Leaf, bark and root	Decoction in water for drinking and bathing	Barreness; river blindness (*nárun ojú*); swollen body (*kókó ara*)
	Bark	Decoction in water for drinking	Tonic
	Leaf, bark	Decoction in water for drinking	Diabetes
	Leaf and bark	Decoction in water for drinking and bathing	Skin diseases
	Leaf	N	Hypertension
	Bark	N	Black tongue (*adó ahón*)
	Bark	Decoction in water for drinking	Cough

N = Not disclosed.

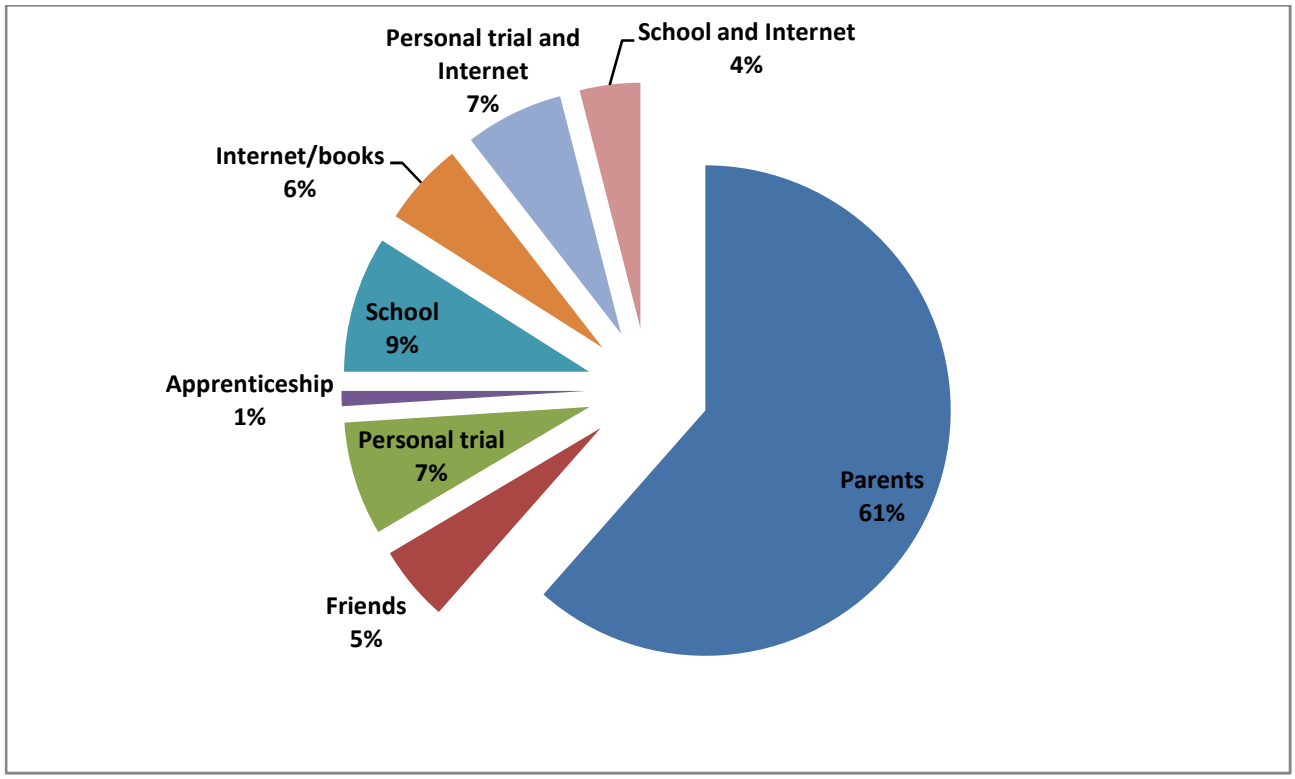


Figure 4.3: Sources of plant medicinal knowledge from respondents in Ibadan

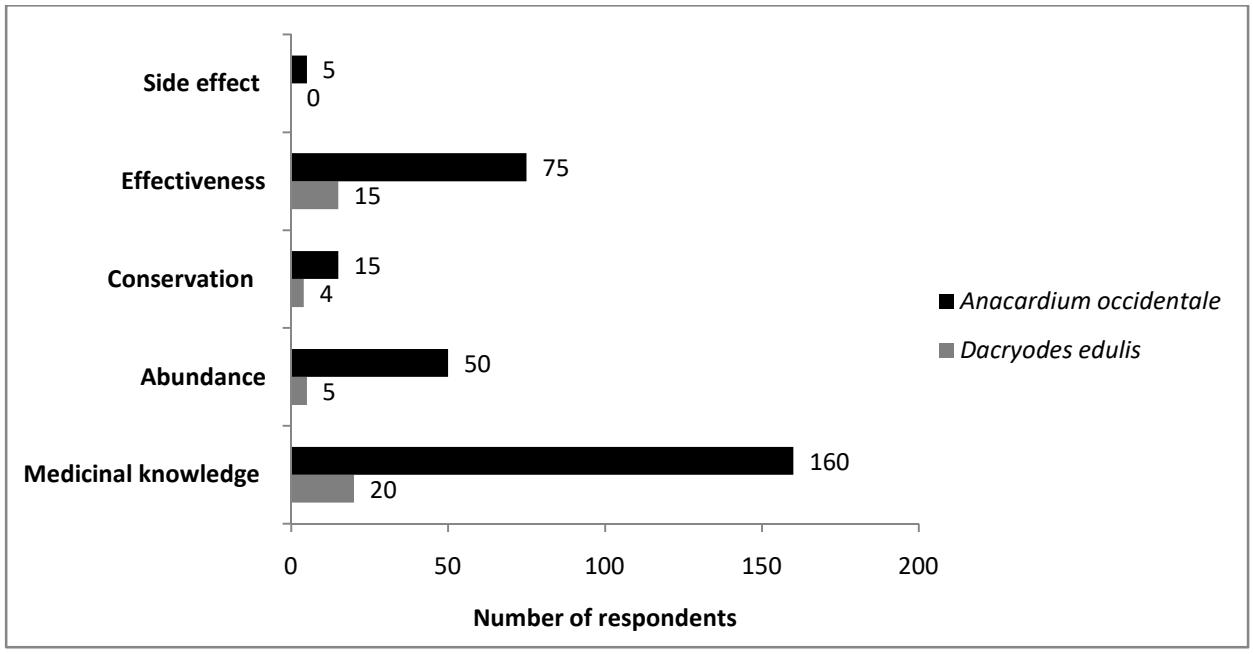


Figure 4.4: Ethnobotanical information on *Dacryodes edulis* and *Anacardium occidentale* in Ibadan

4.2.1 *Dacryodes edulis* leaf extracts using 1, 1 diphenyl-2-picrylhydrazyl method

In *Dacryodes edulis*, there was no significant difference between the treatment means of antioxidant activity obtained for each of the five solvents, from the Randomized Complete Block Design (RCBD). However, there were significant differences in the means of the concentrations (Table 4.2). This implies that variation in concentration of the treatments caused significant differences in the percentage scavenging of the solvents (methanol, hexane, ethyl acetate, butanol, and water). These differences are observed only within the blocks (that is, concentration of extracts) but not significantly different among the treatments (that is, solvents). Thus, there exist differences in at least a pair of the block means, but there were no significant differences in at least a pair of the treatment means. The result of Post-Hoc test (using 95 % confidence interval) showed that there was a significant difference among all the four blocks. This implies there is a significant difference between the efficacies of all possible pairs of the block (Table 4.2).

4.2.2 *Anacardium occidentale* leaf extracts using 1, 1 diphenyl-2-picrylhydrazyl method

In *Anacardium occidentale*, there were significant differences between the treatment means of antioxidant activity obtained for each of the five plant extracts. Also, there were significant differences in concentration means level as shown in Table 4.2. The variation in concentration of the treatments caused significant differences in the percentage scavenging of the solvents (methanol, hexane, ethyl acetate, butanol, and water). These differences were noticed within blocks and between the treatments. Consequently, there exist, differences in at least a pair of the block means and in at least a pair of the treatment means. A Post-Hoc test was conducted to check the block(s) in which the differences exist, because significant differences exist within the solvent concentration level.

The significant differences that exist within the blocks (concentration level of the solvent extracts) were mainly prominent within concentration 0.25 mg/mL and every other concentration – 0.125, 0.0625 and 0.03125 mg/mL. Thus, concentration difference from 0.25 mg/mL with other levels caused significant variations in the percentage scavenging of the DPPH. Similarly, the same difference was also observed within 0.125 mg/mL and 0.03125 mg/mL.

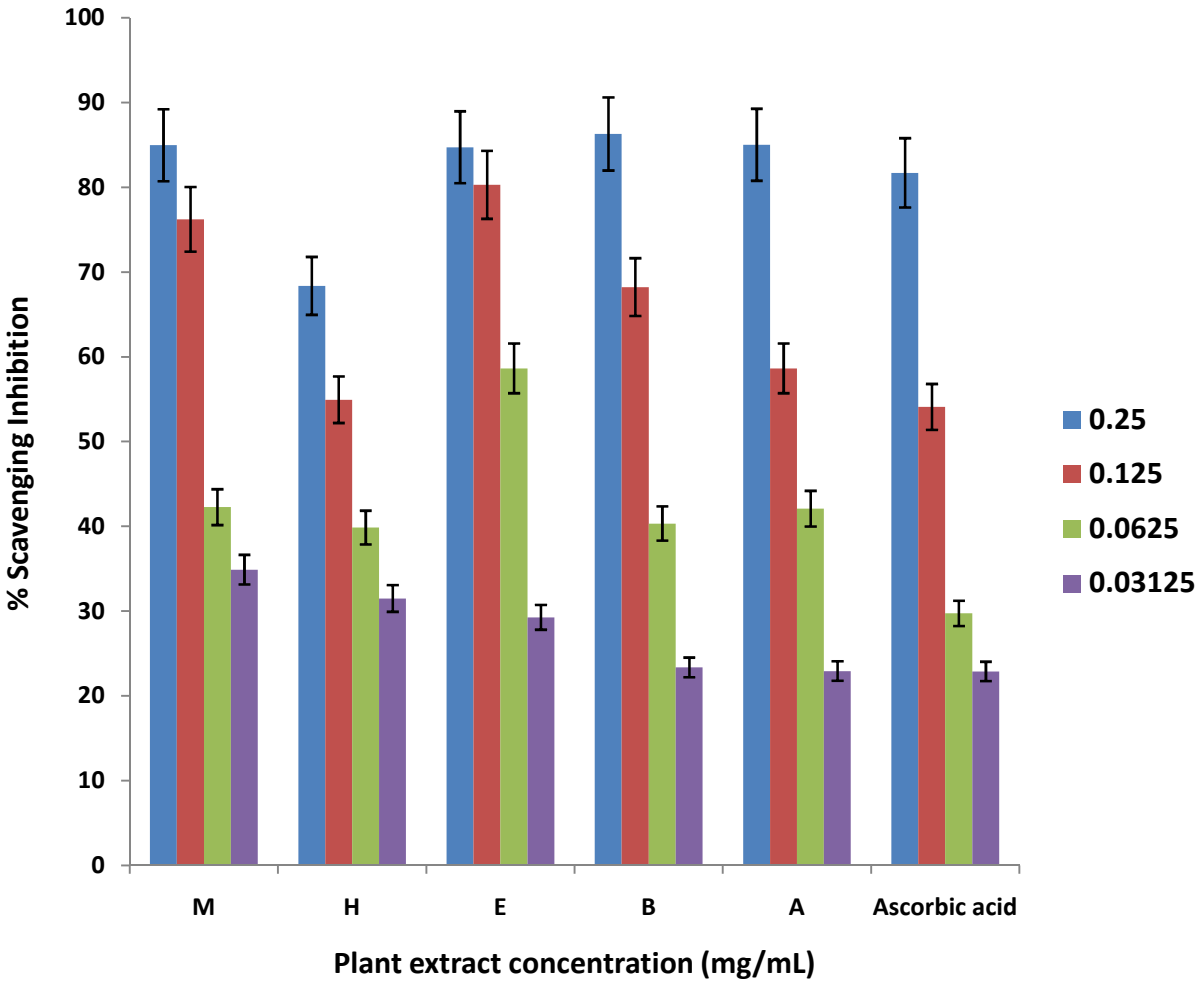


Figure 4.5: Antioxidant activity of *Dacryodes edulis* leaf extracts using 1, 1 diphenyl-2-picrylhydrazyl assay

M = Methanol extract of *D. edulis*, H = Hexane fraction of *D. edulis*, E = Ethyl acetate fraction of *D. edulis*, B = Butanol fraction of *D. edulis*, A = Aqueous fraction of *D. edulis*, Ascorbic acid = Control.

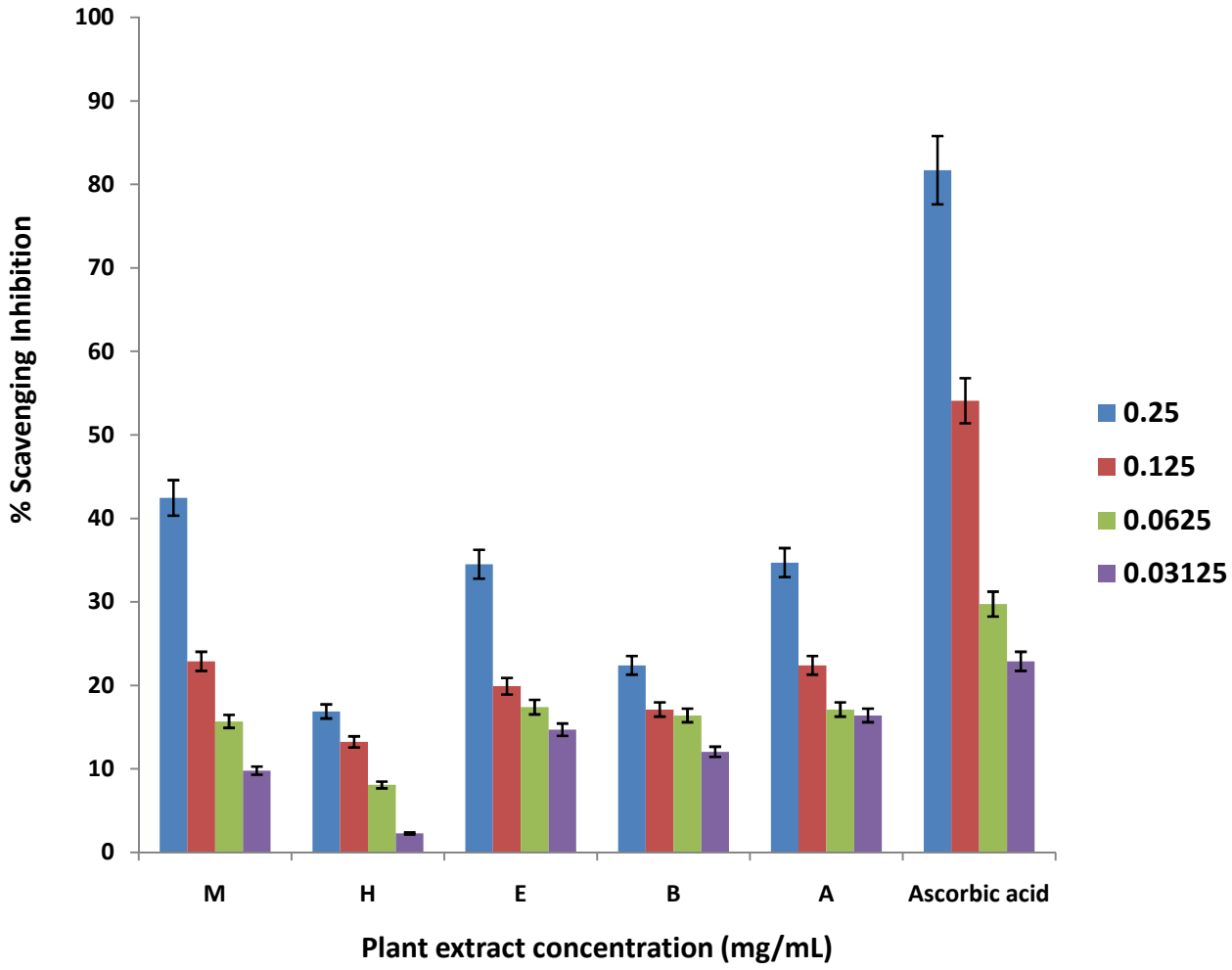


Figure 4.6: Antioxidant activity of *Anacardium occidentale* leaf extracts using 1, 1 diphenyl-2-picrylhydrazyl assay

M = Methanol extract of *A. occidentale*, H = Hexane fraction of *A. occidentale*, E = Ethyl acetate fraction of *A. occidentale*, B = Butanol fraction of *A. occidentale*, A = Aqueous fraction of *A. occidentale*, Ascorbic acid = Control.

The significant differences that exist among the treatments (the solvents) are mainly pronounced between aqueous and hexane. The solvents caused significant variations in the percentage scavenging observed. Additionally, comparable difference was also observed between ethyl acetate and hexane fractions, and between hexane fraction and methanol extract (Table 4.2). The antioxidant data and statistics are in the appendixes III and IV.

4.3 Antioxidant activity of *Dacryodes edulis* and *Anacardium occidentale* leaf extracts using ferrous ion-chelating method

The ethylene diamine tetra-acetate (EDTA) which was the control had the highest inhibition of free radical (49.05 %) at 0.25 mg/mL while hexane fraction of *D. edulis* had the least (28.43 %). However, ethyl acetate fraction of *D. edulis* had the highest (28.74 % and 13.13 %) at 0.125 and 0.03125 mg/mL respectively while hexane fraction of *D. edulis* (12.91 %) was least at 0.125 mg/mL and EDTA (3.44 %) was least at 0.03125 mg/mL. Butanol fraction of *D. edulis* had the highest inhibition (24.72 %) while EDTA (7.61 %) was the least at 0.0625 mg/mL (Fig. 4.7).

The ethylene diamine tetra-acetate had the highest inhibition of free radical for 0.25, 0.125 and 0.03125 mg/mL, while hexane and butanol fractions of *A. occidentale* were least with the respective concentrations. There was difference of 27.38 % between EDTA and aqueous fraction of *A. occidentale*. However, at 0.0625 mg/mL, aqueous fraction of *A. occidentale* had the highest inhibition, while butanol fraction of *A. occidentale* had the least (Fig. 4.8).

Comparing Figures 4.7 and 4.8, the EDTA performed better at 0.25, 0.125 and 0.03125 mg/mL, than *A. occidentale* (except at 0.0625 mg/mL of aqueous and ethyl acetate fractions of *A. occidentale*), thus indicating poor antioxidant ability of the plant. *Dacryodes edulis* performed better than EDTA at 0.0625 and 0.03125 mg/mL (except at 0.25 mg/mL for methanol extract and all the fractions). The ethyl acetate and butanol fractions of *D. edulis* also performed better than EDTA at 0.125 mg/mL, thus indicating an appreciable antioxidant capability of the plant, especially at low concentrations.

4.3.1 *Dacryodes edulis* leaf extracts using ferrous ion-chelating method

In *Dacryodes edulis*, there were significant differences between the treatment means of antioxidant activity obtained for each of the five plant extracts. Similarly, there were significant differences in the level of concentration means (Table 4.2). The variation in concentration of the

treatments caused significant differences in the percentage scavenging (that is, chelating effect) of the solvents (methanol, hexane, ethyl acetate, butanol, and water). These differences are seen within blocks (that is, concentration of extracts) and between the treatments (that is, solvents). Consequently, there exist differences in at least a pair of the block means and in at least a pair of the treatment means.

The Post-Hoc test (using 95 % confidence interval) revealed that the significant differences within the blocks (concentration level of extracts) were mainly pronounced within the concentration 0.25 mg/mL and every other concentration (0.125, 0.0625 and 0.03125 mg/mL). By implication, it means that concentration difference from 0.25 mg/mL with other levels caused significant variations in the percentage scavenging of the ferrozine-Fe^{2+} complex. Moreover, similar difference was also observed within 0.125 mg/mL and 0.03125 mg/mL. The significant differences that exist among the treatments (the solvents) were pronounced mainly between aqueous and hexane fractions. The solvents caused significant variations in the percentage scavenging of the ferrozine-Fe^{2+} complex. In addition, similar difference was observed within butanol and hexane fractions.

4.3.2 *Anacardium occidentale* leaf extracts using ferrous ion-chelating method

In *Anacardium occidentale*, significant differences were obtained between the treatment means of antioxidant activity for each of the five extracts; also, there were significant differences in the concentration levels (Table 4.2). Therefore, variation in concentration of the treatments caused significant differences in the percentage scavenging of the ferrozine-Fe^{2+} complex. These differences were observed within blocks (concentration of extracts) and between the treatments (solvents). Consequently, there exist differences in at least a pair of the block means and in at least a pair of the treatment means.

The Post-Hoc test showed significant differences exist within the blocks (concentration level of the solvent extracts) and were mostly obvious within the concentration 0.25 mg/mL and each other concentration (0.125, 0.0625 and 0.03125 mg/mL). Therefore, the concentration difference from 0.25 mg/mL with other levels caused significant variations in the percentage scavenging of the ferrozine-Fe^{2+} complex. Similar difference was also noticed within 0.125 and 0.0625, 0.125 and 0.03125, 0.0625 and 0.03125 mg/mL.

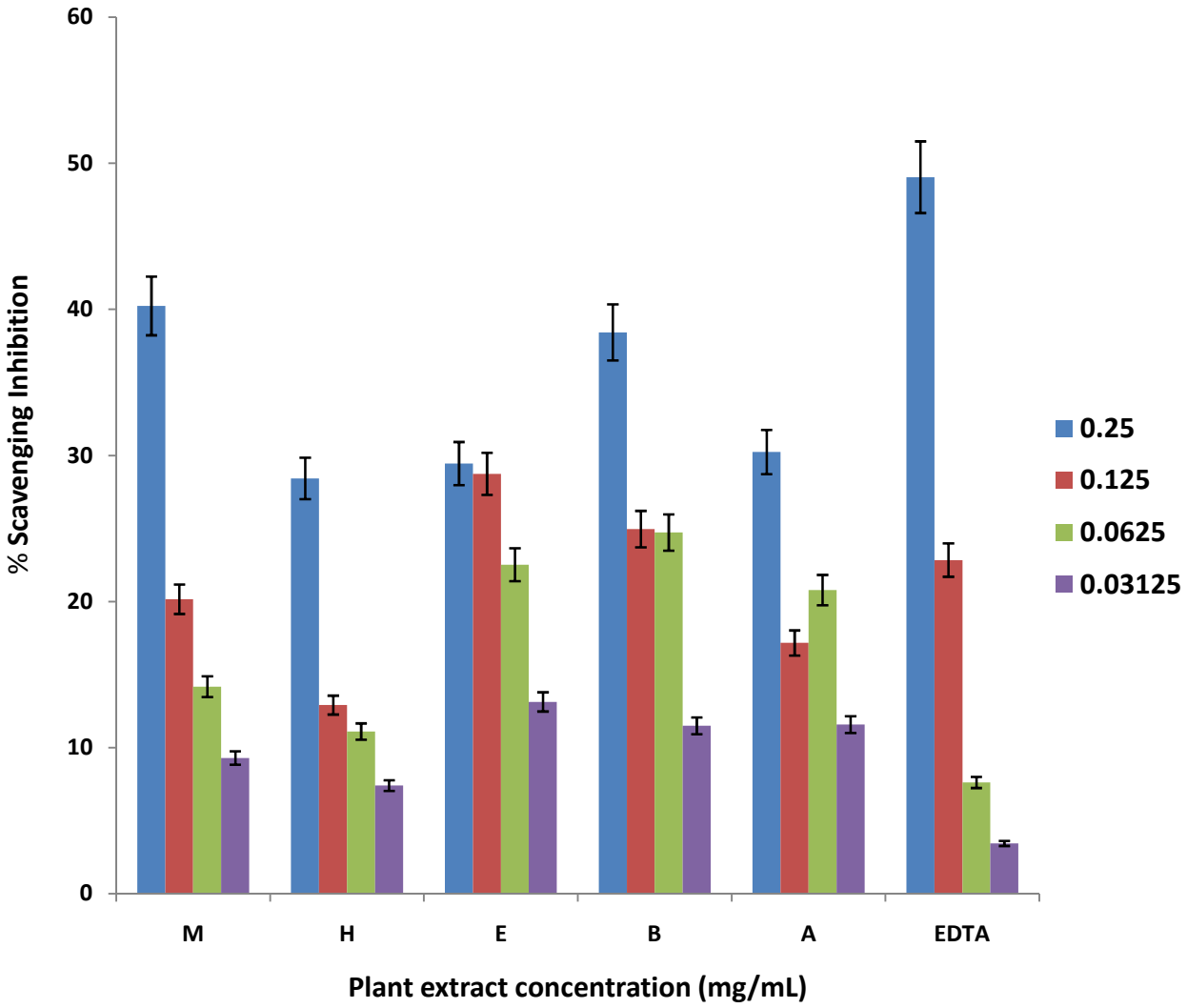


Figure 4.7: Antioxidant activity of *Dacryodes edulis* leaf extracts using ferrous ion-chelating assay

M = Methanol extract of *D. edulis*, H = Hexane fraction of *D. edulis*, E = Ethyl acetate fraction of *D. edulis*, B = Butanol fraction of *D. edulis*, A = Aqueous fraction of *D. edulis*, EDTA = Ethylene diamine tetra-acetate (control).

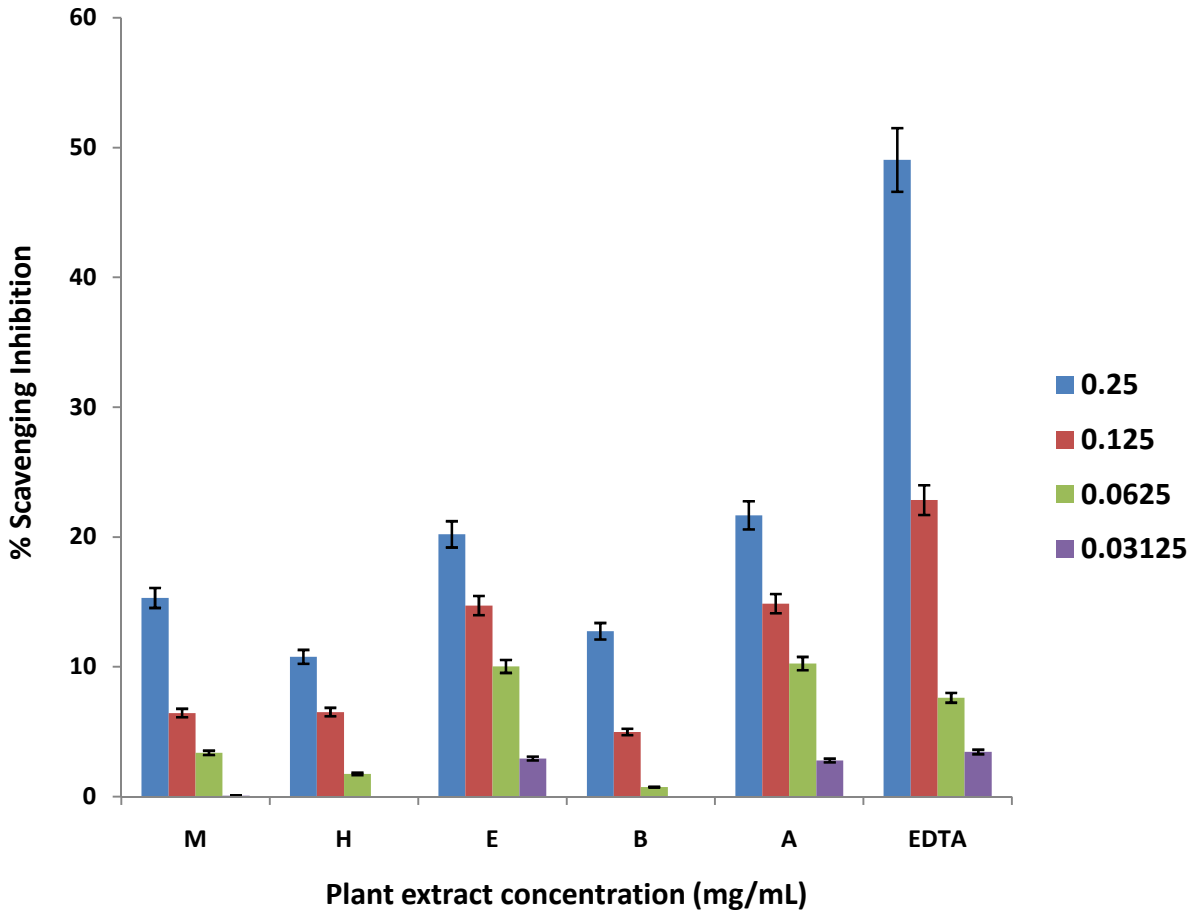


Figure 4.8: Antioxidant activity of *Anacardium occidentale* leaf extracts using ferrous ion-chelating assay

M = Methanol extract of *A. occidentale*, H = Hexane fraction of *A. occidentale*, E = Ethyl acetate fraction of *A. occidentale*, B = Butanol fraction of *A. occidentale*, A = Aqueous fraction of *A. occidentale*, EDTA = Ethylene diamine tetra-acetate (control).

Table 4.2: Mean square variance of *Dacryodes edulis* and *Anacardium occidentale* using 1, 1 diphenyl-2-picrylhydrazyl and ferrous ion-chelating methods

Plants	Source of variation	df	DPPH	FIC
Dacryodes edulis	Treatment	4	135.10^{ns}	80.90*
	Block	3	8482.00**	1374.20***
	Residual	12	43.50	19.10
Anacardium occidentale	Treatment	4	116.80**	76.34***
	Block	3	1024.70***	736.00***
	Residual	12	17.20	3.47

DPPH = 1, 1 diphenyl-2-picrylhydrazyl
FIC = Ferrous ion chelation
df = degree of freedom
ns = not significant
* = significant

The significant differences that exist among the treatments (the solvents) were mainly prominent between aqueous and butanol fractions, aqueous and hexane fractions, aqueous fraction and methanol extract. By implication, it means that the solvents used caused significant variations in the percentage scavenging of the ferrozine-Fe^{2+} complex. Moreover, similar differences were observed between ethyl acetate and butanol fractions, ethyl acetate and hexane fractions, hexane fraction and methanol extract.

4.4 Phytochemical constituents of *Dacryodes edulis* leaf

The phytochemical constituents in *Dacryodes edulis* leaf extract and fractions showed the following trends (Table 4.3). Saponins in *D. edulis* were highest in value for the hexane fraction (1628.33 ± 18.78 mg/100g). This was followed by the ethyl acetate fraction, methanol extract, aqueous fraction, raw leaf, while the butanol fraction showed the least value (13.33 ± 1.67 mg/100g). Tannins in *D. edulis* were highest in the ethyl acetate fraction (1111.67 ± 9.28 mg/100g), followed by the butanol fraction, hexane fraction, methanol extract, and the raw leaf, while the aqueous fraction (13.33 ± 1.67 mg/100g) was least in value.

The methanol extract (961.67 ± 7.26 mg/100g) showed the highest value in alkaloids for *D. edulis*. This was followed by the raw leaf, ethyl acetate fraction, butanol fraction, and aqueous fraction, while the hexane fraction (416.67 ± 6.01 mg/100g) gave the least value. The hexane fraction (92.33 ± 0.15 GAE/g) gave the highest value in phenolics for *D. edulis*. This was followed by the butanol fraction, ethyl acetate fraction, methanol extract, and aqueous fraction, while the raw leaf has the least value (8.57 ± 0.15 GAE/g).

Anthraquinones in *D. edulis* were highest in the butanol fraction (831.67 ± 10.14 mg/100g), followed by the ethyl acetate fraction, methanol extract, hexane fraction, and aqueous fraction, while the raw leaf was least in value (116.67 ± 6.01 mg/100g). Flavonoids in *D. edulis* were highest in the raw leaf (1505.00 ± 10.41 mg/100g), followed by the butanol fraction, ethyl acetate fraction, methanol extract, aqueous fraction, while the hexane fraction (183.33 ± 3.33 mg/100g) gave the least value.

The butanol fraction (673.33 ± 7.26 mg/100g) showed the highest value in steroids for *D. edulis*. This was followed by the ethyl acetate fraction, methanol extract, raw leaf, and aqueous fraction, while the hexane fraction (345.00 ± 8.66 mg/100g) produced the least value. Terpenoids in *D.*

edulis were highest in the butanol fraction (948.33 ± 10.14 mg/100g), followed by the aqueous fraction, hexane fraction, methanol extract, and ethyl acetate fraction, while the raw leaf was least in value (81.67 ± 4.41 mg/100g).

The methanol extract (33.33 ± 1.67 mg/100g) showed the highest value in cardiac glycosides for *D. edulis*, followed by the hexane fraction, aqueous fraction, and ethyl acetate fraction, while the butanol fraction (11.67 ± 1.67 mg/100g) produced the least value. Carotenoids in *D. edulis* were highest in the butanol fraction (2141.67 ± 6.01 µg/100g), followed by the aqueous fraction, ethyl acetate fraction, hexane fraction, and methanol extract, with the raw leaf showing the least value (720.00 ± 2.89 µg/100g).

4.5 Phytochemical constituents of *Anacardium occidentale* leaf

The phytochemical components in *Anacardium occidentale* leaf extract and fractions showed the following sequences (Table 4.4). The butanol fraction (1750.00 ± 7.64 mg/100g) of *A. occidentale* had the highest value for saponins, followed by the hexane fraction, methanol extract, ethyl acetate fraction, aqueous fraction, while the raw leaf (control) had the least value (438.33 ± 6.01 mg/100g). The highest value for tannins in *A. occidentale* was shown by the butanol fraction (860.00 ± 10.41 mg/100g). It was followed by the ethyl acetate fraction, hexane fraction, methanol extract, and aqueous fraction, while the raw leaf was least in value (18.33 ± 1.67 mg/100g).

The raw leaf (1105.00 ± 10.41 mg/100g) of *A. occidentale* was highest in alkaloids, followed by the methanol extract, aqueous fraction, ethyl acetate fraction, hexane fraction, while the least value was observed in the butanol fraction (91.67 ± 1.67 mg/100g). Phenolics were highest in the methanol extract (98.30 ± 0.15 GAE/g) of *A. occidentale*, followed by the butanol fraction, hexane fraction, ethyl acetate fraction, aqueous fraction, but the raw leaf (21.43 ± 0.18 GAE/g) produced the least amount of phenolics. Anthraquinones in *A. occidentale* were highest in the ethyl acetate fraction (598.33 ± 4.41 mg/100g), followed by the methanol extract, aqueous fraction, butanol fraction, and hexane fraction, while the raw leaf had the least value (93.33 ± 1.67 mg/100g).

The raw leaf (2196.67 ± 8.82 mg/100g) of *A. occidentale* was highest in flavonoids, the methanol extract, ethyl acetate fraction, aqueous fraction, and hexane fraction followed it, while

the least value was in the butanol fraction (155.00 ± 7.64 mg/100g). Steroids were highest in the aqueous fraction (528.33 ± 6.01 mg/100g) of *A. occidentale*, followed by the methanol extract, butanol fraction, ethyl acetate fraction, and hexane fraction; the raw leaf produced the least value (261.67 ± 6.01 mg/100g).

The aqueous fraction of *A. occidentale* yielded the highest value of terpenoids (581.67 ± 6.01 mg/100g). This was followed by the butanol fraction, ethyl acetate fraction, hexane fraction, and methanol extract. The lowest value was observed in the raw leaf with a value of 115.00 ± 5.00 mg/100g. The ethyl acetate fraction (43.33 ± 4.41 mg/100g) of *A. occidentale* had the highest value for cardiac glycosides, followed by the aqueous fraction, methanol extract, butanol fraction, and hexane fraction (11.67 ± 1.67 mg/100g). The aqueous fraction of *A. occidentale* was highest in carotenoids (2126.67 ± 7.26 µg/100g). It was followed by the butanol fraction, methanol extract, ethyl acetate fraction, and hexane fraction, while the raw leaf showed the least value (711.67 ± 4.41 µg/100g). The phytochemical data are in the appendix IV.

4.6 Antibacterial activities of *Dacryodes edulis* and *Anacardium occidentale*

The microbial susceptibility to the plant extracts were shown by clear zones of growth inhibition around the holes, therefore revealing the relative activity of the test plant extracts against the bacteria. The highest zone of inhibition was observed at the highest concentration (100 mg/mL). None of the blank solvents (methanol, hexane, ethyl acetate, and water) showed a zone of inhibition.

4.6.1 Antibacterial activity of *Dacryodes edulis*

The ethyl acetate fraction of *D. edulis* was active for all the concentrations, giving its highest zone of inhibition as 17.0 mm for 100 mg/mL against *Staphylococcus aureus*; followed by its methanol extract: 13.0 mm for 100 mg/mL. The hexane and aqueous fractions showed no zone of inhibition against *S. aureus* (Table 4.5). Also, the ethyl acetate fraction showed the highest zone of inhibition of 12.0 mm for 100 mg/mL against *Serratia marcescens*. The methanol extract, hexane and aqueous fractions of *D. edulis* showed no zone of inhibition against *S. marcescens*. Also, the ethyl acetate fraction has the highest zone of inhibition at 100 mg/mL against *Bacillus cereus*: 20.0 mm, followed by the methanol extract: 14.0 mm, and hexane fraction: 4.0 mm. Its aqueous fraction showed no zone of inhibition against *B. cereus*.

Table 4.3: Phytochemical constituents of *Dacryodes edulis* leaf extracts

Parameters	Quantity per solvent of extraction					
	M	**H**	**E**	**B**	**A**	**J**
Saponins (mg/100g)	571.67 ± 7.26^{c}	1628.33 ± 18.78^{a}	636.67 ± 9.28^{b}	13.33 ± 1.67^{f}	358.33 ± 7.26^{d}	231.67 ± 7.26^{e}
Tannins (mg/100g)	76.67 ± 4.41^{d}	91.67 ± 1.67^{c}	1111.67 ± 9.28^{a}	168.33 ± 6.01^{b}	13.33 ± 1.67^{f}	50.00 ± 2.87^{e}
Alkaloids (mg/100g)	961.67 ± 7.26^{a}	416.67 ± 6.01^{f}	808.33 ± 4.41^{c}	630.00 ± 10.00^{d}	480.00 ± 10.41^{e}	916.67 ± 10.14^{b}
Phenolics (GAE/g)	26.67 ± 0.15^{d}	92.33 ± 0.15^{a}	62.27 ± 0.15^{c}	79.47 ± 0.12^{b}	25.33 ± 0.09^{e}	8.57 ± 0.15^{f}
Anthraquinones (mg/100g)	435.00 ± 7.64^{c}	235.00 ± 10.41^{d}	578.33 ± 9.28^{b}	831.67 ± 10.14^{a}	165.00 ± 8.66^{e}	116.67 ± 6.01^{f}
Flavonoids (mg/100g)	355.00 ± 8.66^{d}	183.33 ± 3.33^{f}	855.00 ± 7.64^{c}	1435.00 ± 10.41^{b}	230.00 ± 7.64^{e}	1505.00 ± 10.41^{a}
Steroids (mg/100g)	551.67 ± 6.01^{b}	345.00 ± 8.66^{e}	650.00 ± 7.64^{a}	673.33 ± 7.26^{a}	381.67 ± 10.93^{d}	418.33 ± 4.41^{c}
Terpenoids (mg/100g)	471.67 ± 4.41^{d}	525.00 ± 7.64^{c}	381.67 ± 6.01^{e}	948.33 ± 10.14^{a}	670.00 ± 7.64^{b}	81.67 ± 4.41^{f}
Cardiac glycosides (mg/100g)	33.33 ± 1.67^{a}	20.00 ± 0.00^{b}	13.33 ± 1.67^{c}	11.67 ± 1.67^{d}	20.00 ± 2.89^{b}	ND
Carotenoids (µg/100g)	1083.33 ± 7.26^{d}	1535.00 ± 8.66^{c}	1718.33 ± 10.14^{b}	2141.67 ± 6.01^{a}	1726.67 ± 6.01^{b}	720.00 ± 2.89^{e}

n = 3; Values = Means ± S.E., superscripts are level of significance ($p < 0.05$) for rows, M = Methanol extract of *D. edulis*, H = Hexane fraction of *D. edulis*, E = Ethyl acetate fraction of *D. edulis*, B = Butanol fraction of *D. edulis*, A = Aqueous fraction of *D. edulis*, J = Raw leaf (control) of *D. edulis*. ND = Not detected.

*1000 µg = 1mg.

Table 4.4: Phytochemical constituents of *Anacardium occidentale* leaf extracts

Parameters	Quantity per solvent of extraction					
	M	**H**	**E**	**B**	**A**	**K**
Saponins (mg/100g)	1625.00 ± 8.66^{c}	1703.33 ± 6.01^{b}	1455.00 ± 10.41^{d}	1750.00 ± 7.64^{a}	691.67 ± 10.14^{e}	438.33 ± 6.01^{f}
Tannins (mg/100g)	26.67 ± 1.67^{d}	151.67 ± 4.41^{c}	635.00 ± 8.66^{b}	860.00 ± 10.41^{a}	23.33 ± 1.67^{d}	18.33 ± 1.67^{d}
Alkaloids (mg/100g)	683.33 ± 9.28^{b}	216.67 ± 4.41^{e}	258.33 ± 7.26^{d}	91.67 ± 1.67^{f}	326.67 ± 6.01^{c}	1105.00 ± 10.41^{a}
Phenolics (GAE/g)	98.30 ± 0.15^{a}	67.27 ± 0.15^{c}	48.70 ± 0.12^{d}	76.63 ± 0.12^{b}	36.43 ± 0.12^{e}	21.43 ± 0.18^{f}
Anthraquinones (mg/100g)	523.33 ± 6.01^{b}	380.00 ± 7.64^{e}	598.33 ± 4.41^{a}	413.33 ± 4.41^{d}	461.67 ± 4.41^{c}	93.33 ± 1.67^{f}
Flavonoids (mg/100g)	366.67 ± 6.01^{b}	175.00 ± 7.64^{c}	345.00 ± 7.64^{b}	155.00 ± 7.64^{c}	176.67 ± 9.28^{c}	2196.67 ± 8.82^{a}
Steroids (mg/100g)	446.67 ± 7.26^{b}	366.67 ± 6.01^{c}	385.00 ± 7.64^{c}	426.67 ± 9.28^{b}	528.33 ± 6.01^{a}	261.67 ± 6.01^{d}
Terpenoids (mg/100g)	345.00 ± 10.41^{e}	393.33 ± 6.01^{d}	421.67 ± 4.41^{c}	521.67 ± 7.26^{b}	581.67 ± 6.01^{a}	115.00 ± 5.00^{f}
Cardiac glycosides (mg/100g)	25.00 ± 2.87^{c}	11.67 ± 1.67^{e}	43.33 ± 4.41^{a}	16.67 ± 1.67^{d}	30.00 ± 2.89^{b}	ND
Carotenoids (µg/100g)	1935.00 ± 7.64^{c}	925.00 ± 7.64^{e}	1848.33 ± 9.28^{d}	2076.67 ± 6.01^{b}	2126.67 ± 7.26^{a}	711.67 ± 4.41^{f}

n = 3; Values = Means ± S.E., superscripts are level of significance ($p < 0.05$) for rows, M = Methanol extract of *A. occidentale*, H = Hexane fraction of *A. occidentale*, E = Ethyl acetate fraction of *A. occidentale*, B = Butanol fraction of *A. occidentale*, A = Aqueous fraction of *A. occidentale*, K = Raw leaf (control) of *A. occidentale*. ND = Not detected. *1000 µg = 1mg.

The ethyl acetate fraction had the highest zone of inhibition at 100 mg/mL against *Klebsiella pneumoniae*: 21.0 mm, followed by the methanol extract 13.0 mm, and hexane fraction: 2.0 mm. The aqueous fraction showed no zone of inhibition against *K. pneumoniae*. Also, the ethyl acetate and hexane fractions revealed the highest zone of inhibition at 100 mg/mL against *Proteus vulgaris*: 8.0 mm. The methanol extract and aqueous fraction showed no zone of inhibition against *P. vulgaris*. However, the aqueous fraction of *D. edulis* had the highest zone of inhibition at 100 mg/mL against *Pseudomonas aeruginosa*: 12.0 mm, followed by the ethyl acetate fraction: 9.0 mm. The methanol extract and hexane fraction showed no zone of inhibition against *Pseudomonas aeruginosa* (Table 4.5).

4.6.2 Antibacterial activity of *Anacardium occidentale*

Ethyl acetate fraction showed the highest zone of inhibition for *A. occidentale*: 11.0 mm for 100 mg/mL against *Staphylococcus aureus*. Moreover, the methanol extract, aqueous and hexane fractions showed no zone of inhibition against *S. aureus*. Ethyl acetate fraction showed the highest zone of inhibition of 10.0 mm for 100 mg/mL against *Serratia marcescens*. The methanol extract, hexane and aqueous fractions revealed no zone of inhibition against *S. marcescens* (Table 4.6).

The ethyl acetate fraction had the highest of zone of inhibition at 100 mg/mL for *A. occidentale* against *Bacillus cereus*: 11.0 mm, followed by the aqueous fraction: 7.0 mm. The methanol extract and hexane fraction showed no zone of inhibition against *B. cereus*. Also, the ethyl acetate fraction produced the highest zone of inhibition at 100 mg/mL against *Klebsiella pneumoniae*: 10.0 mm, followed by the aqueous fraction: 7.0 mm. The methanol extract and hexane fraction showed no zone of inhibition against *K. pneumonia*.

The ethyl acetate fraction of *A. occidentale* had the highest zone of inhibition against *Proteus vulgaris*: 10.0 mm for 100 mg/mL. The methanol extract, aqueous and hexane fractions showed no zone of inhibition against *P. vulgaris*. Moreover, the ethyl acetate fraction revealed the highest zone of inhibition against *Pseudomonas aeruginosa*: 7.0 mm for 100 mg/mL. The methanol extract, hexane and aqueous fractions showed no zone of inhibition against *P. aeruginosa* (Table 4.6).

4.6.3 Comparing the antibacterial activities of *Dacryodes edulis* and *Anacardium occidentale*

Comparatively, the ethyl acetate fraction of *D. edulis* showed higher zones of inhibition at 100 mg/mL than *A. occidentale* against *Staphylococcus aureus*, *Serratia marcescens*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *Proteus vulgaris*. However, the aqueous fraction of *A. occidentale* was only higher than the ethyl acetate fraction of *D. edulis* against *Pseudomonas aeruginosa* (Tables 4.5 and 4.6).

The methanol extract of *D. edulis* was only active against *S. aureus*, *B. cereus* and *K. pneumoniae*. The aqueous fraction of *A. occidentale* was active against *K. pneumoniae*, unlike the aqueous fraction of *D. edulis*. The hexane fraction of *D. edulis* was only active against *B. cereus*, *K. pneumoniae*, and *P. vulgaris*. The order of antibacterial activity for *D. edulis*: ethyl acetate fraction > methanol extract > aqueous fraction > hexane fraction. However, the order of antibacterial activity for *A. occidentale*: ethyl acetate fraction > aqueous fraction. In *D. edulis*, *Proteus vulgaris* was the most resistible, while *K. pneumoniae* was the most susceptible; but in *A. occidentale*, *P. aeruginosa* was the most resistible, while *B. cereus* was the most susceptible (Tables 4.5 and 4.6).

4.7 Antifungal activities of *Dacryodes edulis* and *Anacardium occidentale*

Organisms that were resistant to the plant extract grew right up to the ditch, while susceptible ones showed a zone of inhibition adjacent to the ditch, thus indicating the relative activity of the test plant extract against the molds. None of the blank solvents (methanol, hexane, ethyl acetate, and water) showed a zone of inhibition.

The methanol extract and all the fractions of *Anacardium occidentale* did not show any antifungal acivity on all the tested molds. However, only the ethyl acetate and aqueous fractions of *D. edulis* showed antifungal activities. The ethyl acetate fraction showed the highest zone of inhibition at 100 mg/mL against *Trichophyton mentagrophytes* (54.0 mm), followed by *Cladosporium herbarum* (44.0 mm) and *Trichoderma* species (19.0 mm), while the aqueous fraction showed a zone of inhibition of 47.0 mm against *C. herbarum* at 100 mg/mL (Table 4.7). *Dacryodes edulis* and *A. occidentale* leaves did not show antifungal activity against *Penicillium camemberti*, *Aspergillus flavus* and *Rhizopus stolonifer*.

4.8 Antimicrobial potentials of the leaf ethyl acetate fractions of *Dacryodes edulis* and *Anacardium occidentale*

Anacardium occidentale had the highest zone of inhibition against *Candida albicans* (24 mm) at 200 mg/mL. This was followed by *D. edulis* against *C. albicans* (20 mm), *A. occidentale* against *Tricophyton rubrum* (18 mm), and *D. edulis* against *Pseudomonas aeruginosa* clinical isolate (16 mm). The same zone of inhibition (14 mm) at 200 mg/mL was recorded for *D. edulis* against *P. aeruginosa* (ATCC 29213) and *T. rubrum*, and *A. occidentale* against *P. aeruginosa* clinical isolate and *P. aeruginosa* (ATCC 29213). Likewise at 200 mg/mL, the same zone of inhibition (10 mm) was recorded for *A. occidentale* against *Staphylococcus aureus* clinical isolate and *S. aureus* (ATCC 27853). The same and least zone of inhibition (8 mm) at 200 mg/mL was recorded for *D. edulis* against *S. aureus* clinical isolate, *S. aureus* (ATCC 27853), *Epidermophyton* species, and also for *A. occidentale* against *Epidermophyton* species (Table 4.8). The antimicrobial plates are in the appendix IV.

4.9 Functional groups in *Dacryodes edulis* and *Anacardium occidentale* leaves

The Fourier transform infrared (FTIR) spectra generated for the ethyl acetate fractions of *D. edulis* and *A. occidentale* leaves are shown in Figures 4.9 and 4.10 respectively, while the interpretations of their peaks are shown in Tables 4.9 and 4.10 respectively. The classes of the highest peak (758.29) and lowest peak (3366.00) for *D. edulis* are aromatics and carboxylic acids respectively, while the classes of the highest peak (719.86) and lowest peak (3386.00) for *A. occidentale* are alkenes and carboxylic acids respectively. The data for FTIR are in appendix V.

4.10 Acute toxicity of *Dacryodes edulis* and *Anacardium occidentale* leaves on rats

No mortality was recorded during the experiment, showing that the ethyl acetate and aqueous fractions of *D. edulis* and *A. occidentale* leaves were not toxic. The photomicrographs of the livers and kidneys of the Wistar rats are shown in Plates 1 - 4 for *D. edulis*, and Plates 5 - 8 for *A. occidentale*. There were a little weight losses in the rats given aqueous fractions of *A. occidentale* and *D. edulis* when compared with the rats administered only 20 % Dimethyl sulphoxide (control), having the highest value (Table 4.11). Moreover, there was a significant weight loss in the rats administered with ethyl acetate fraction of *D. edulis* and those given ethyl acetate fraction of *A. occidentale*, compared with the control.

Table 4.5: Antimicrobial activity of *Dacryodes edulis* leaf extracts on selected bacteria

Microorganism	Extract concentration (mg/mL)					
	3.125	**6.25**	**12.5**	**25**	**50**	**100**
	Zones of inhibition (mm)					
Staphylococcus aureus (NCIB 8588)	E (4) M (2)	E (7) M (4)	E (8) M (7)	E (10) M (8)	E (12) M (10)	E (17) M (13)
Serratia marcescens (NCIB 1377)	-	-	E (1)	E (3)	E (7)	E (12)
Bacillus cereus (NCIB 6349)	E (2)	E (9) M (4)	E (12) M (5)	E (14) M (8)	E (17) M (10) H (2)	E (20) M (14) H (4)
Klebsiella pneumoniae (NCIB 418)	E (1)	E (9) M (3)	E (15) M (5)	E (17) M (7)	E (19) M (9) H (1)	E (21) M (13) H (2)
Proteus vulgaris (NCIB 67)	-	-	-	E (1)	E (6) H (2)	E (8) H (8)
Pseudomonas aeruginosa (NCIB 950)	-	-	W (7)	W (8) E (2)	W (10) E (4)	W (12) E (9)

M = methanol extract, H = hexane fraction, E = ethyl acetate fraction, W = aqueous fraction, - = No inhibition, NCIB = National Collection of Industrial Bacteria, Scotland.

Table 4.6: Antimicrobial activity of *Anacardium occidentale* leaf extracts on selected bacteria

Microorganism	Extract concentration (mg/mL)					
	3.125	6.25	12.5	25	50	100
	Zones of inhibition (mm)					
Staphylococcus aureus (NCIB 8588)	-	-	E (3)	E (4)	E (6)	E (11)
Serratia marcescens (NCIB 1377)	-	-	E (1)	E (4)	E (6)	E (10)
Bacillus cereus (NCIB 6349)	-	-	E (1)	E (3) W (2)	E (7) W (5)	E (11) W (7)
Klebsiella pneumoniae (NCIB 418)	-	-	E (1)	E (4) W (3)	E (5) W (5)	E (10) W (7)
Proteus vulgaris (NCIB 67)	-	-	E (1)	E (3)	E (5)	E (10)
Pseudomonas aeruginosa (NCIB 950)	-	E (1)	E (2)	E (4)	E (5)	E (7)

E = ethyl acetate fraction, W = aqueous fraction, - = No inhibition, NCIB = National Collection of Industrial Bacteria, Scotland.

Table 4.7: Antimicrobial activity of *Dacryodes edulis* leaf extracts on selected fungi

Microorganism	Extract concentration (mg/mL)					
	3.125	6.25	12.5	25	50	100
	Zones of inhibition (mm)					
Penicillium camemberti	-	-	-	-	-	-
Trichophyton mentagrophytes	-	-	-	E (14)	E (21)	E (49)
Aspergillus flavus	-	-	-	-	-	-
Rhizopus stolonifer	-	-	-	-	-	-
Trichoderma sp.	-	-	-	-	-	E (14)
Cladosporium herbarum	-	-	-	-	-	W (42)
	-	-	-	E (13)	E (25)	E (39)

E = ethyl acetate fraction, W = aqueous fraction, - = No inhibition.

Table 4.8: Antimicrobial activity of *Dacryodes edulis* and *Anacardium occidentale* leaf ethyl acetate fractions

Microorganism	Extract concentration (mg/mL)										Controls	
	De	Ao	De	Ao	De	Ao	De	Ao	De	Ao	AmT	KeT
	12.5		25		50		100		200		20 µg/mL	50 mg/mL
	Zones of inhibition (mm)											
Staphylococcus aureus	4	4	4	6	6	6	6	8	8	10	8	NA
Staphylococcus aureus (ATCC 27853)	-	4	-	6	4	8	6	8	8	10	10	NA
Pseudomonas aeruginosa	8	6	10	8	12	10	14	12	16	14	8	NA
Pseudomonas aeruginosa (ATCC 29213)	-	6	4	8	6	10	8	12	14	14	10	NA
Tricophyton rubrum	-	6	4	8	6	10	12	14	14	18	NA	10
Epidermophyton sp.	-	-	-	-	-	4	4	6	8	8	NA	10
Candida albicans	-	4	4	8	4	18	6	20	20	24	NA	10

ATCC = American Type Culture Collection. De = *Dacryodes edulis*, Ao = *Anacardium occidentale*, AmT = Amoxillin tablet, KeT = Ketoconazole tablet, NA = Not applicable, - = No inhibition.

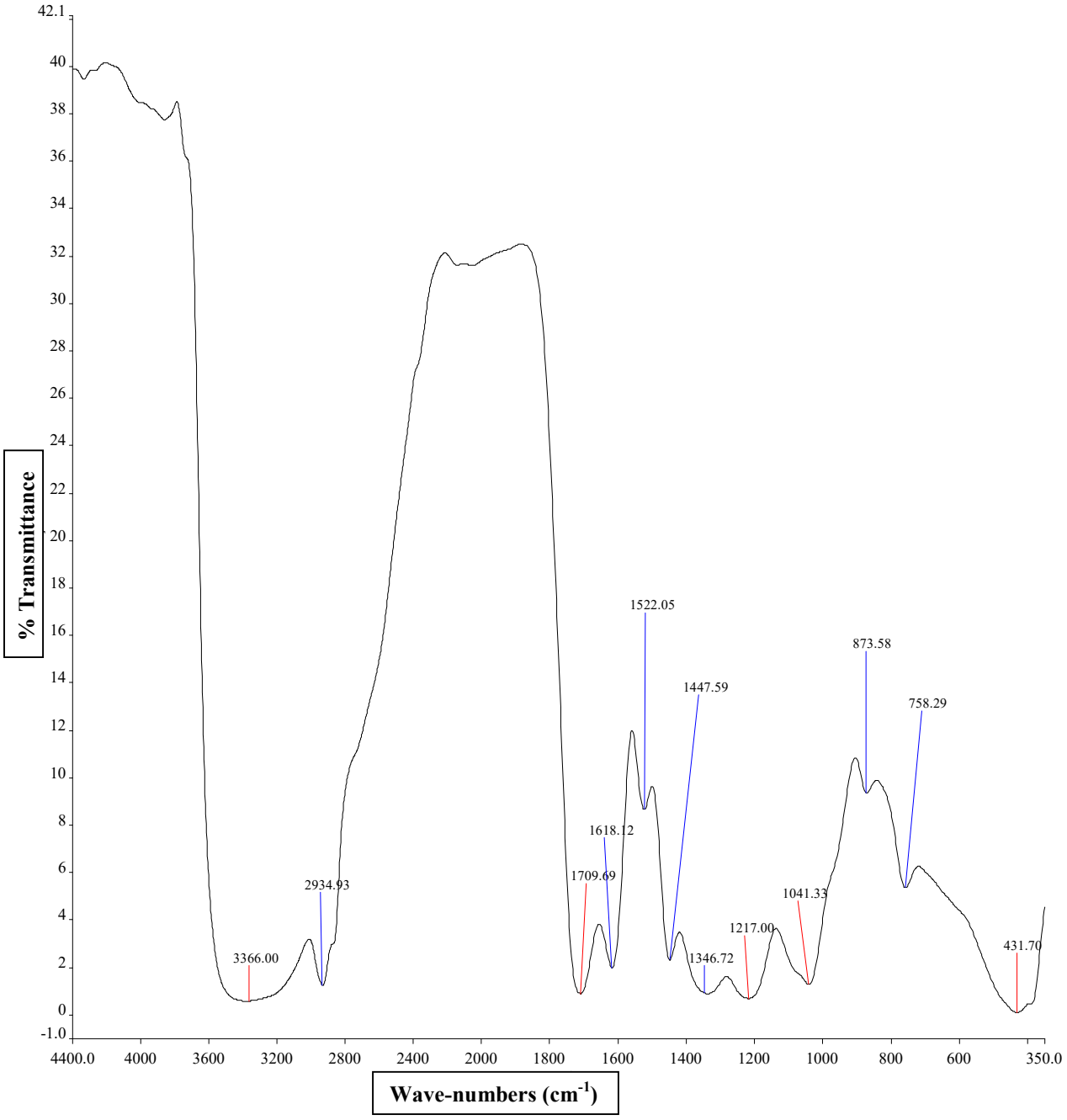


Figure 4.9: Fourier transform infrared spectrum of *Dacryodes edulis* leaf ethyl acetate fraction

Table 4.9: **Functional groups in *Dacryodes edulis* leaf ethyl acetate fraction**

S/N	Peak	Class	Structure	Intensity	Assignment
1	758.29	Aromatics	Monosubst. ; ortho-disub.	M	C-H out of plane
2	873.58	Aromatics	1, 2, 3, 4, 5-pentasub.	W	C-H out of plane
3	1041.33	Misc.	P-OR esters	S	P-OR esters
4	1217.00	Amines	RNH_2; R_2NH	M	C-N stretch
5	1346.72	Alkyl halides	R-F	S	C-F stretch
6	1447.59	Misc.	S=O sulphate	S	S=O sulphate ester
7	1522.05	Misc.	N-O nitro comp.	m, s	Arom. Nitro
8	1618.12	Amides	$RCONH_2$	S	NH out of plane
9	1709.69	Carboxylic acids	RCO-OH	s	Dimer C=O
10	2934.93	Alkanes	RCH_2CH_3	S	CH stretch
11	3366.00	Carboxylic acids	RCO-OH	s (broad)	Dimer OH

Misc. = miscellaneous, m = medium, s = strong, w = weak.

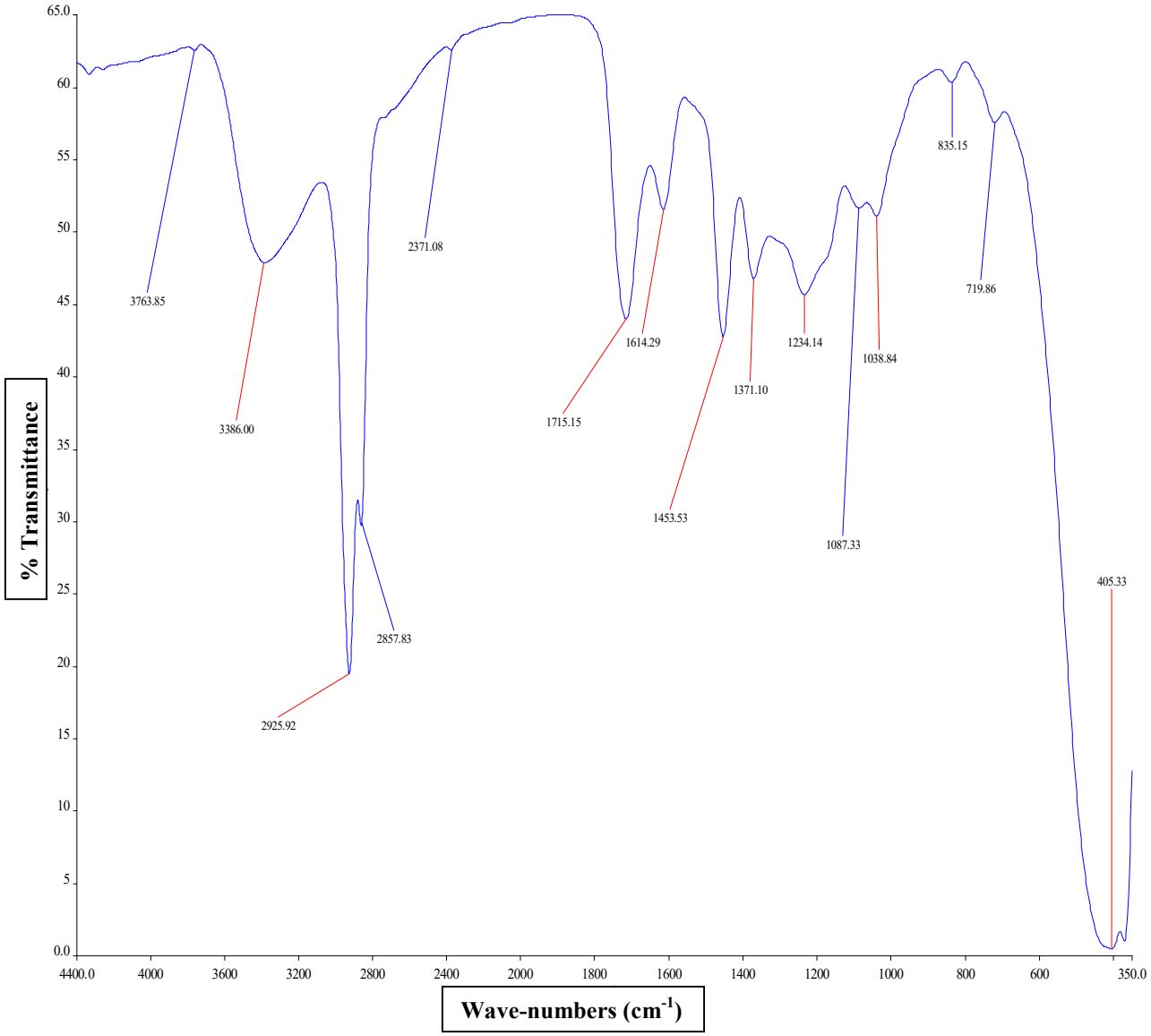


Figure 4.10: Fourier transform infrared spectrum *Anacardium occidentale* leaf ethyl acetate fraction

Table 4.10: **Functional groups in *Anacardium occidentale* leaf ethyl acetate fraction**

S/N	Peak	Class	Structure	Intensity	Assignment
1	719.86	Alkenes	Trans RCH=CHR	M	=CH out of plane
2	835.15	Alkenes	R_2C=CHR	S	=CH out of plane
		Aromatics	Para-disub.	M	C-H out of plane
3	1038.84	Misc.	P-OR esthers	S	P-OR esthers
4	1087.33	Ethers	R-O-R	S	C-O stretch
5	1234.14	Esthers	Ar-O-R	S	C-O stretch
6	1371.10	Alkanes	RCH_2CH_3	S	CH_2 and CH_3
7	1453.53	Alkanes	RCH_2CH_3	S	CH_2 and CH_3
8	1614.29	Alkenes	5-ring	W	C=C stretch
9	1715.15	Ketones	R_2CO	s	C=O stretch
10	2371.08	Misc.	P-H phosphine	M	P-H phosphine sharp
11	2857.83	Alkanes	RCH_2CH_3	S	CH stretch
12	2925.92	Alkanes	RCH_2CH_3	m, s	-CH_2-
13	3386.00	Carboxylic acids	RCO-OH	s (broad)	Dimer OH

Misc. = miscellaneous, m = medium, s = strong, w = weak.

Rats administered with the aqueous fraction of *D. edulis* had the highest splenosomatic index; while those given the aqueous and ethyl acetate fractions of *A. occidentale* were almost similar in indices. The least and same index was observed in rats administered with the ethyl acetate fraction of *D. edulis* and the control. There were no significant differences in the indices of all the plant extracts, compared with the control, therefore non-toxic to their spleens. Moreover, rats administered with the aqueous fraction of *A. occidentale* had the highest renosomatic index followed by the ethyl acetate fraction of *A. occidentale*, aqueous fraction of *D. edulis*, ethyl acetate fraction of *D. edulis*, while the control had the least index. There was no significant difference in the indices of all the plant fractions when compared with the control. Similarly, no visible lesion was observed on their kidneys (Plates 2, 4, 6 and 8); therefore they were non-toxic to their kidneys.

Rats administered aqueous fraction of *D. edulis* had the highest cardiosomatic index followed by the ethyl acetate fraction of *A. occidentale*, aqueous fraction of *A. occidentale*, ethyl acetate fraction of *D. edulis*, while the control had the least index. There was no significant difference in the indices of all the plant fractions, compared with the control, therefore non-toxic to their hearts. Moreover, rats administered with the aqueous fraction of *A. occidentale* produced the highest hepatosomatic index, followed by the aqueous fraction of *D. edulis*, ethyl acetate fraction of *D. edulis*, ethyl acetate fraction of *A. occidentale*, while the control had the least index. When compared with the control, there were significant differences of 2.22, 1.64, 1.48 and 1.33 g, respectively (Table 4.11). There was no visible lesion on their livers, consequently they were non-toxic to their livers (Plates 3 and 7), except at 5,000 mg/kg of ethyl acetate fractions of *D. edulis* and *A. occidentale* (Plates 1 and 5).

4.11 Physicochemical properties of *Dacryodes edulis* and *Anacardium occidentale* creams

All the formulated creams elicited a pleasant visual outlook with the colours for *D. edulis* creams ranging from light green to dark green and dark yellow to deep brown for *A. occidentale* creams. The colour change was based on the concentration of the fraction and their interaction with the base (Plate 9). The odour of each formulation was characteristic and could not be related to known odours. They were oil-in-water (O/W) creams that were less greasy and easily washed off with water. The evaluated organoleptic properties of the formulated creams are shown in Table 4.12. The physicochemical statistics are in appendix V.

Table 4.11: Toxicity of *Dacryodes edulis* and *Anacardium occidentale* leaves on rats

Parameters	CE	FE	PO	ZO	CX
Initial weights (g)	56.67 ± 3.47	48.89 ± 3.64	46.11 ± 5.80	41.11 ± 2.00	34.44 ± 5.46
Change in weights (g)	-3.67 ± 3.48	3.93 ± 2.43	-0.99 ± 2.90	4.09 ± 0.98	4.41 ± 0.06
Splenosomatic indices (g)	0.73 ± 0.07	1.01 ± 0.17	0.96 ± 0.07	0.96 ± 0.10	0.73 ± 0.07
Renosomatic indices (g)	1.10 ± 0.10	1.16 ± 0.09	1.19 ± 0.12	1.22 ± 0.11	1.04 ± 0.05
Cardiosomatic indices (g)	0.47 ± 0.01	0.63 ± 0.08	0.55 ± 0.05	0.47 ± 0.03	0.44 ± 0.03
Hepatosomatic indices (g)	4.90 ± 0.38	5.09 ± 0.36	4.75 ± 0.45	5.64 ± 0.62	3.42 ± 0.32

n = 5, Values = Means ± S.E ($p < 0.05$). Change in weight of rat (g) = final (carcass) weight – initial weight; CE = Rats administered *D. edulis* leaf ethyl acetate fraction, FE = Rats administered *D. edulis* leaf aqueous fraction, PO = Rats administered *A. occidentale* leaf ethyl acetate fraction, ZO = Rats administered *A. occidentale* leaf aqueous fraction, CX = Rats administered 20 % Dimethyl sulphoxide (Control). Dosage range: 10 – 1,000 mg/kg.

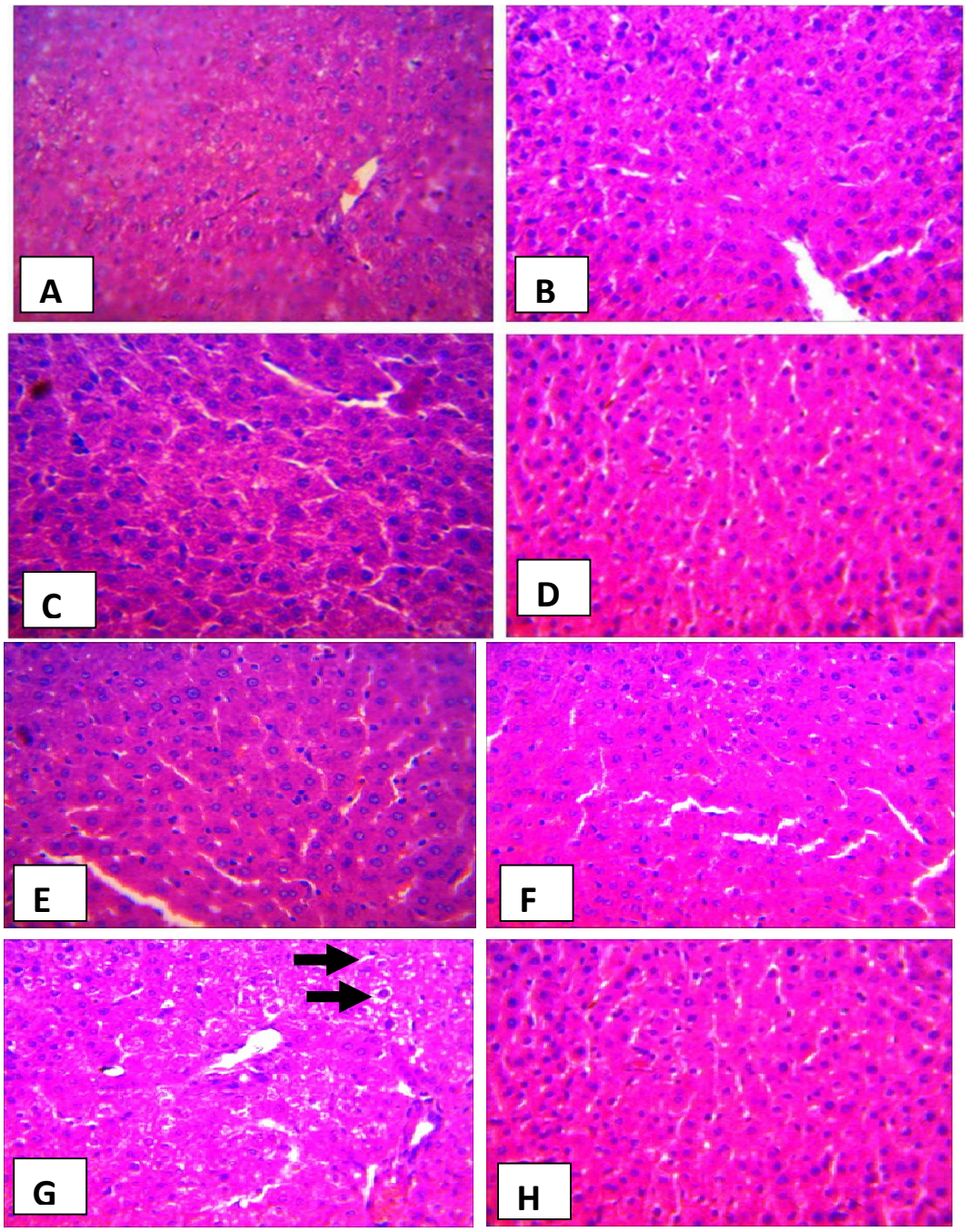


Plate 1: Liver photomicrographs (x400) of rats administered *Dacryodes edulis* leaf ethyl acetate fraction

A = Rat administered 10 mg/kg; B = Rat administered 100 mg/kg; C = Rat administered 1000 mg/kg; D = Rat administered 20 % Dimethyl sulphoxide (control I); E = Rat administered 1,600 mg/kg; F = Rat administered 2,900 mg/kg; G = Rat administered 5,000 mg/kg: showing mild vacuolar degeneration of hepatocytes (arrows); H = Rat without dose (control II). A – F and H show no visible lesions.

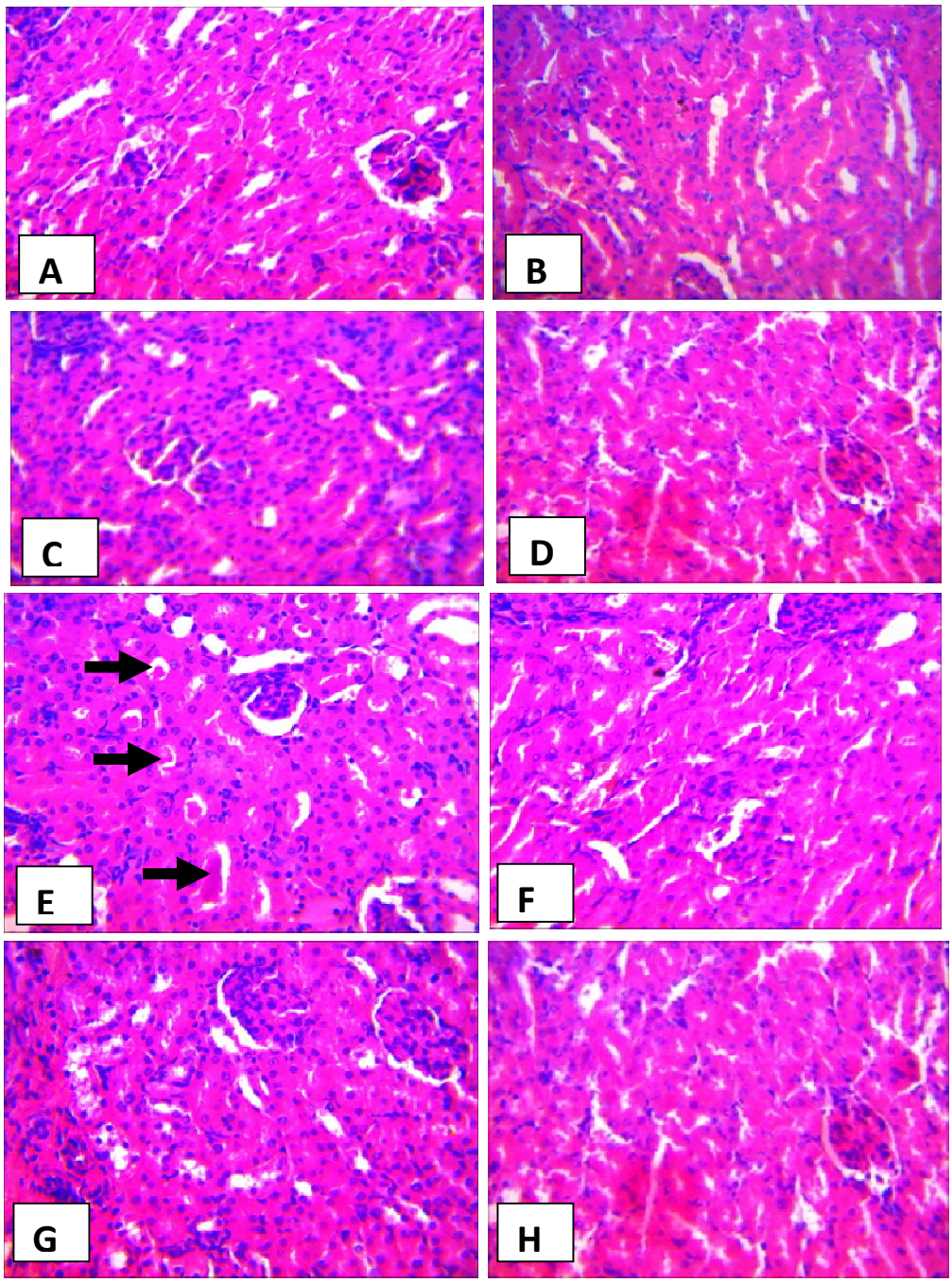


Plate 2: Kidney photomicrographs (x400) of rats administered *Dacryodes edulis* leaf ethyl acetate fraction

A = Rat administered 10 mg/kg; B = Rat administered 100 mg/kg; C = Rat administered 1000 mg/kg; D = Rat administered 20 % Dimethyl sulphoxide (control I); E = Rat administered 1,600 mg/kg: showing pink staining casts in the tubular lumen of the renal tubules (arrows); F = Rat administered 2,900 mg/kg; G = Rat administered 5,000 mg/kg; H = Rat without dose (control II). All show no visible lesions.

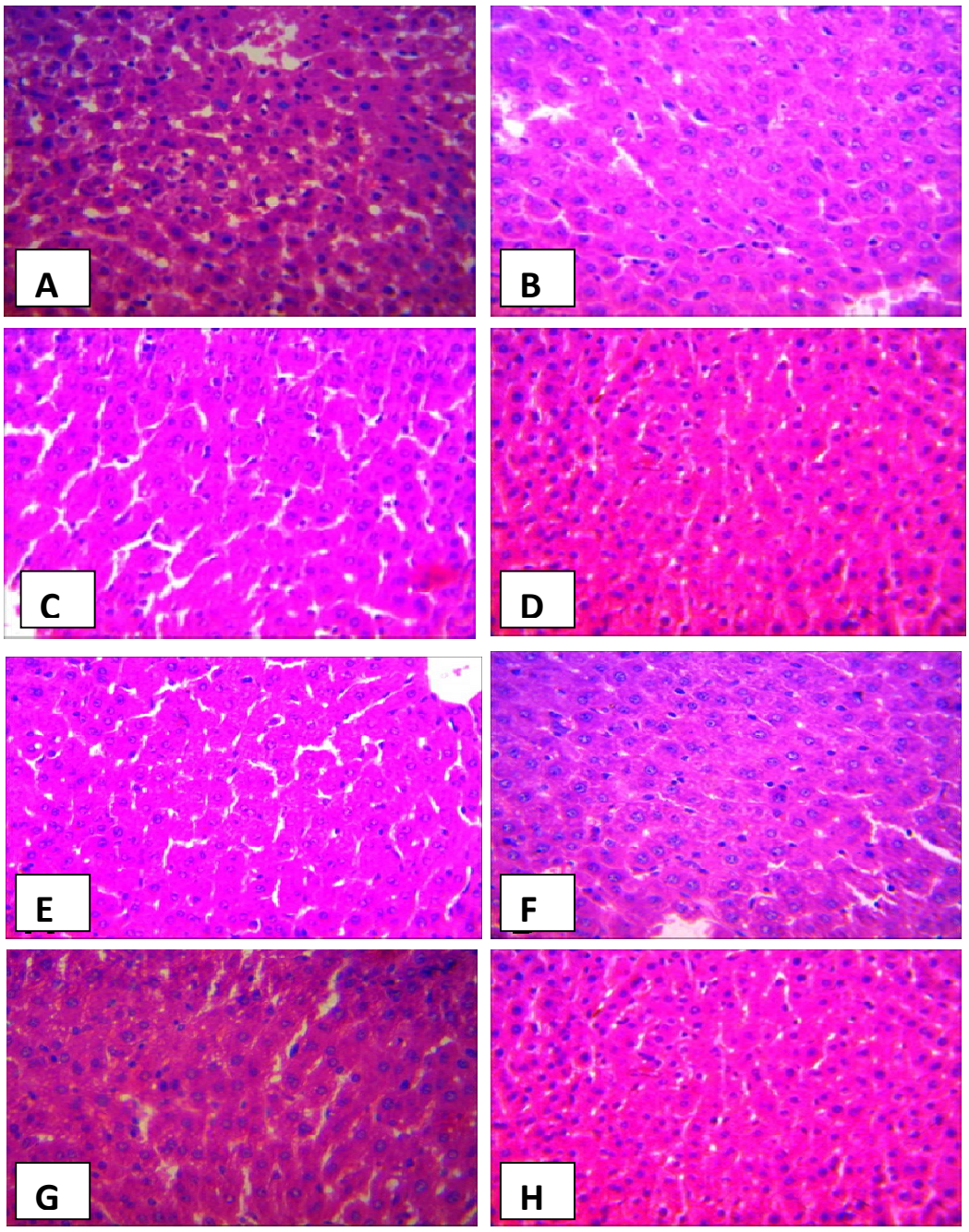


Plate 3: Liver photomicrographs (x400) of rats administered *Dacryodes edulis* leaf aqueous fraction

A = Rat administered 10 mg/kg; B = Rat administered 100 mg/kg; C = Rat administered 1000 mg/kg; D = Rat administered 20 % Dimethyl sulphoxide (control I); E = Rat administered 1,600 mg/kg; F = Rat administered 2,900 mg/kg; G = Rat administered 5,000 mg/kg; H = Rat without dose (control II). All show no visible lesions.

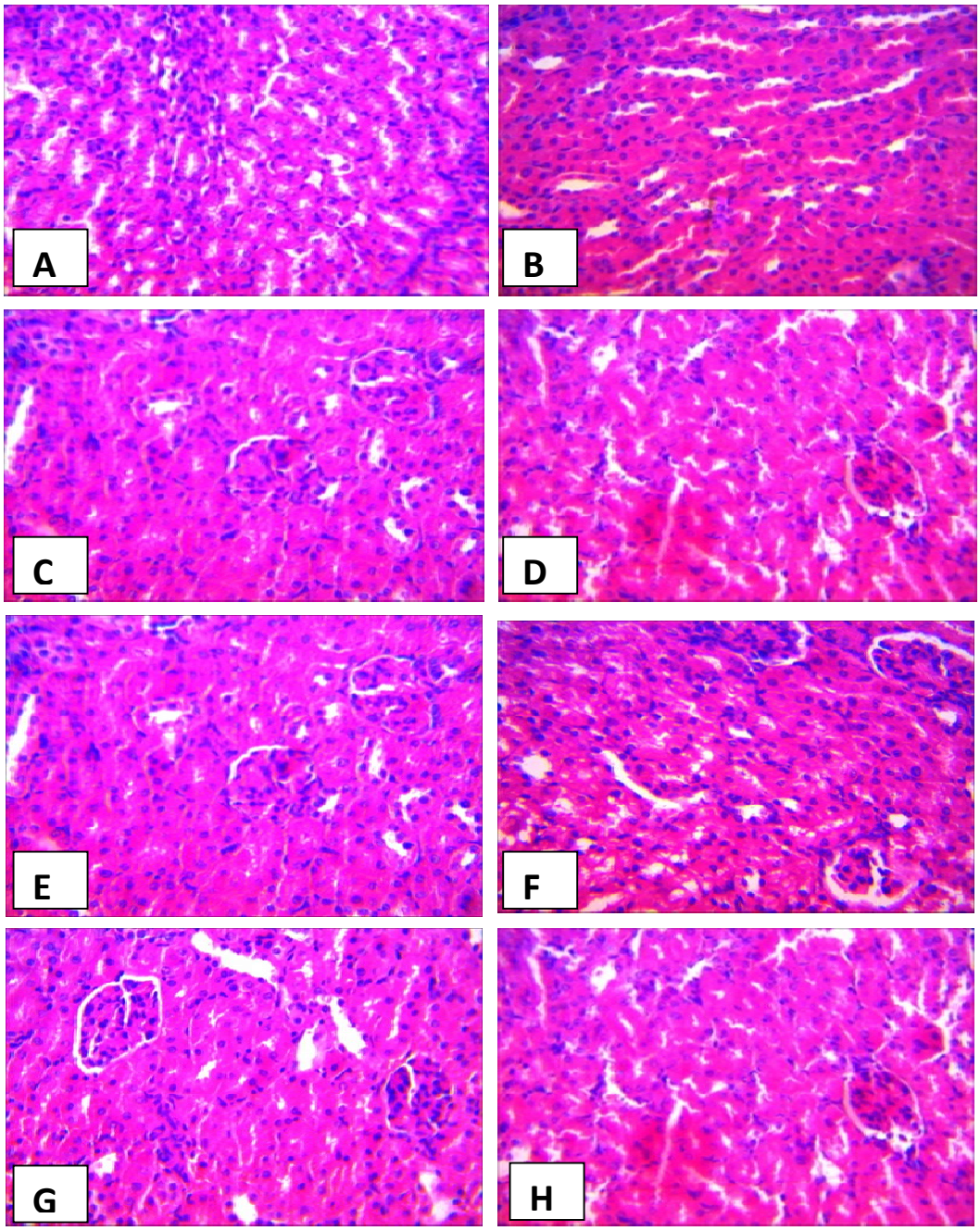


Plate 4: Kidney photomicrographs (x400) of rats administered *Dacryodes edulis* leaf aqueous fraction

A = Rat administered 10 mg/kg; B = Rat administered 100 mg/kg; C = Rat administered 1000 mg/kg; D = Rat administered 20 % Dimethyl sulphoxide (control I); E = Rat administered 1,600 mg/kg; F = Rat administered 2,900 mg/kg; G = Rat administered 5,000 mg/kg; H = Rat without dose (control II). All show no visible lesions.

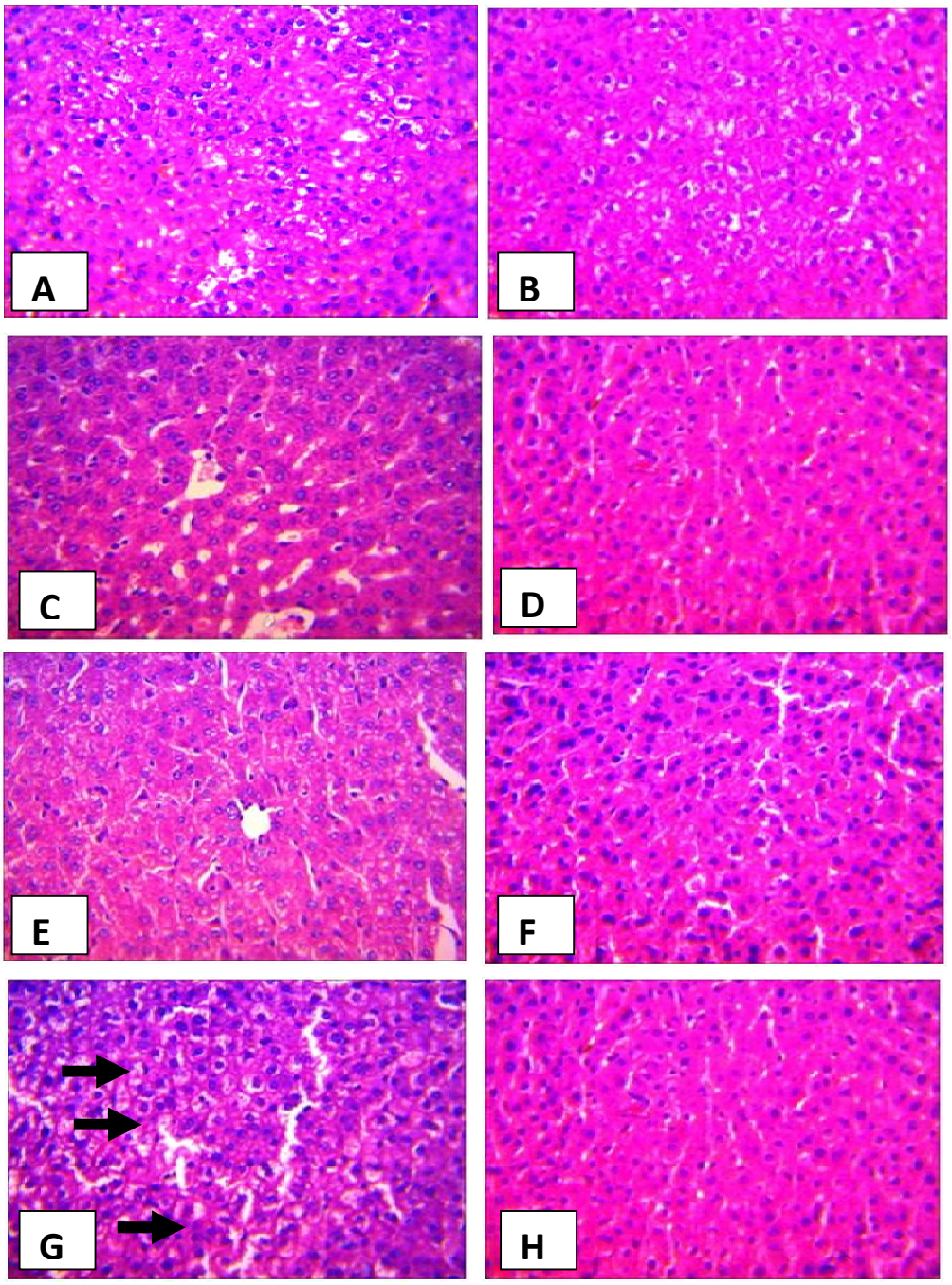


Plate 5: Liver photomicrographs (x400) of rats administered *Anacardium occidentale* leaf ethyl acetate fraction

A = Rat administered 10 mg/kg; B = Rat administered 100 mg/kg; C = Rat administered 1000 mg/kg; D = Rat administered 20 % Dimethyl sulphoxide (control I); E = Rat administered 1,600 mg/kg; F = Rat administered 2,900 mg/kg; G = Rat administered 5,000 mg/kg: there is a severe diffuse vacuolar degeneration of hepatocyte; H = Rat without dose (control II). All (except G) show no visible lesions.

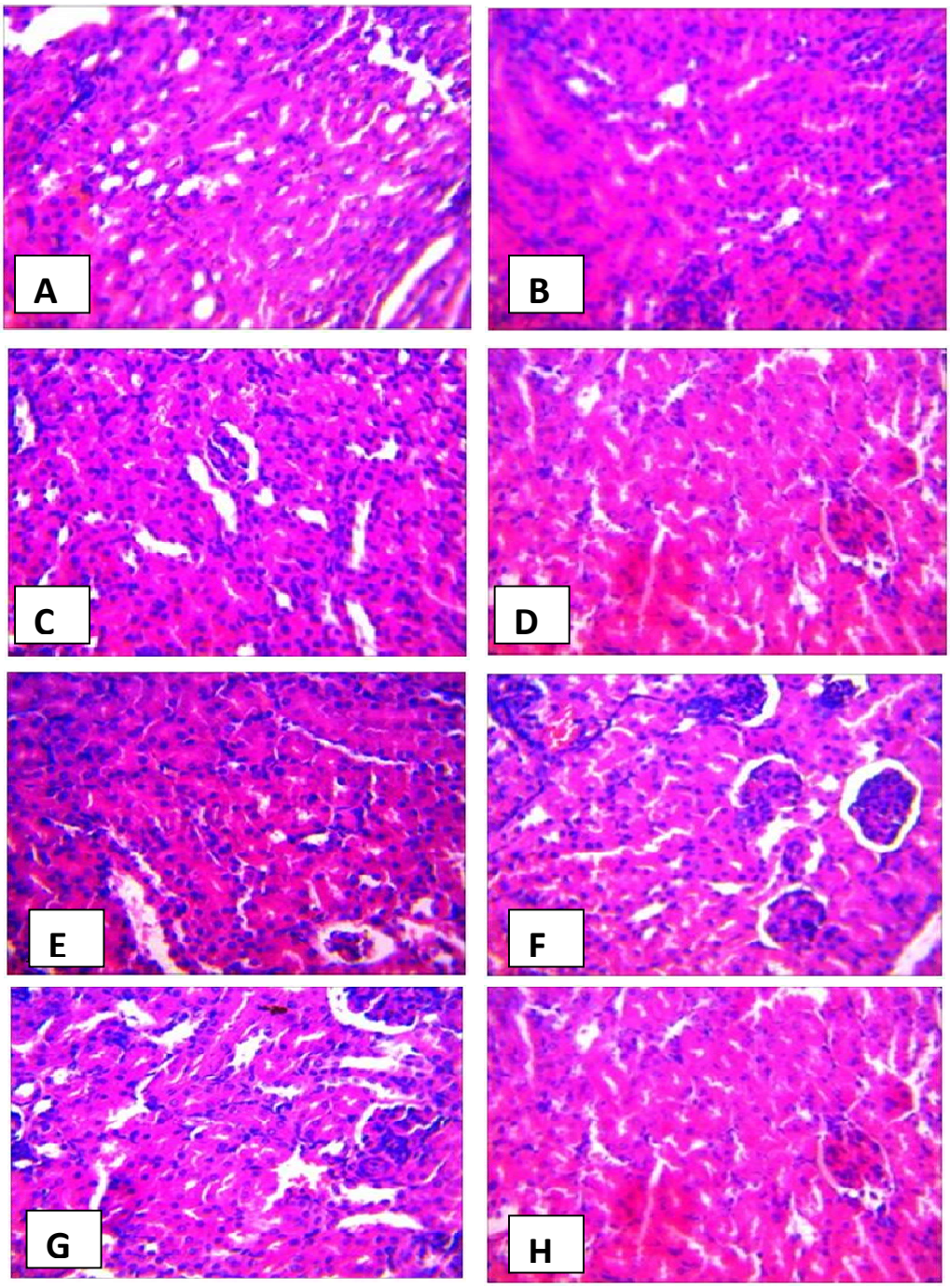


Plate 6: Kidney photomicrographs (x400) of rats administered *Anacardium occidentale* leaf ethyl acetate fraction

A = Rat administered 10 mg/kg; B = Rat administered 100 mg/kg; C = Rat administered 1000 mg/kg; D = Rat administered 20 % Dimethyl sulphoxide (control I); E = Rat administered 1,600 mg/kg; F = Rat administered 2,900 mg/kg; G = Rat administered 5,000 mg/kg; H = Rat without dose (control II). All show no visible lesions.

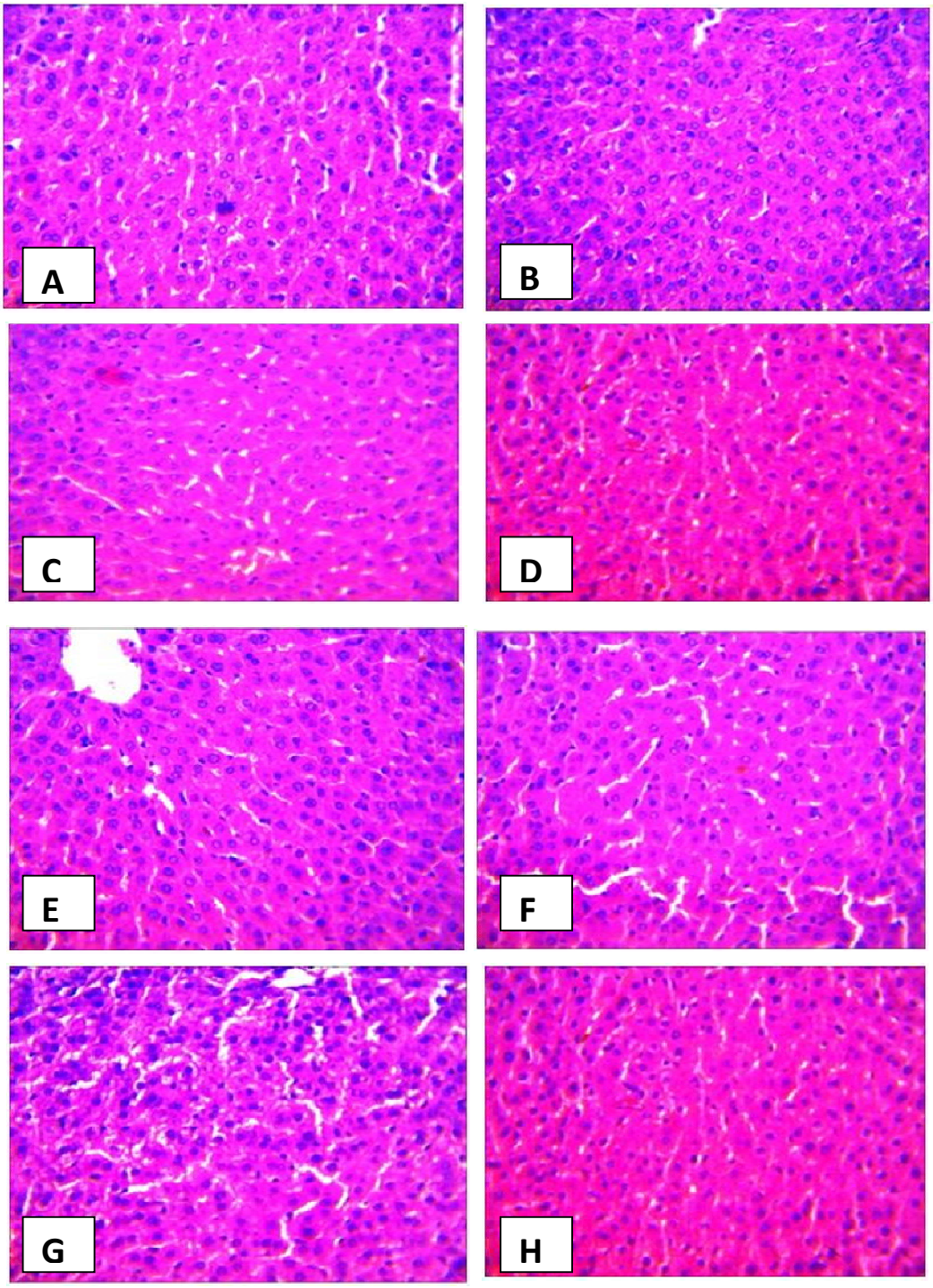


Plate 7: Liver photomicrographs (x400) of rats administered *Anacardium occidentale* leaf aqueous fraction

A = Rat administered 10 mg/kg; B = Rat administered 100 mg/kg; C = Rat administered 1000 mg/kg; D = Rat administered 20 % Dimethyl sulphoxide (control I); E = Rat administered 1,600 mg/kg; F = Rat administered 2,900 mg/kg; G = Rat administered 5,000 mg/kg; H = Rat without dose (control II). All show no visible lesions.

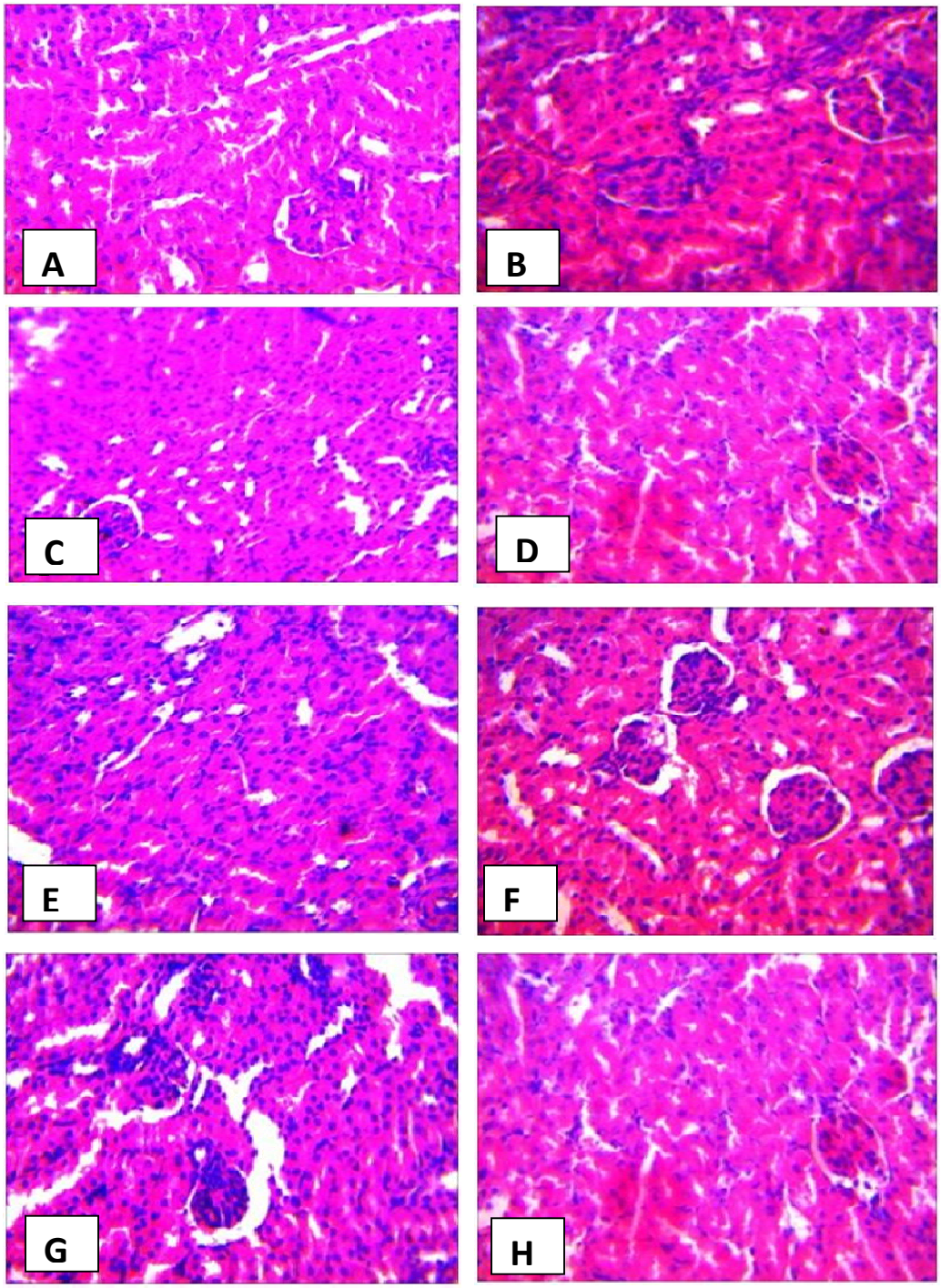


Plate 8: Kidney photomicrographs (x400) of rats administered *Anacardium occidentale* leaf aqueous fraction

A = Rat administered 10 mg/kg; B = Rat administered 100 mg/kg; C = Rat administered 1000 mg/kg; D = Rat administered 20 % Dimethyl sulphoxide (control I); E = Rat administered 1,600 mg/kg; F = Rat administered 2,900 mg/kg; G = Rat given 5,000 mg/kg; H = Rat without dose (control II). All show no visible lesions.

4.11.1 Physicochemical properties of *Dacryodes edulis* and *Anacardium occidentale* formulated creams

The density of the formulated creams increased, as the concentrations of the extracts increased (Table 4.12). The 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3) had the highest density (0.95 ± 0.02 g/cm^3), while 2.5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe1) was the least (0.90 ± 0.02 g/cm^3). The 10 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo3) had the highest density (0.98 ± 0.01 g/cm^3), while 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo1) was the least (0.78 ± 0.10 g/cm^3) as shown in Table 4.12.

The 2.5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe1) had the highest extruding time (5.87 ± 0.78 sec.); while the 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3) was the lowest (5.57 ± 0.64 sec.). Likewise, the 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo1) had the highest extruding time (6.01 ± 0.23 sec.), while 10 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo3) was the least (4.81 ± 0.27 sec.). The highest spreading length was recorded in the 2.5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe1) (4.93 ± 0.81 cm), while the 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3) was the least (3.53 ± 0.75 cm). Similarly, the 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo1) had the highest spreading length (4.28 ± 0.61 cm), while the 10 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo3) had the least (3.93 ± 1.03 cm).

The 2.5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe1) had the highest pH value (4.52 ± 0.08), while the 5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe2) was the least (3.24 ± 0.05). However, the 5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo2) had the highest pH value (4.01 ± 0.03), while the 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo1) was the least (3.58 ± 0.02). The 2.5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe1) had the highest rate of diffusion (3.33 mm/hr), while the 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3) was the least (1.58 mm/hr). The 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo1) had the highest rate of diffusion (3.00 mm/hr), while the 10 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo3) was the least (2.11 mm/hr).

The highest viscosity value was observed in the 2.5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe1) (1815.00 ± 148.49 cP), while the 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3) was the least (360.00 ± 16.33 cP). The 2.5 % leaf ethyl acetate fraction of *A.*

occidentale cream (FAo1) had the highest viscosity value (1277.50 ± 307.59 cP), while the 10 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo3) was the least (622.50 ± 3.54 cP). The 5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe2) had the largest globule size (95.99 ± 75.56 μm), while the 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3) was the least (29.12 ± 15.00 μm). Likewise, the 5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo2) had the largest diameter for the globule size (188.40 ± 98.88 μm), while the 10 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo3) was the least (55.38 ± 42.65 μm).

4.11.2 Comparing the physicochemical property of *Dacryodes edulis* and *Anacardium occidentale* creams

Comparing the creams in Table 4.12, the density showed thus trend FAo3 > FDe3 > FAo2 > FDe2 > FDe1 > FAo1.

The extruding time of all the creams showed that FAo3 > FDe3 > FDe2 > FAo2 > FDe1 > FAo1.

The spread lengths of all the creams showed that FAo1 > FDe3 > FDe2 > FAo2 > FDe1 > FAo3.

The pH values of the creams ranked thus: FDe1 > FAo2 > FAo3 > FAo1 > FDe3 > FDe2.

The diffusion rates for the creams are: FDe1 > FAo1 > FAo2 > FAo3 > FDe2 > FDe3.

The viscosities of the creams are; FDe1 > FDe2 > FAo1 > FAo2 > FAo3 > FDe3.

The globule sizes of the creams are; FAo2 > FDe2 > FAo1 > FAo3 > FDe1 > FDe3.

4.11.3 Viscosity and rotational speed of *Dacryodes edulis* and *Anacardium occidentale* creams

The values of viscosity were inversely proportional to those of the rotational speed. The viscosities of the 2.5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe1) and the 5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe2) are closer and higher than those of the 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3) (Figure 4.11). Likewise, the viscosities of the 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo1) and the 5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo2) are closer and higher than those of the 10 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo3) (Figure 4.12). Comparing the viscosity against

rotational speed (rheological profiles) for the creams in Figures 4.11 and 4.12, FDe1 > FAo1 (except at 100 rpm), FDe2 > FAo2, while FAo3 > FDe3 for all the revolutions per minute.

4.12 Stability profiles of the formulated creams

4.12.1 *Dacryodes edulis* creams viscosity at different temperatures

The mean viscosity for the 2.5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe1) stored at room temperature (29 ± 4 ^{o}C) had the highest value of mean viscosity (5060 ± 1590 cP), while the cream stored at high temperature (46 ^{o}C) produced the least (4085 ± 722.4 cP) as indicated in Figure 4.13. Likewise, for the 5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe2), the cream stored at room temperature (29 ± 4 ^{o}C) had the highest value of mean viscosity (4939 ± 1398 cP), while the cream stored at high temperature (46 ^{o}C) was the least (4050 ± 1108 cP). However, in the 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3), the cream stored at cold temperature (0 ^{o}C) had the highest value of mean viscosity (3705 ± 1107 cP), while the cream stored at high temperature (46 ^{o}C) produced the least (3134 ± 780.4 cP).

In FDe1 and FDe2, the cream stored at room temperature (R) had the highest viscosity while the cream stored in a hot place (H) produced the least. However in FDe3, the cream stored inside freezer (C) had the highest viscosity, while the cream stored in a hot place (H) gave the least value. However, there was no significant difference between the cream stored at room temperature (R), compared with the one stored inside freezer (C), likewise for R compared with H, and also for C compared with H, thus showing their stability (Figure 4.13).

4.12.2 *Anacardium occidentale* creams Viscosity at different temperatures

The mean viscosity for the 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo1) stored at room temperature (29 ± 4 ^{o}C) had the highest value of mean viscosity (5495 ± 1098 cP), while the cream stored at cold temperature (0 ^{o}C) produced the least value (3640 ± 914.9 cP) as shown in Figure 4.14. Likewise, the 5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo2) stored at room temperature (29 ± 4 ^{o}C) had the highest value of mean viscosity (4721 ± 1512 cP), while the cream stored at cold temperature (0 ^{o}C) gave the least value (3615 ± 1576 cP). Similarly, the 10 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo3) stored at room temperature (29 ± 4 ^{o}C) had the highest value of mean viscosity (3854 ± 1117 cP), while the cream stored at cold temperature (0 ^{o}C) had the least value (2510 ± 728.4 cP).

Table 4.12: Physicochemical properties of *Dacryodes edulis* and *Anacardium occidentale* formulated creams

Creams	Density (g/cm^3)	Extruding time (sec.)	Spreading length (cm)	pH	Viscosity (cP)	Globule size (µm)	Diffusion rate (mm/hr)	Colour	Texture
FDe1	0.90 ± 0.02	5.87 ± 0.78	4.93 ± 0.81	4.52 ± 0.08	1815.00 ± 148.49	53.21 ± 35.02	3.33	Light green	Smooth
FDe2	0.92 ± 0.01	5.73 ± 0.65	4.15 ± 0.61	3.24 ± 0.05	1725.75 ± 239.36	95.99 ± 75.56	1.92	Green	Smooth
FDe3	0.95 ± 0.02	5.57 ± 0.64	3.53 ± 0.75	3.46 ± 0.09	360.00 ± 16.33	29.12 ± 15.00	1.58	Dark green	Smooth
FAo1	0.78 ± 0.10	6.01 ± 0.23	4.28 ± 0.61	3.58 ± 0.02	1277.50 ± 307.59	74.41 ± 72.24	3.00	Dark yellow	Smooth
FAo2	0.93 ± 0.03	5.81 ± 2.78	3.93 ± 1.16	4.01 ± 0.03	1257.50 ± 251.02	188.40 ± 98.88	2.89	Light brown	Smooth
FAo3	0.98 ± 0.01	4.81 ± 0.27	3.93 ± 1.03	3.77 ± 0.03	622.50 ± 3.54	55.38 ± 42.65	2.11	Deep brown	Smooth

n = 4, *n = 100 for globule size, Mean ± SD, FDe1 = 2.5 % leaf ethyl acetate fraction of *D. edulis* cream, FDe2 = 5 % leaf ethyl acetate fraction of *D. edulis* cream, FDe3 = 10 % leaf ethyl acetate fraction of *D. edulis* cream, FAo1 = 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream, FAo2 = 5 % leaf ethyl acetate fraction of *A. occidentale* cream, FAo3 = 10 % leaf ethyl acetate fraction of *A. occidentale* cream. The pH values of leaf ethyl acetate fraction of *D. edulis* and *A. occidentale* are 3.59 ± 0.06 and 4.92 ± 0.14, respectively.

FDe1, FDe2 and FDe3

FAo1, FAo2 and FAo3

Aqueous cream (base)

Plate 9: Macroscopic view of *Dacryodes edulis* and *Anacardium occidentale* formulated creams and the aqueous cream

FDe1 = 2.5 % leaf ethyl acetate fraction of *D. edulis* cream, FDe2 = 5 % leaf ethyl acetate fraction of *D. edulis* cream, FDe3 = 10 % leaf ethyl acetate fraction of *D. edulis* cream, FAo1 = 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream, FAo2 = 5 % leaf ethyl acetate fraction of *A. occidentale* cream, FAo3 = 10 % leaf ethyl acetate fraction of *A. occidentale* cream.

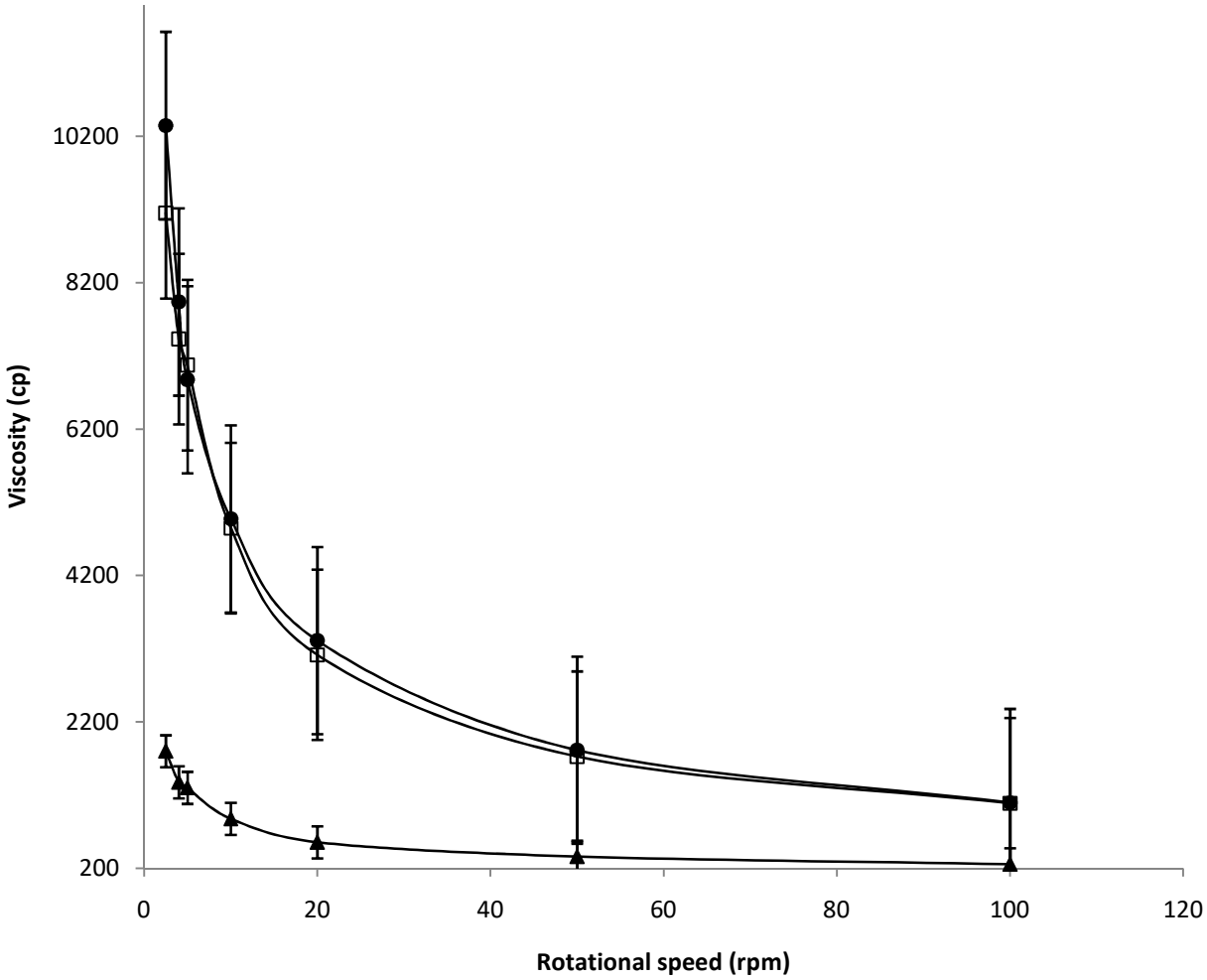


Figure 4.11: Viscosity profiles of *Dacryodes edulis* formulated creams

● FDe1 □ FDe2 ▲FDe3
FDe1 = 2.5 % leaf ethyl acetate fraction of *D. edulis* cream, FDe2 = 5 % leaf ethyl acetate fraction of *D. edulis* cream, FDe3 = 10 % leaf ethyl acetate fraction of *D. edulis* cream.

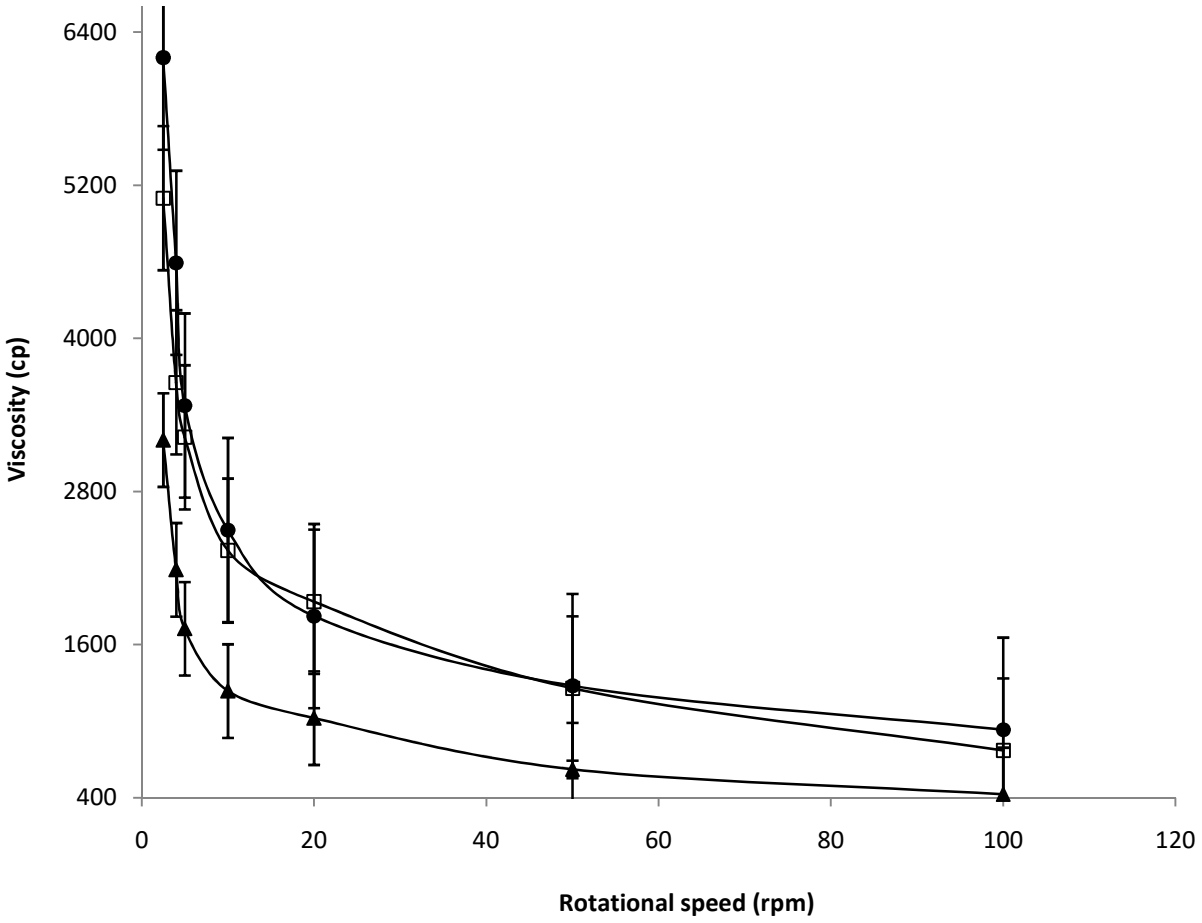


Figure 4.12: Viscosity profiles of *Anacardium occidentale* formulated creams

● FAo1 □ FAo2 ▲ FAo3
FAo1 = 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream, FAo2 = 5 % leaf ethyl acetate fraction of *A. occidentale* cream, FAo3 = 10 % leaf ethyl acetate fraction of *A. occidentale* cream.

In FAo1 and FAo3, the cream stored at room temperature (R) produced the highest viscosity, while the cream stored in freezer (C) had the least value. However in FAo2, the cream stored in freezer (C) yielded the highest viscosity while the cream stored at room temperature (R) had the least value. There were significant differences between R and C of FAo1 and FAo3, unlike their R compared to H, and C compared to H. In FAo2, there was no significant difference between R and C, R and H, C and H, thus showing stability as indicated in Figure 4.14.

4.12.3 Organoleptic properties of *Dacryodes edulis* creams at different temperatures

There was no observable change in the state, colour, texture, and odour of all *D. edulis* formulated creams stored at 29 ± 4 ^{o}C and 0 ^{o}C. Moreover, there was no observable change in the state, texture, and odour of the cream stored at 46 ^{o}C, but a gradual colour change (dark patches) was noticed from day 49 (Plate 10).

4.12.4 Organoleptic properties of *Anacardium occidentale* creams at different temperatures

There was no noticeable change in the state, colour, texture, and odour of all *A. occidentale* formulated creams stored at 29 ± 4 ^{o}C and 0 ^{o}C. Additionally, there was no noticeable change in the state, texture, and odour of the cream stored at 46 ^{o}C, however there was a slow colour change (dark patches) observed from day 49 (Plate 11).

4.13 Antimicrobial activity of *Dacryodes edulis* and *Anacardium occidentale* creams

In *Dacryodes edulis* creams, the 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3) had the highest zone of inhibition (21.0 ± 1.0 mm) on *Staphylococcus aureus* (clinical isolate), while the 2.5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe1) had the least inhibition (5.0 ± 1.2 mm) as shown in Table 4.13. The FDe3 also had the highest zone of inhibition on *S. aureus* (ATCC 27853), *Pseudomonas aeruginosa* (ATCC 29213) and *P. aeruginosa* clinical isolate (19.0 ± 1.0, 17.0 ± 1.0 and 16.0 ± 2.0 mm, respectively). Furthermore, only the 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3) revealed a zone of inhibition on *Tricophyton rubrum* and *Epidermophyton* species (17.7 ± 0.6 and 11.0 ± 1.0 mm, respectively). The FDe3 also had the highest zone of inhibition against *Candida albicans* (16.0 ± 2.0 mm).

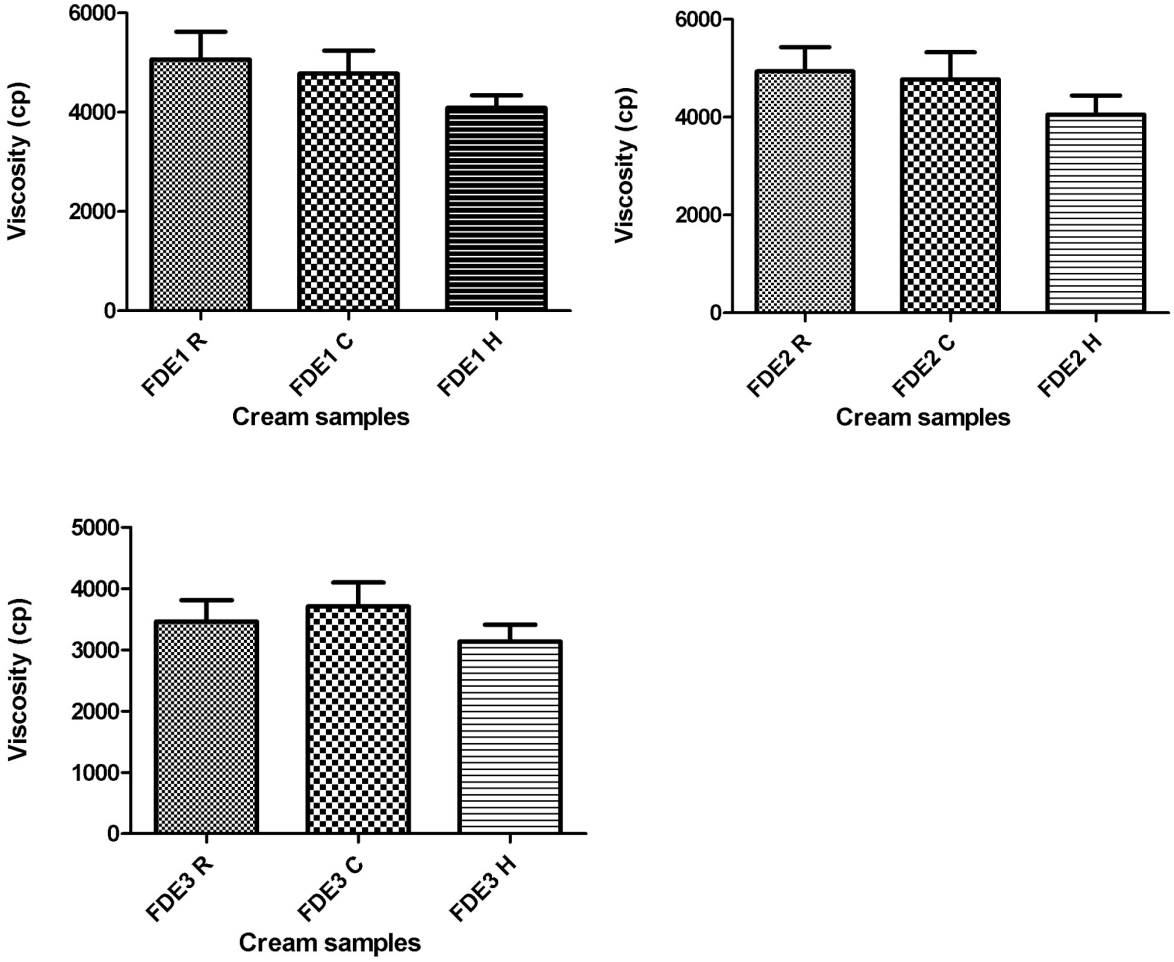


Figure 4.13: Mean viscosity of *Dacryodes edulis* creams of 120 days at different temperatures

FDE1 = 2.5 % leaf ethyl acetate fraction of *D. edulis* cream, FDE2 = 5 % leaf ethyl acetate fraction of *D. edulis* cream, FDE3 = 10 % leaf ethyl acetate fraction of *D. edulis* cream, R = 29±4 ^{o}C, C = 0 ^{o}C, H = 46 ^{o}C.

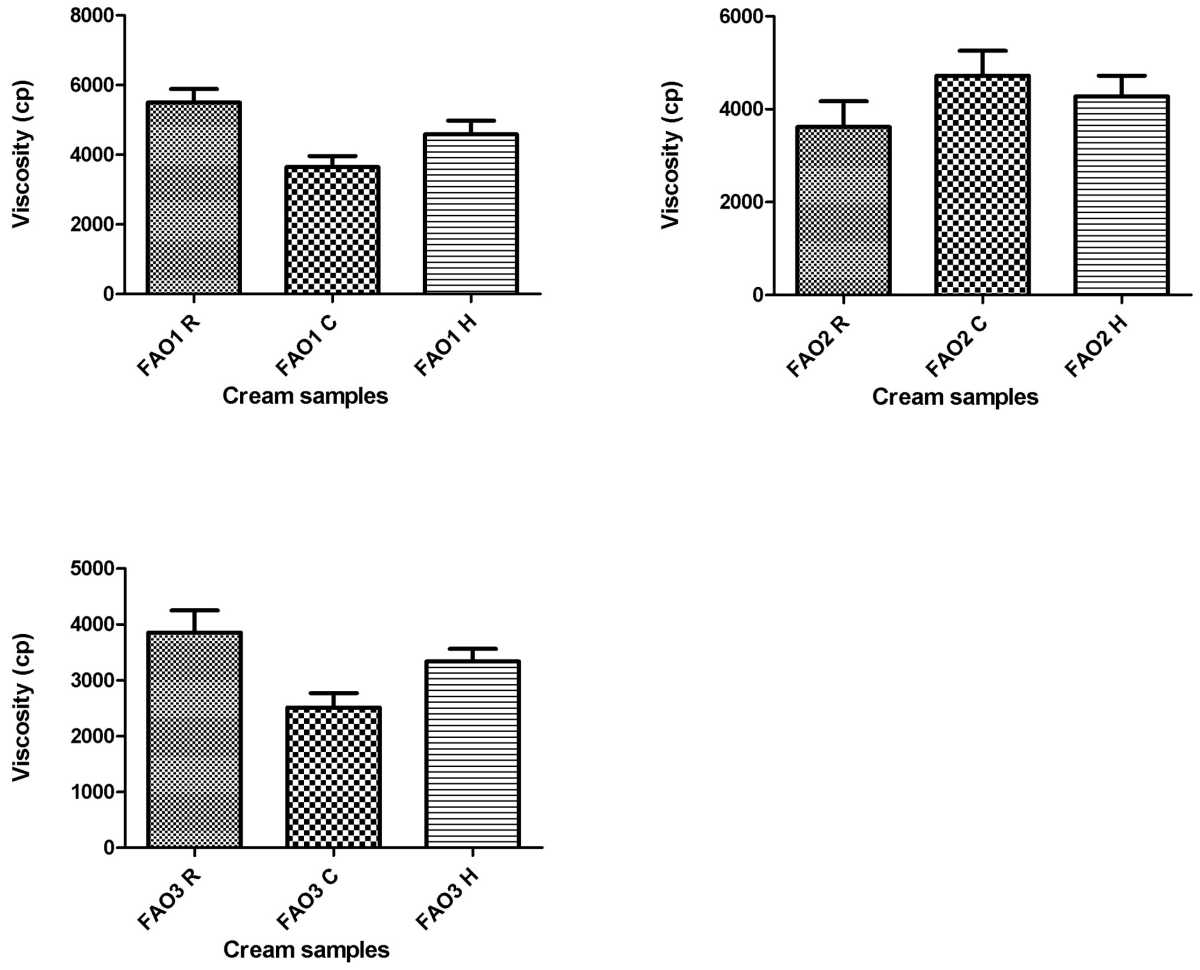


Figure 4.14: Mean viscosity of *Anacardium occidentale* creams of 120 days at different temperatures

FAO1 = 2.5 % leaf ethyl acetate fraction *A. occidentale*, FAO2 = 5 % leaf ethyl acetate fraction *A. occidentale*, FAO3 = 10 % leaf ethyl acetate fraction *A. occidentale*, R = 29±4 ^{o}C, C = 0 ^{o}C, H = 46 ^{o}C.

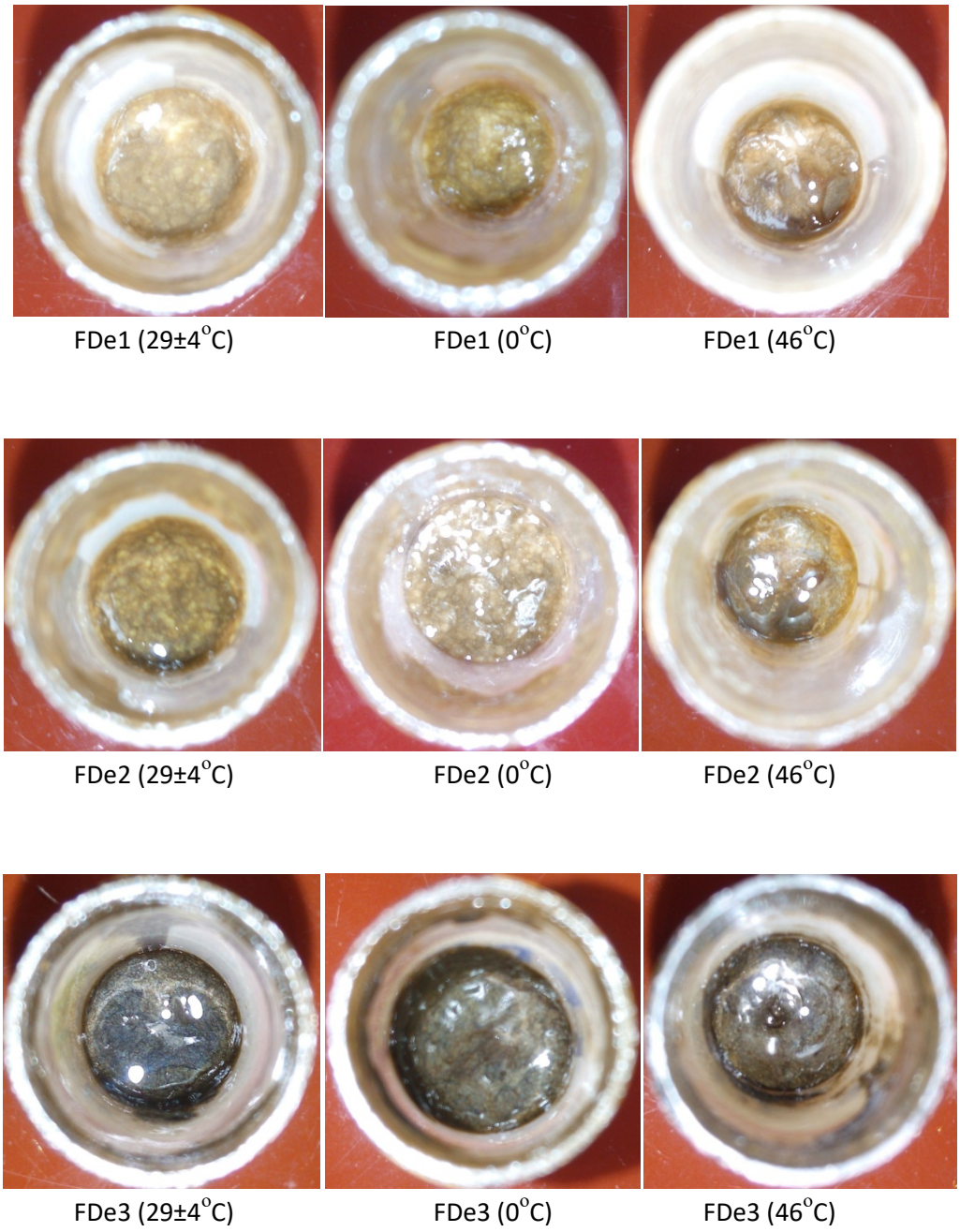
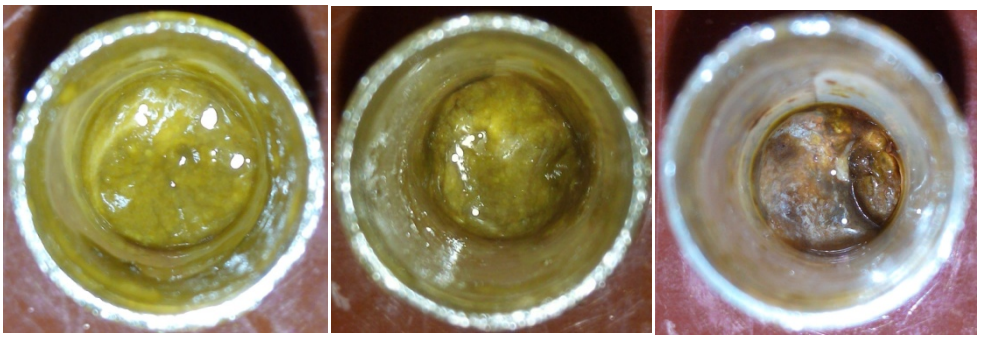
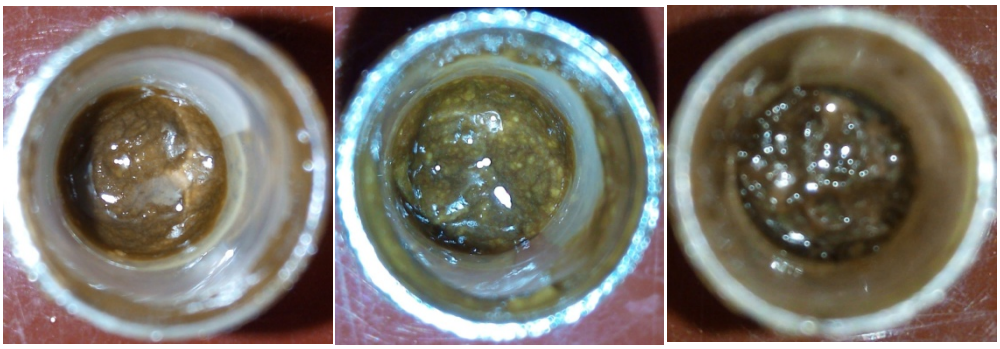


Plate 10: *Dacryodes edulis* creams at day 120 showing organoleptic stability profiles

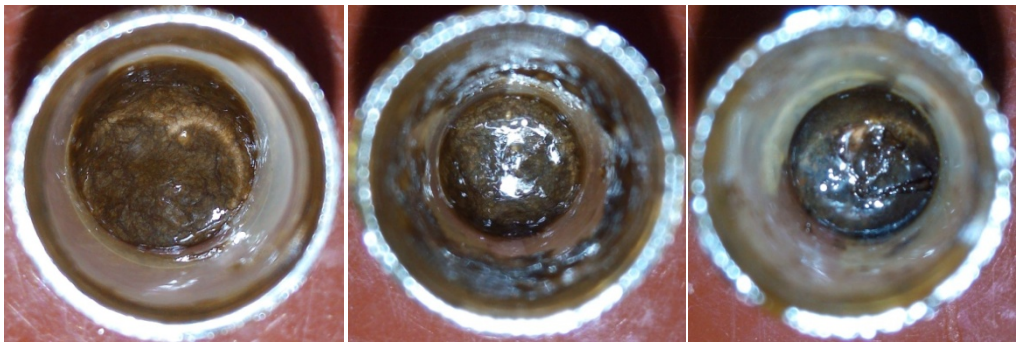
FDe1 = 2.5 % leaf ethyl acetate fraction of *D. edulis* cream, FDe2 = 5 % leaf ethyl acetate fraction of *D. edulis* cream, FDe3 = 10 % leaf ethyl acetate fraction of *D. edulis* cream.



FAo1 (29±4^{o}C) FAo1 (0^{o}C) FAo1 (46^{o}C)



FAo2 (29±4^{o}C) FAo2 (0^{o}C) FAo2 (46^{o}C)



FAo3 (29±4^{o}C) FAo3 (0^{o}C) FAo3 (46^{o}C)

Plate 11: *Anacardium occidentale* creams at day 120 showing organoleptic stability profiles

FAo1 = 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream, FAo2 = 5 % leaf ethyl acetate fraction of *A. occidentale* cream, FAo3 = 10 % leaf ethyl acetate fraction of *A. occidentale* cream.

In *Anacardium occidentale* creams, the 10 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo3) had the highest zone of inhibition on *S. aureus* clinical isolate (10.7 ± 1.2 mm), whereas the 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo1) produced the least inhibition (8.0 ± 0.0 mm). The 5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo2) had the highest zone of inhibition on *S. aureus* (ATCC 27853) and *P. aeruginosa* (ATCC 29213) (10.3 ± 2.1 and 9.0 ± 1.0 mm, respectively), while the 10 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo3) gave the least inhibition on the two microbes (8.0 ± 0.0 and 7.3 ± 3.1 mm, respectively). The FAo3 and FAo2 gave the same zone of inhibition against *P. aeruginosa* clinical isolate (8.0 ± 0.0 mm). In addition with respect to *A. occidentale* creams, the 5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo2) had the highest zone of inhibition on *T. rubrum* and *Epidermophyton* species (6.0 ± 0.0 and 4.0 ± 0.0 mm, respectively). The 10 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo3) gave the highest zone of inhibition (8.0 ± 0.0 mm) on *C. albicans*.

4.13.1 Comparing the antimicrobial activity of *Dacryodes edulis* and *Anacardium occidentale* creams

Comparatively in Table 4.13, *D. edulis* and *A. occidentale* creams showed these trends:

Tydineal cream performed better than FDe3 > FAo3 > FAo2 > FAo1 > FDe2 > FDe1 against *Staphylococcus aureus*.

Tydineal cream inhibited *S. aureus* (ATCC 27853) than FDe3 > FAo2 > FAo1 > FAo3 > FDe1.

Tydineal cream performed better than > FDe3 > FAo2 = FAo3 > FAo1 > FDe1 against *Pseudomonas aeruginosa*.

Tydineal cream inhibited *Pseudomonas aeruginosa* (ATCC 29213) than FDe3 > FDe2 > FAo2 > FAo1 > FAo3 > FDe1.

Mycozoral cream inhibited *Tricophyton rubrum* than FDe3 > FAo2 > FAo3 = FAo1.

Mycozoral cream performed better than FDe3 > FAo2 > FAo1 against *Epidermophyton* species.

Mycozoral cream inhibited *Candida albicans* than FDe3 > FDe2 > FAo3 > FDe1 > FAo2 > FAo1.

4.14 Antibacterial evaluations of *Dacryodes edulis* and *Anacardium occidentale* creams in mice

The effects of *Dacryodes edulis* and *Anacardium occidentale* creams in the albino mice injected with *Staphylococcus aureus* (clinical isolate) are shown in Table 4.14. Among the *D. edulis* creams, the 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3) performed best (85.89 %), while the 2.5 % leaf ethyl acetate fraction of *D. edulis* cream (FDe1) was the least in performance (85.03 %). Among the *A. occidentale* creams, the 5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo2) performed best (85.39 %), whereas the 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream (FAo1) yielded the least performance (85.23 %). Arranging the *in vivo* antibacterial activities in order of effectiveness, Tydineal cream (control) performed better than FDe3 > FAo2 > FAo3 > FAo1 > FDe2 > FDe1. Mice skin injected but untreated and those treated with Aqueous cream (controls) were too numerous for viable count.

4.15 Dermal irritation and sub-acute toxicity of *Dacryodes edulis* and *Anacardium occidentale* creams in mice

No abnormal behaviour was observed in the mice after the application of *D. edulis* and *A. occidentale* creams; moreover, the visual observation of their skins after 24 hours did not show any redness or oedema. The continual application of *D. edulis* and *A. occidentale* creams on the mice for twenty-one days did not result into any abnormal behaviour or skin aberration. Additionally, the histological results of the mice' skins treated with *D. edulis* creams and *A. occidentale* creams are shown in Plates 12 and 13, respectively.

Table 4.13: The effects of *Dacryodes edulis* and *Anacardium occidentale* formulated creams against some microorganisms

Creams	*S. aureus*	*S. aureus* (ATCC 27853)	*P. aeruginosa*	*P. aeruginosa* (ATCC 29213)	*T. rubrum*	*Epidermophyton* sp.	*C. albicans*
	Zones of inhibition (mm)						
FDe1	5.0 ± 1.2	6.0 ± 0.0	6.6 ± 1.2	5.0 ± 1.0	-	-	9.7 ± 0.6
FDe2	6.0 ± 0.0	-	-	12.0 ± 2.0	-	-	12.0 ± 0.0
FDe3	21.0 ± 1.0	19.0 ± 1.0	16.0 ± 2.0	17.0 ± 1.0	11.7 ± 0.6	11.0 ± 1.0	16.0 ± 2.0
FAo1	8.0 ± 0.0	9.3 ± 1.2	7.3 ± 1.2	8.3 ± 2.1	4.0 ± 0.0	3.7 ± 0.6	5.7 ± 0.6
FAo2	9.3 ± 1.2	10.3 ± 2.1	8.0 ± 0.0	9.0 ± 1.0	6.0 ± 0.0	4.0 ± 0.0	8.0 ± 0.0
FAo3	10.7 ± 1.2	8.0 ± 0.0	8.0 ± 0.0	7.3 ± 3.1	4.0 ± 0.0	-	10.0 ± 0.0
TyC	27.7 ± 0.7	29.7 ± 0.6	27.7 ± 0.6	27.7 ± 0.6	NA	NA	NA
MyC	NA	NA	NA	NA	19.7 ± 0.6	21.7 ± 0.6	21.7 ± 0.6

n = 3, Mean ± SD, FDe1 = 2.5 % leaf ethyl acetate fraction of *D. edulis* cream, FDe2 = 5 % leaf ethyl acetate fraction of *D. edulis* cream, FDe3 = 10 % leaf ethyl acetate fraction of *D. edulis* cream, FAo1 = 2.5 % leaf ethyl acetate fraction of *A. occidentale* cream, FAo2 = 5 % leaf ethyl acetate fraction of *A. occidentale* cream, FAo3 = 10 % leaf ethyl acetate fraction of *A. occidentale* cream, *S. aureus* = *Staphylococcus aureus*, *P. aeruginosa* = *Pseudomonas aeruginosa*, *T. rubrum* = *Tricophyton rubrum*, *C. albicans* = *Candida albicans*, ATCC = American Type Culture Collection, TyC = Tydineal cream (reference cream), MyC = Mycozoral cream (reference cream), - = no inhibition, NA = Not applicable.

Table 4.14: Antibacterial evaluations of *Dacryodes edulis* and *Anacardium occidentale* formulated creams in mice

Creams	Viable count	Viable count (CFU/ml)	Log (CFU/ml)	Bacteria survival (%)	Bactericidal effect (%)
FDe1	206	1030×10^2	5.013	14.97	85.03
FDe2	180	900×10^2	4.954	14.79	85.21
FDe3	106	530×10^2	4.724	14.11	85.89
FAo1	177	885×10^2	4.947	14.77	85.23
FAo2	156	780×10^2	4.892	14.61	85.39
FAo3	164	820×10^2	4.914	14.68	85.32
TyC	22	110×10^2	4.041	12.07	87.93

FDe1 = 2.5 % leaf ethyl acetate fraction of *Dacryodes edulis* cream, FDe2 = 5 % leaf ethyl acetate fraction of *D. edulis* cream, FDe3 = 10 % leaf ethyl acetate fraction of *D. edulis* cream, FAo1 = 2.5 % leaf ethyl acetate fraction of *Anacardium occidentale* cream, FAo2 = 5 % leaf ethyl acetate fraction of *A. occidentale* cream, FAo3 = 10 % leaf ethyl acetate fraction of *A. occidentale* cream, TyC = Tydineal cream (reference cream), bacteria = *Staphylococcus aureus*, CFU/ml = colony forming unit per millimetre.

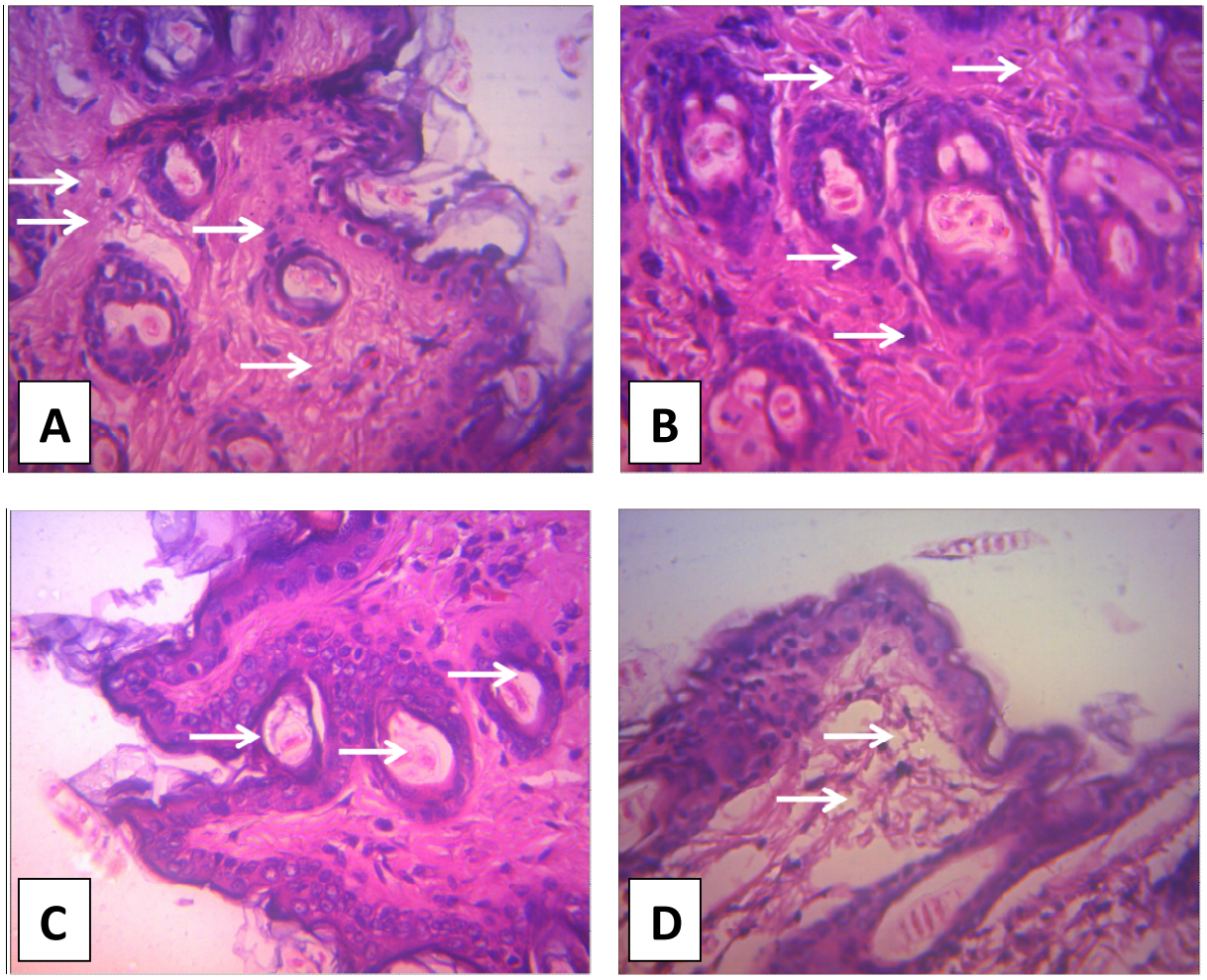


Plate 12: Photomicrographs of mice skins (x400) treated with *Dacryodes edulis* formulated creams

A, B, C treated with creams having 2.5, 5, 10 % ethyl acetate leaf fraction of *D. edulis* respectively, D = Untreated skin (control). A = There is high density of hair follicle sections and sebaceous glands. There is a mild to moderate dermal cellular infiltration (arrows), especially within the dense collagen fibers and around the base of the hair follicles. B = There is a severe dermal cellular infiltration, especially around the base of the hair follicles (arrows). C = The hair follicle and sebaceous sections are dense within the collagen fibers. The hair follicle sections are prominent and appear enlarged (arrows). D = The dermal collagen is very sparse and loose (arrows). All creams show no visible lesions.

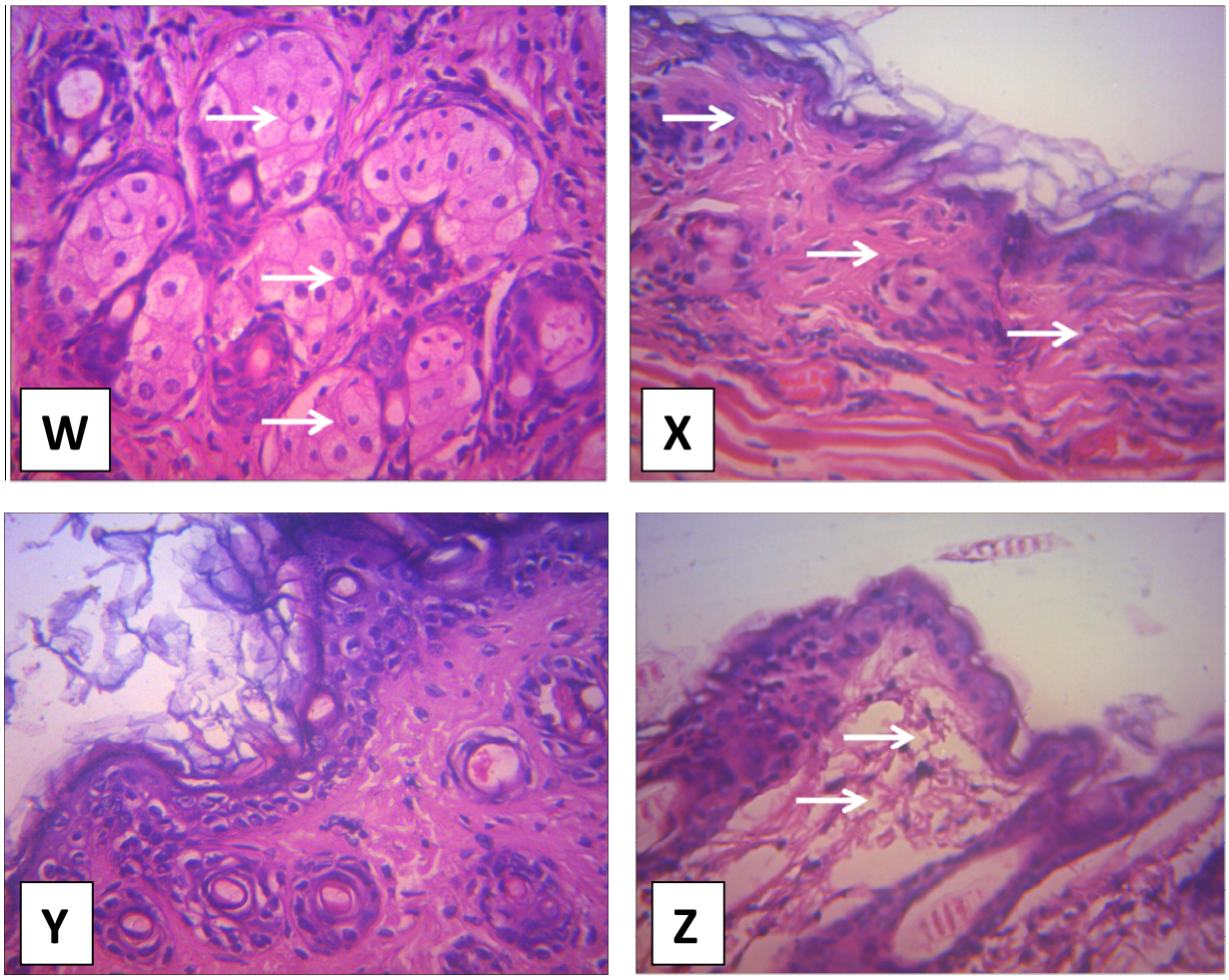


Plate 13: Photomicrographs of mice skins (x400) treated with *Anacardium occidentale* formulated creams

W, X, Y treated with creams having 2.5, 5, 10 % ethyl acetate leaf fraction of *A. occidentale* respectively, Z = Untreated skin (control). Sebaceous glands are clumped together in W; dermal connective tissue is thin, congested and infiltrated in X; very scanty adipose layer in Y; very sparse and loose dermal collagen in Z. All creams show no visible lesions.

CHAPTER FIVE
DISCUSSIONS

In the hierarchy of existence, plant came before man and every society of the world has its cultural linkage with the basic uses of plants for food, medicine, shelter, and clothing. Dutta (2010) reported that the economic uses of plants are varied and the scope for improvement to meet man's ever increasing need is immense. It is observed that, since global civilization began, synthetic products are gaining grounds, causing drastic loss of the indigenous knowledge of ethno-medicinal usage of plants, usually transferred orally and scantily documented, especially in Nigeria. However, increasing resistance of pathogens to conventional antibiotics and undesirable side effects to some drugs is globally reviving the use of herbal medicine, as an alternative therapy (WHO, 2002b).

Majority of the respondents in Ibadan irrespective of their ages (from 21 years and above), occupations (as civil servants, artisans, farmers, herbalists, and students), educational statuses (uneducated, primary, secondary, and tertiary), and religions (Christianity, Islam, Traditional, and those without religion) knew about medicinal herbs. Many of the people using medicinal herbs do not mix them together with orthodox drugs; this might be from their experiences or information on hazard resulting from such combination for treatment. Moreover, majority of the respondents in Ibadan were more familiar with *Anacardium occidentale* than *Dacryodes edulis*. *Anacardium occidentale* is locally used for treating ailments and diseases such as cough, malaria, typhoid, blood parasites, and skin diseases; while *D. edulis* is used for treating child fever, body ache, joint pains, skin itching, measles, and also for skin smoothness. The tradomedicinal uses of the two plants agreed with the earlier reports of Okwu and Nnamdi (2008), Orwa *et al*. (2009), Omonhinmin (2012), Omonhinmin (2014) and Adeniji *et al*. (2018).

The traders in Bode market in Ibadan are popularly known for selling tradomedical materials, including various plant and animal parts. It is frequently patronized by the people within Ibadan, Oyo State. It is more or less likened to an ethnomedicinal centre, where wholesalers and retailers transact businesses. Suppliers from different parts of the Oyo State, transport their goods to the market for sale. The herb sellers in the market were unwilling to entertain interviewers, let alone

of divulging information about the medicinal uses of any plant for free. They protested that information given free of charge to learned people in the past had been used in getting promotion in their work places, and also scientifically refined in making some products.

Leaves and especially barks of *Anacardium occidentale* are usually supplied and thus, always available in Bode market. A pack of leaves (150 g) is sold for fifty naira while a pack of barks (450 g) is sold for one hundred naira, but its roots are very scarce; implying the death of this economically useful plant. However, no part of *Dacryodes edulis* is sold in the market, it was called a strange leaf (*ewékéwé*); and if not that the plant was shown to them, many mistook it for *Persea americana* Mill. (Avocado pear) when asked about Igbo pear. In contrast, Adeniji *et al*. (2018) reported that the bark of *D. edulis* is available and sold for thirty naira in some popular herbal markets in Osun State, Nigeria. Surprisingly, two of the respondents in Bode market had a prior knowledge about *D. edulis*, some of its uses and mode of preparations. However, only its fruits are sold in other places like Agbowo area, Bodija, and Bere markets in Ibadan during its season.

Ethnobotanical studies and communications with local people frequently expose the cultural manner and views of such people on the utilization of bio-resources inside their communal boundaries (Omonhinmin, 2014). Properly documented ethnobotanical researches of any community would be significantly useful in academic course, as well as preserving loss of indigenous traditional healthcare knowledge. Moreover, ethnobotanical surveys could be useful in discovering crude or new drugs for pharmaceutical industries.

The ethyl acetate fraction of *D. edulis* leaf with 1, 1 diphenyl-2-picrylhydrazyl (DPPH) performed better than the aqueous and hexane fractions at 0.25 mg/mL; than aqueous and butanol at 0.03125 mg/mL; than methanol extract, hexane, butanol and aqueous fractions at 0.125 mg/mL and 0.0625 mg/mL. This is similar to Onocha and Oloyede (2011), who reported that the ethyl acetate fraction of *D. edulis* leaf showed higher antioxidant ability than the hexane and butanol fractions at 1.0 mg/mL with DPPH.

In this study, *D. edulis* leaf is rich in tannins and has high antioxidant capability, therefore supports the reports of many previous workers that *D. edulis* has strong antioxidant potentials (Tee *et al*., 2014; Oboh *et al*., 2015). The implication is that *D. edulis* leaf might be useful in

combating ailments and diseases associated with oxidative stress. A concise report by Lobo *et al*. (2010) presented that an antioxidant acts as electron donor, hydrogen donor, singlet oxygen quencher, peroxide decomposer, radical scavenger, synergist, metal-chelating agents, and enzyme inhibitor. Free radical scavenging assay is used in selecting medicinal plants with high antioxidant properties (Aponjolosun, 2010).

Akinmoladun *et al*. (2007) reported that the curative effects of some vegetables and plants used in traditional medicine are typically ascribed to their antioxidant constituents. Moreover, some epidemiological researches have revealed that intake of plant foods having antioxidants, are helpful to health, because it down-regulates several degenerative processes and could efficiently reduce the occurrence of cardio-vascular diseases and cancer; they are also considered useful in hypertension therapy (Aderiye *et al*., 2015). The leaf of *D. edulis* in this study showed good antioxidant ability, thus might serve as natural antioxidant against cancer and cadio-vascular diseases.

Free radicals cause oxidative changes in collagen, elastin material and membrane features which stimulate polymerization responses (Saini *et al*., 2016). Herbal cosmetics should contain natural antioxidant, such as vitamin C, which is required for the hydroxylation of proline, procollagen, as well as lysine. It effectively stimulates collagen repair and therefore eradicates many outcomes of photo-aging on the skin (Kadam *et al*., 2013). The leaf of *D. edulis* is rich in antioxidant property and thus might be useful in formulations for retarding or preventing premature ageing of the skin.

Saponins possess useful effect by binding cholesterol and diminish its quantity in the body, thus reduce the danger of atherosclerosis and consequent problems. They are essential for the action of cardiac glycosides; facilitate the absorption of foods and medicine; and also have anticancer activity (Okanlawon *et al*., 2015). The methanol extracts, all the fractions (except butanol fraction of *D. edulis*) of *D. edulis* and *A. occidentale* leaves are rich in saponins and might consequently be used as anti-cholesterol agents.

Tannins terminate the growth of microorganisms by precipitating their protein, thus causing dietary protein inaccessible to them (Okanlawon *et al*., 2015). As a result of their considerable toxicity against fungi and bacteria, they may presume pharmacological importance in the future

(Oyesomi and Ajao, 2011; Aderiye *et al*., 2015). Medicinal plants that are rich in tannins are utilized as antiseptic, because of the presence of the phenolic group. In Ayurveda, tannin-rich plants are formulated for treating diseases like leucorrhoea, rhinnorhoea and diarrhea. Moreover, tannins are applied in the dyeing, photography, brewery and wine industries, and astringent in medicinal preparations (Belonwu *et al*., 2014). The hexane, ethyl acetate, and butanol fractions of *D. edulis* and *A. occidentale* are rich in tannins.

Alkaloids have pharmacological uses as anesthetics, central nervous system stimulants, antimalarials, and analgesics. However, plants with alkaloids are not usually used in herbal medicine because of their high toxicity, though they are frequently significant in allopathy where the dose is stringently restricted and in homoepathy where the dose-rate is safely low (Okanlawon *et al*., 2015). The methanol extracts, all the fractions and the raw leaves of *D. edulis* and *A. occidentale* are rich in alkaloids but not toxic as shown in the acute toxicity results, therefore the quantity is considered moderate and might be useful in drug production. Phenols have gained significant awareness as protecting substances against heart diseases and cancer, owing to their antioxidant effectiveness (Gordana *et al*., 2007). The hexane, ethyl acetate and butanol fractions of *D. edulis* and methanol extract, hexane and butanol fractions of *A. occidentale* leaves are rich in phenolics and could be relevant in treating heart diseases and cancer.

Saponins, tannins, alkaloids, phenolics were higher in values for the methanol extract than the aqueous fraction in *A. occidentale* leaf, therefore agrees with Ojezele and Agunbiade (2013), who reported that tannins, polyphenols, alkaloids and saponins had higher values in the ethanol extract than the water extract in *A. occidentale* leaf. Additionally, the high values of alkaloids and phenolics in *A. occidentale* leaf in this study supported Belonwu *et al*. (2014) who reported highest concentrations of alkaloids and phenolics in the leaf of red fruited species and yellow fruited species of *A. occidentale* than in the stem barks, fruits and roots.

Anthraquinones have anti-inflammatory, neuro-protective and anti-atherogenesis activities, and also act as powerful antioxidants with similar ability to neutralize free radicals linked to oxidative stress disorders (Belonwu *et al*., 2014). The methanol extracts, all the fractions and the controls of *D. edulis* and *A. occidentale* leaves are rich in anthraquinones, and thus might be beneficial as anti-inflammatory and neuro-protective drugs. Flavonoids display anti-

inflammatory, anti-allergic, anti-cancer activities, and as powerful antioxidants. They inhibit tumor growth, protect gastrointestinal infections and are of pharmacognostic importance in ethnomedicine. Flavonoids and phenolic compounds have extensively been stated as antioxidant agents, and associated with the treatment of cardiovascular diseases, due to their anti-hypertensive attributes (Belonwu *et al*., 2014; Aderiye *et al*., 2015). They have antibacterial, antimultagenic, antiviral, antineoplatic, antithrombotic and vasodilatory activities (Alan and Miller, 1996; Okanlawon *et al*., 2015). The methanol extracts, all the fractions of *D. edulis* and *A. occidentale* leaves are rich in flavonoids, and therefore might be useful in combating inflammation, tumor and cancer.

In this study, the leaf of *D. edulis* is rich in flavonoids and this supported Tee *et al*. (2014) who reported that its fruits and leaves have flavonols. The leaves also have saponins, tannins, alkaloids, anthraquinones and flavonoids, and similar to Anyam *et al*. (2015) findings who reported the same phytochemical compounds for hexane, chloroform, ethyl acetate and methanol extracts of boiled seed of *D. edulis*.

Cardiac glycosides are principally steroids with an intrinsic capability to give a definite and powerful action, mostly on the cardiac muscle, when administered through injection into animals or humans. However, Kar, 2007 expressed that caution must be taken when using steroidal glycosides, as its little quantity would cause desirable stimulation on a diseased heart, and even too much dose may result into death. The methanol extracts, all the fractions and the raw leaves of *D. edulis* and *A. occidentale* are moderately rich in steroids, but very low in cardiac glycosides, and this is pharmacologically relevant.

Terpenes have sedative, insecticidal, cytotoxic or anti-inflammatory activity and are used in treating ovarian and breast cancer. They are major constituents of many essential oils that are employed in medicine and aromatherapy. The methanol extracts, all the fractions and the controls of *D. edulis* and *A. occidentale* leaves are rich in terpenoids, and probably useful in treating inflammation. Carotenoids are antioxidants and helpful in preventing certain cancer, macular degeneration, prostate problems, and asthma. The methanol extracts, all the fractions and the raw leaves of *D. edulis* and *A. occidentale* are low in carotenoids when compared with other phytochemicals tested, and might not be beneficial in combating these health problems.

Comparing the two plants, *D. edulis* had the highest phytochemical values for tannins in ethyl acetate fraction, anthraquinones in butanol fraction, steroids in butanol fraction, terpenoids in butanol fraction and carotenoids in butanol fraction, while *A. occidentale* had the highest values for saponins in butanol fraction, alkaloids in raw leaf, phenolics in methanol extract, flavonoids in raw leaf and cardiac glycosides in ethyl acetate fraction. However, the overall highest and least values are flavonoids and carotenoids in raw leaf of *A. occidentale* respectively. Consequently, a phytochemical of interest from the plants could be abundantly extracted with a specific solvent of extraction for drug production and effective therapy.

Dacryodes edulis leaf demonstrated significant antimicrobial potency that might be linked to its high content in tannins in ethyl acetate fraction. This corroborated the findings of Ajibesin *et al*. (2011b), that two compounds; ethyl gallate and quercitrin, were previously found to be accountable for antibacterial activity and these were also identified in the leaves of *D. edulis*.

Anacardium occidentale leaf has the highest values for saponins (butanol fraction) and flavonoids (raw leaf), that supported the results of Abubakar *et al*. (2015) that the antidiarrhoeal activity of *A*. *occidentale* aqueous and fractions of the leaf might be due to the quantity of flavonoids and saponins. It also agreed with the findings of Ranjith *et al*. (2017) who reported that the powdered leaf dust of *A*. *occidentale* has flavonoids, saponins and terpenoids.

Concalves *et al*. (2005) reported that the antimicrobial action of *A. occidentale* has been credited to its saponins; while Aderiye *et al*. (2015) reported that flavonoids and tannins are responsible for the anti-microbial activities in *A. occidentale* extracts by inactivation of microbial adhesion enzymes and cell envelop transport cells. However, *A. occidentale* leaf extracts have high content of saponins in an order: methanol extract > hexane fraction > ethyl acetate fraction > water fraction > raw leaf, than tannins as ordered: hexane fraction > ethyl acetate fraction > raw leaf. Moreoso, it was practically observed that the ethyl acetate fraction showed better and broad spectrum of antimicrobial activity than hexane fraction. This suggests that the antimicrobial potency is actually much more in tannins than in saponins, but might not be linked to flavonoids order: raw leaf > methanol extract and all the fractions.

Medicinal plants, which are believed to possess various phytochemicals, have been utilized in traditional medicine for some centuries for their therapeutic properties. Nowadays, they are

processed into conventional drugs. However, the results of phytochemical screening on a plant might vary between different parts of the same plant and from one plant to another and also due to their various geographical locations, climatic changes, and biochemical variations within species, extraction technique, and type of extraction solvent employed.

Plants with medicinal values usually have antioxidant, antimicrobial and phytochemical properties that have been traditionally harnessed for healthcare till date, and are believed to be useful as food supplements, healthcare and for drug development. However, modern medicine prefers single constituents for dosage quantification, requiring comprehensive analysis of phytochemicals. On the other hand, traditional medicine uses plants without the isolation of specific phytochemical constituents, leading to synergistic actions and perhaps reduction of any toxicity of such combined materials.

The methanol extract of *D. edulis* leaf was not active against *S. aureus*. This however contradicted the study of Okwu and Ighodaro (2009), who reported antibacterial inhibition of the oil from the stem bark of *D. edulis* against *S. aureus*. This contradiction might have resulted from the different plant parts used. The ethyl acetate fraction of *D. edulis* leaf showed activities against *S. aureus*, *K. pneumoniae* and *P. aeruginosa*, therefore supported the report of Anyam *et al*. (2015) that the ethyl acetate fraction of *D. edulis* seed was active against the same set of microbes despite the different plant parts employed.

The antibacterial activity of *A. occidentale* supported the report of Ayepola and Ishola (2009) in that *A. occidentale* extracts have been efficiently applied for treating bacterial infections. It however did not agree with Chaithra *et al*. (2013), who reported that methanol leaf extract of *A. occidentale* was more effective in inhibiting bacterial isolates than its methanol bark extract. Varghese *et al*. (2013) reported that *A. occidentale* leaf aqueous extract was effective than its leaf methanol extract against *Porphyromonas gingivalis* and *Prevotella intermedia* that cause certain dental infections. This might have been caused by seasonal metabolic differences while harvesting the plant.

The aqueous fraction of *A. occidentale* leaf showed zones of inhibition against *Klebsiella pneumoniae*; therefore similar to Aiswarya *et al*. (2011) who reported inhibition of water extract of *A. occidentale* apple against *K. pneumoniae*. Although according to Aiswarya *et al*. (2011),

the methanol extract was insensitive to *K. pneumonia* in contrary to the alcohol extract sensitivity to *K. pneumoniae*. This might probably be due to different parts of the plant utilized, since methanol and ethanol solvents generally pull similar phytoconstituents during extraction.

The methanol extract and water fraction of *A. occidentale* leaf were not active against *S. aureus* and *P. aeruginosa*; this is contrary to the activities of ethanol and aqueous extracts of *A. occidentale* bark reported by Arekemase *et al*. (2011). This could have also resulted from different parts of the botanical used.

In this study, the methanol extract of *A. occidentale* leaf was inactive against *S. aureus*, *P. aeruginosa* and *K. pneumonia*. This is however contrary to the activities of the ethanol extract of *A. occidentale* seed coat against the microbes as reported by Vijayakumar and Kalaichelvan (2011). The different plant parts utilized might also account for the variation.

The methanol extract of *A. occidentale* leaf was inactive against *Bacillus cereus* and same was reported by Akinpelu (2001) for methanol extract of *A. occidentale* bark. Akinpelu (2001) also reported activities of *A. occidentale* leaf against *K. pneumoniae, P. vulgaris*, *P. aeruginosa*, and *S. aureus*. Contrarily, these organisms were insensitive to methanol extract of *A. occidentale* leaf in this study; although same standard strains were used, yet the difference might also be due to different plant parts used. The ethyl acetate fraction of *A. occidentale* leaf did not show antifungal activity against *Aspergillus flavus*. This is contrary to the report of Rajesh *et al*. (2009), in that the ethyl acetate fraction of *A. occidentale* nut showed activity againt *A. flavus*. This was also probably be influenced by the different botanical parts employed.

The methanol extract of *A. occidentale* leaf in this finding was inactive against *Staphylococcus aureus* (NCIB 8588), *Bacillus cereus* (NCIB 6349) and *Serratia marcescens* (NCIB 1377). Contrarily, Rajesh *et al*. (2015) reported zones of inhibition for the methanol leaf extract of *A. occidentale* for *S. aureus* (MTCC 96), *B. cereus* (MTCC 456), and *S. marcescens* (MTCC 2708). Athough the same solvent was employed; the difference probably resulted from the different standard strains of bacteria involved. The activities of the ethyl acetate fraction of *A. occidentale* leaf, correlated with Chabi *et al*. (2014) who reported that the ethyl acetate fraction of *A. occidentale* leaf showed bacteriostatic and bactericidal effects on nineteen strains of bacteria.

The inhibition of *Bacillus cereus* and *Pseudomonas aeruginosa* by *A. occidentale* agreed with the findings of Tan and Chan (2014) who reported inhibition of *A. occidentale* leaf against *P. aeruginosa.* The antibacterial activities of *A. occidentale* supported Aderiye *et al*. (2015), who reported that the phenolic compounds such as cardols, triterpenoids, cardanols, methylcardols, xantoprotein and anacardic acids in *A. occidentale* extracts were responsible for cell wall inhibition in bacteria by preventing the cell wall synthesis in growing cells and blocking the deoxyribose nucleic acid, ribose nucleic acid and protein pathways.

The preliminary antimicrobial results are comparable to the work of Ajileye *et al*. (2015) who reported that the ethyl acetate fraction of *A. occidentale* leaf produced the broadest spectrum of inhibitions against some microbes including *S. aureus, P. aeruginosa* and *K. pneumoniae*, as there was no inhibition against the tested fungal strains.

There was a consistency in the outcomes of the preliminary and the repeated antimicrobial tests (that is, antimicrobial properties of leaf ethyl acetate fractions of *Dacryodes edulis* and *Anacardium occidentale*) for *P. aeruginosa* in which *D. edulis* were higher than *A. occidentale*. However, there were few contradictions between the preliminary and the repeated antimicrobial results for the ethyl acetate fractions. *Dacryodes edulis* was higher than *A. occidentale* in the preliminary antimicrobial results for *S. aureus* but was otherwise in the repeated work. However, the ethyl acetate fraction of *A. occidentale* has antifungal activity in the repeated antimicrobial results, contrary to the preliminary antimicrobial results. It was even higher than that of *D. edulis* for *T. rubrum*, *Epidermophyton* species and *C. albicans* at 100 mg/mL; although equal for *Epidermophyton* species, but still greater than *T. rubrum* and *C. albicans* at 200 mg/mL. This might be due to different sources of the microorganisms, genetic differences and resistance among other factors.

The ethyl acetate fraction of *A. occidentale* leaf was active against *T. rubrum*, *Epidermophyton* species and *C. albicans*, therefore contradicting the report of Ajileye *et al*. (2015), that the ethyl acetate fraction of *A. occidentale* leaf has no anti-fungal property. The inhibition of *A. occidentale* leaf against candida corroborated the report of Shetty *et al*. (2014), as *A. occidentale* leaf showed anti-candidal activity. This is also supported by Chan *et al*. (2017) who affirmed that its leaf possesses antibacterial and antifungal properties. Moreover, Chabi *et al*. (2014) speculated that the main antimicrobial ingredients are concentrated in its leaves than in the barks.

The essential oil extracted from its leaf shoots also showed antimicrobial action, while Silva *et al*. (2016) added that its flower extract was more potent than the leaf extract.

Kotzekidou *et al.* (2008) explained the mechanism of action exhibited by plant extracts and essential oils against bacterial strains. This involves the intrusion with the phospholipids bilayer of the cell membrane that causes permeability increase, loss of cellular components, destruction of enzymes concerned with the cellular energy production and structural components synthesis; and inactivation or damage of genetic material.

Functional groups are various components of drug molecules. Chemically, every functional group has an electronic effect, a solubility effect, and a steric effect which are considered in assessing the general pharmacodynamic and pharmacokinetic properties of any drug molecule (Harrold and Zavod, 2013). The functional groups showed that only the ethyl acetate fraction of *D. edulis* has amines, alkyl halides and amides, while only the ethyl acetate fraction of *A. occidentale* has esthers, alkenes and ketones. However, both have aromatics, alkanes and carboxylic acids in their functional groups.

Alkaloids are amines that are produced by plants. The Fourier transform infrared (FTIR) results corroborated the phytochemical results, showing the alkaloids in the ethyl acetate fraction of *D. edulis* about four times higher than that of ethyl acetate fraction of *A. occidentale*. The amine functionality is the most frequent functional group found in drug molecules. The principal physicochemical property of significance in the drug chemistry of the amino group is its basicity. Since the majority of amines are basic, salt forms can be produced to help water solubility or solubility in other media for drug administration. Emphatically, most of the drugs that are invented in the recent century are prodrugs of amines, showing their importance in the medical field (Chandy *et al*., 2013).

Toxicity is the extent to which a material can harm an organism. Toxicity can refer to the consequence on a whole organism and the effect on a substructure of the organism, like a cell (that is, cytotoxicity) or an organ like the liver (that is, hepatotoxicity). Liver injury ranges from mild elevation of liver enzymes to liver failure (Woolf, 2003).

Acute toxicity is the adverse effect of any substance at higher quantity from either single or multiple exposures within a short time, usually in twenty-four hours; while chronic toxicity is

repeated exposure at lower quantity for a longer time, usually weeks, months or years (Walum, 1998). Most reports of toxicity related to herbal drugs and nutritional supplements use, are connected with hepatotoxicity, while other toxic effects on the blood, kidney, nervous, cardiovascular and dermatological systems may result to mutagenicity and carcinogenicity (Woolf, 2003).

The non-toxicity of *D. edulis* leaf corroborated Ajibesin (2011) who reported that no part of *D. edulis* is identified as being toxic. He also added that there was no other information on the toxicity study of *D. edulis* in experimental animals. However, this study has been able to conduct the toxicity study on rats and has further proven the safety of *D. edulis*.

There was no significant increase in the weights of rats administered with the leaf aqueous fraction of *A. occidentale*. This is supported the report of Dare *et al*. (2011), who submitted that there was no significant increase in the body weights of rats treated with aqueous extract of *A. occidentale* leaves. The ethyl acetate fraction of *A. occidentale* leaf in this study reduced the body weights of the Wistar rats. However, Konan and Bacchi (2007) reported that there was an increase in weight of mice treated with ethanol extract of *A. occidentale* leaf. Offor *et al*. (2014) also reported that the ethanol leaf-extract of *A. occidentale* produced significant dose-dependent decrease in the haemoglobin levels, packed cell volume (PVC) and body weights of albino rats. The differences in these reports could be due to different solvents of extraction, influence of location, time of collection and seasonal changes in plant constituents.

Konan and Bacchi (2007) reported that there were no signs of acute toxicity or apparent changes in the organs, as swiss mice were treated with ethanol extract of *A. occidentale* leaf, at 2,000 mg/kg body weight. Moreover, the ethyl acetate fraction of *A. occidentale* leaf was not toxic to the albino rats at 2,900 mg/kg body weight, therefore showing its safety with the solvents (ethanol and ethyl acetate) of extraction.

Traditional medicine has a challenge with subjective, unequal, inaccurate or unprecised measurement in their prescriptions. Therefore, the determination of dosages and toxicity of *D. edulis* and *A. occidentale* leaves reveals the danger or intrinsic hazard in consuming great amount within a short interval of time.

Medicinal plants have curative properties because of secondary plant metabolites in them (Awad *et al*., 2015). They are applied in diverse ways like poultices, concoctions of assorted plants, infusions as teas or tinctures, or as constituent mixtures in porridges and soups, and also as oral, rectal (enemas), nasal (snuffing, smoking or steaming), and topically applied in form of creams, lotions or oils (Kar, 2007).

The most frequently used cosmetic delivery systems is oil-in-water emulsions, because they supply moisture to the skin and also enhance the state of the skin by forming occlusive obstruction on it. The colours of the formulated creams deepened as the extract concentrations increased. Humectants are hydrophilic substances added to aqueous phases to absorb water from the atmosphere and prevent degradation. The humectant (propylene glycol) added shinning property to the formulation. Pleasant colour of a formulation can improve patient compliance to medication (Ajala, 2014).

All the formulated creams have good spreading values and this might have been influenced by the presence of the base and the humectant. The bioavailability efficiency of a formulation also depends on its spreading value. Moreover, semi solid formulations should spread easily without much drag or generating friction in the rubbing process. Generally and according to Ajala, 2014, creams containing humectants spread faster than those without them.

The pH values of the ethyl acetate fractions of *D. edulis* and *A. occidentale* were 3.59 ± 0.06 and 4.92 ± 0.14, respectively. The addition of the humectant (propylene glycol) and the base (aqueous cream) to the plant fractions significantly decreased the pH of FAo1, FAo2, and FAo3, while FDe1 was significantly increased. However, the reduction in FDe2 and FDe3 were insignificant. Isa *et al*. (2000) reported that the stratum corneum is resistant to changes in pH, ranging 3 - 9. Thus, the pH of *D. edulis* and *A. occidentale* creams were within the acceptable range.

The humectant (propylene glycol) diluted the ethyl acetate fractions of *D. edulis* and *A. occidentale* and therefore, might have aided their diffusion rate. Ajala *et al*. (2016) stated that the presence of humectant (glycerin) facilitated diffusion in the *Phyllanthus amarus* creams.

Generally, the humectant lowers the viscosity, making each formulation more fluid in consistency. It was however observed that the viscosity of FDe3 and FAo3 were significantly

smaller when compared with the other cream formulations. This was due to the quantities of the ethyl acetate fractions of *D. edulis* and *A. occidentale* in the formulations. The rheological profiles of the creams showed that the formulations have non-Newtonian flow property (that is, they did not follow Newton's law of viscosity). The creams showed pseudoplastic (viscoelatic) behaviour, because their viscosities decreased with the rotational speed. High viscosity shows that the attractive forces between the molecules are high. Kryscio *et al*. (2008) reported that high viscosity makes formulations to have high yield stress which keeps them from running off the skin surface after application. Interestingly, Adeyeye *et al*. (2002) reported that high viscosity assists in extending drug delivery at the site of application. This means that the creams produced will adhere to the skin surface to deliver their therapeutic purpose.

Horney layer (stratum corneum) is the outmost layer of skin and the principal barrier for penetration. Rolland (1993) reported that micro-particles with diameters greater than 10 μm remain on the skin surface, while those less than 3 μm are randomly spread into hair follicle and horney layer. He also stated that the maximum globule size for a conventional cream is 100 μm. The globule sizes of *D. edulis* and *A. occidentale* creams (except FAo2) are less than 100 μm, and this implies that they are within the satisfactory limit.

Skin, the soft outer covering of vertebrates, usually serves as an indicator in measuring the health status of any organism. Therefore, impairment or malignant growth in skin, nail or hair could serve as diagnostic tool to determine ailment, sickness and diseases in man or animal. Every infection is caused by a pathogen, while a disease may or may not be caused by a pathogen. Skin infections seem not to be life-threatening challenges and are therefore under-emphasized, but in the real sense, it could lead to depression and social isolation resulting from skin discomfort, discolouration, or disfigurement. Therefore, it is becoming a global issue that needs serious attention and intervention.

Plants are significant in formulating non-chemical, natural cosmetic products (Chermahini and Majid (2011). However, formulation of cosmetics from only natural raw materials is a cumbersome work. The challenge does not only lie in substituting synthetic base for naturals, but also in getting same functional effects acquired from synthetic ones (Kapoor and Saraf, 2010). Hence, the reason for the chemical base (aqueous cream) used in this research. Farahmand *et al*.

(2016) reported that herbal medicine as an independent treatment method or in combination with western medicine could be useful in treating fungal infections.

The two reference creams showed the highest antimicrobial activities. However, FDe3 has the optimum antimicrobial activities among the herbal cream formulations. Surprisingly, FDe2 was not active against *S. aureus* (ATCC 27853), *P. aeruginosa, T. rubrum* and *Epidermophyton* species. Likewise, FDe1 was not active against *T. rubrum* and *Epidermophyton* species, while FAo3 was not active against *Epidermophyton* species. This might be that the phytochemicals in *D. edulis* ethyl acetate fraction at the highest concentration (10 %) for FDe3, has better compatibility with the base than FAo3.

The ethyl acetate fractions of *D. edulis* and *A. occidentale* have noticeable antimicrobial properties against *S. aureus* and *P. aeruginosa* and the dermatophytes (*T. rubrum, Epidermophyton* species and *C. albicans*). However, when the antimicrobial activities of the formulated creams (FDe3 and FAo3) were compared to their ethyl acetate fractions at 200 mg/mL, FDe3 is preferred better formulation, especially against *S. aureus* (clinical isolate), *S. aureus* (ATCC 27853), *P. aeruginosa* (ATCC 29213) and *Epidermophyton* species.

The antimicrobial activities of *D. edulis* and *A. occidentale* creams are in support of Ajala *et al*. (2016) who reported antimicrobial activities for *Phyllanthus amarus* creams against *S. aureus*, *P. aeruginosa*, *T. rubrum* and *C. albicans*. Although, different plants were used in the formulations, yet antimicrobial activities were recorded.

The use of mice as model animals to study human biology is predicated on the physiological and genetical resemblance between mice and man (Perlman, 2016). The *in vitro* and *in vivo* antibacterial activities of *D. edulis* and *A. occidentale* creams against *Staphylococcus aureus*, confirmed the potency of the formulated creams. The FAo2 performed better than FAo3 in *in vivo* study, while it was otherwise in *in vitro* study. The *in vivo* antibacterial activity of the creams in mice was similar to the findings of Ajala *et al*. (2016) who used rats in similar experiment.

There were insignificant changes in the organoleptic properties of *D. edulis* and *A. occidentale* creams stored under room temperature (29 ± 4 ^{o}C) and cold (0 ^{o}C) on day 120. However,

significant changes of dark patches were observed in those stored at higher temperature (46 ^{o}C) from day 49. Therefore, the former showed stability while the latter showed degradation of products. The outcome was similar to Goncalves and Gobbo (2012) who developed cosmetic formulations from apple extracts of *A. occidentale*, and reported gradual darkening of the formulations stored between 40 ^{o}C and 60 ^{o}C after twenty-eight days. Derick (2000) explained phase separation, as a type of instability that occurs in emulsified systems, sometimes called creaming, resulting into reduction of viscosity and increase in liquefaction caused by time and temperature. Abdurahman and Rosli (2006) added that an emulsion with smaller particles have higher viscosity and thus decrease the rate of creaming.

Akhtar *et al*. (2011) reported that a change in temperature also has an indirect effect on the stability of an emulsified system, including creams. This occurs by changing the interfacial tension, viscosity, nature of surfactant, vapour pressure of the liquid phases and thermal agitation of the molecules. However, there was no phase separation of *D. edulis* and *A. occidentale* creams stored at different temperatures (29 ± 4 ^{o}C and 0 ^{o}C), thus showing their stability for 120 days of observation.

The mean viscosity range for *D. edulis* creams stored at different temperatures was 3134 ± 780.4 – 5050 ± 1590 cP, while for *A. occidentale* creams stored at different temperatures was 2510 ± 728.4 – 5495 ± 1098 cP. Generally, there were significant increases in the viscosities of *D. edulis* and *A. occidentale* creams stored at different temperatures over time. Therefore, the stored creams showed rheopectic behaviour. This opposed Ajala (2014), who reported that there were insignificant changes in the viscosities of *Phyllantus amarus* creams at different temperatures over time. This might be due to the different plants and humectants used for the cream preparations. The observed stability of *D. edulis* and *A. occidentale* creams could be because of the compatibility of the extracts with the base. Moreover, Huang *et al*. (2001) reported that small oil globules, high viscosity and compatibility of base with the active principles in plant extract, generally contribute to the stability of any cream.

Rawlins (2004) reported that temperature affects viscosity of fluids and semi-solids in an indirect relationship by the Arhenius equation. Therefore, this study supports that creams should be stored at lower temperature (not more than 29 ± 4 ^{o}C), in order to prolong stability and shelf life; because higher temperatures accelerate degradation.

Contamination with heavy metals, pesticides, microbes, toxins or adulteration with conventional drugs, may be the factors responsible for toxicity of some formulations. Consequently in the cosmetic industry, assessment of irritancy potential to human skin of any chemical or formulation is compulsory (Nair *et al*., 2012a).

The skin irritation test of the formulated creams was purposively done on the skin surfaces of the mice, in order to verify the risk of irritation or safety of the creams. There was no behavioural abnormality in the mice, after the application of *D. edulis* and *A. occidentale* creams; no oedema or erythema on their skins, thus showing no dermal irritation to the mice.

There was no abnormal behaviour or anomalous colour change in the newly grown furs of the mice for the twenty-one days of application of *D. edulis* and *A. occidentale* creams. Moreover, no visible lesions were found with *D. edulis* creams and *A. occidentale* creams. A lesion as described by Lademann *et al*., 1999, is any localized abnormal structural change in a body part and could be a tubercle, an ulcer or any type of injury; it is any pathological or traumatic change in a cell.

Laxmi and Pranita (2014) reported that an ideal topical product is one that achieves a sufficient concentration in the target tissue, so as to produce a desired pharmacological response, in addition to having an acceptable safety profile, thereby leaving the skin harmless. Therefore, *D. edulis* creams and *A. occidentale* creams, whether in use for short or long period, could be regarded as safe for topical applications.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Only very few people living in Ibadan were familiar with *Dacryodes edulis* and its medicinal uses, but many have prior knowledge about *Anacardium occidentale* and its medicinal uses, that includes treatment of skin infections in Ibadan, Southwest, Nigeria.

The leaf of *D. edulis* had a strong antioxidant capability, while *A. occidentale* leaf has very poor antioxidant ability, thus the leaf of *D. edulis* might be useful in formulating antioxidant drugs and skin anti-ageing creams.

Dacryodes edulis and *A. occidentale* have rich phytochemical constituents. Therefore, phytochemicals of interest might be optimally extracted from the plants based on their respective solvents of extraction for drug production.

Dacryodes edulis and *A. occidentale* leaf extracts had antimicrobial property. Therefore, active ingredients for drugs against skin infections causing boil, carbuncles, folliculitis, and candidiasis could be isolated and formulated from the plants.

The functional groups such as amines, alkyl halides and amides in *D. edulis* ethyl acetate fraction; and esthers, alkenes and ketones in *A. occidentale* ethyl acetate fraction showed their drug ability.

Ethyl acetate and aqueous fractions of *D. edulis* and *A. occidentale* leaves were non-toxic to kidneys and livers of albino rats at 10 - 2,900 mg/kg body weight. Only the ethyl acetate of *D. edulis* and *A. occidentale* leaves showed mild and severe toxicity on rats' liver at 5,000 mg/kg respectively.

The formulated creams from *D. edulis* (FDe1, FDe2, and FDe3) and *A. occidentale* (FAo1, FAo2, and FAo3) elicited satisfactory physicochemical properties. The 10 % leaf ethyl acetate fraction of *D. edulis* cream (FDe3) was the most potent against the tested microorganisms. All the creams were more stable at lower temperatures and not irritating or toxic to the skins.

Summarily, this research revealed and compared the antioxidant power, phytochemical contents, antimicrobial activity and toxicity of *D. edulis* and *A. occidentale* leaves, therefore supporting some ethnomedicinal uses against some skin infections causing microorganisms. Furthermore, creams were formulated and the formulated creams have antimicrobial abilities that might be developed in treating skin infections such as boils, carbuncles, breast abscess, infant-impetigo, folliculitis, candidiasis and dermatophytosis. The creams also have acceptable physicochemical properties which are stable, safe and could be commercially developed for use.

6.2 Recommendations

More medicinal assays should be done on the leaves and other parts of *D. edulis* and *Anacardium occidentale*. This is in order to have a comprehensive herbal profile for reference which could be useful for both local and industrial drug producers. Moreover, polyherbal antimicrobial cream formulations from the two plants and other medicinal plants should be also investigated.

Conventional drugs usually indicate the possible contra-indications on the drugs' labels. However, people traditionally assume many plants to be non-toxic and frequently use them either for self medication; or concocted and packaged for sale without any toxicological verification of the state of the plants before use. It is therefore recommended that the toxicity level of a non-toxic plant should be re-affirmed, before any herbal preparation is taken, in order to ensure it is toxic free.

Plants are the main medicinal sources to treat infectious diseases in some developing countries, therefore plant extracts should be continuously researched to know their medicinal compounds and find new drugs, especially those with potentials against multi-resistant microbes thus challenging pharmaceutical industries globally. In addition, isolation and characterization of active ingredients from *D. edulis* and *A. occidentale* leaves against serious pathogens are essential.

More cultivation of the plants and commercial drug production from them might reduce the problem of unemployment and alleviate poverty and in addition to solving health problems.

The study of medicinal plants and herbal product formulations should be encouraged, promoted and intensified nationally by all the stakeholders.

Finally, the National Agency for Food and Drug Administration and Control (NAFDAC) should work more at both local and national levels on continual monitoring and testing for quality control of all herbal products.

6.3 Contribution to knowledge

This research scientifically proved that if *Dacryodes edulis* and *Anacardium occidentale* leaves are used singly in treating any of the tested microbes, the inactivity or effectiveness is dependent on the solvent of extraction.

The information on antioxidant, phytocemical, antimicrobial, functional group and toxicity of *D. edulis* and *A. occidentale* leaves in this study would be useful in the standardization of herbal medicine and products involving *D. edulis* and *A. occidentale* leaves in Nigeria.

This work is the first to incorporate *Dacryodes edulis* leaf (an indigenous plant) and *Anacardium occidentale* leaf into conventional cream formulations in the treatment of some skin infections.

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