

**MICROBIAL EVALUATION AND ANTIBACTERIAL PROPERTIES OF  
COCONUT WATER FROM THREE MARKET IN BENIN CITY**

**BY**

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**LSC2007249**

**DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY**

**FACULTY OF LIFE SCIENCES**

**UNIVERSITY OF BENIN**

**BENIN CITY.**

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY  
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## CERTIFICATION

It is certified that this project work was carried out by **Emmanuel Osadebamwen Osarenmwinda** with Matriculation number LSC2007249 in the Department of Plant Biology and Biotechnology, Faculty of Life sciences, University of Benin, Benin city, Edo state Nigeria.

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**Date**

## **DEDICATION**

This project work is dedicated to GOD ALMIGHTY.

## ACKNOWLEDGEMENT

I am highly grateful to GOD ALMIGHTY for his infinite love and mercy which saw me throughout this research.

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## ABSTRACT

Coconut is one of the most nutritionally and economically valuable foods known to humankind. Its various edible products further enhance its significance, making the coconut palm a highly valued tree, especially in tropical regions where it is widely cultivated. Due to the increasing resistance of microorganisms to commonly used antibiotics, there is a growing need for alternative antimicrobial agents. This study investigated the antibacterial activity of coconut water obtained from both young and mature coconut fruits against selected gastrointestinal bacteria. Gastrointestinal bacteria are known to cause infections that primarily affect the digestive system, leading to symptoms such as diarrhea and vomiting. For this study, coconuts were sourced from three locations in Benin: Oluku Market, NIFOR, and Iguosa Market. Coconut water contains lauric acid, a bioactive compound with antibacterial, antifungal, and antiviral properties, which helps strengthen the immune system. Additionally, coconut water plays a role in improving digestion and nutrient absorption while offering a low-calorie alternative to other beverages. Given its natural antimicrobial potential, coconut water could serve as a functional food with therapeutic benefits, particularly in managing gastrointestinal infections. Further research into its mechanisms of action and potential applications in clinical settings could provide new insights into its role as a natural remedy for bacterial infections.

## CHAPTER ONE

### 1.0

### INTRODUCTION

Raw fruits provide proteins, carbohydrates, many vitamins, and minerals essential to healthy human life. These fruits are convenient meals for contemporary lifestyles (Abdulelah, *et al.*, 2011). In many countries, including Nigeria, the rate of consumption of raw fruit is increasing day by day. To prevent cancer, diabetes, heart disease, obesity, and micronutrient deficiencies, the WHO Expert Consultation Panel recommends an adequate daily intake of 400–500 g of fruit (WHO, 2004). Fresh produce may be contaminated with different kinds of microorganisms (Taura and Habibu, 2009; Lynch *et al.*, 2009). Microbial contamination can occur during any steps of the farm-to-table and this contamination can occur from environmental, animal, human sources, and technological applications. Microorganisms could spread through direct contact, with air, and water (Jay, 2000).

Raw fruits are consumed without enough heating process, and therefore the possibility of food poisoning and food-borne infections always exists (Brandl, 2006). The number of reported food-borne disease outbreaks associated with raw fruits has increased in recent years. The identified outbreak's etiology has a primarily bacteriological origin (Frank and Waribor, 2006). However, most farmers do not have enough information about hygienic production and good agricultural practices. This poses a high potential risk to public health. Increasing consumption of fresh produce has been associated with outbreaks of foodborne illnesses. Fresh produce can be a vehicle for the contamination of enterotoxigenic and enterohemorrhagic organisms, *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, thermotolerant *Campylobacter* spp., parasitic and viral pathogens capable of causing human illness and many reports refer to raw vegetables and fruits harbouring potential foodborne pathogens (Hamilton *et al.*, 2006; Hanning, 2009; Miedes and Lorences, 2012).

The coconut tree (*Cocos nucifera*) is a member of the family Arecaceae (palm family) (Neto *et al.*, 2007). The coconut is known for its great versatility and uses of its different parts, it is also found throughout the tropics and subtropics (Pummer *et al.*, 2001). Nutrients in Coconut water include Lauric acid, Chloride, and Iron, as well as important

electrolytes such as Potassium, Magnesium, Calcium, Sodium, and Phosphorous (Scalbert and Saltmarsh, 2005).

Coconut fruit helps to remove toxins from the body and aids digestion, Coconut fruit has amazing anti-viral, anti-fungal, and anti-microbial properties that help to cure diseases and heal wounds (Ajibogun and Oboma, 2013). Coconut water is the nutritious clear liquid inside the coconut fruit which is packed with vitamins and minerals. There is usually more water in a young coconut, since the water is replaced by the white coconut flesh as it matures. Therefore, for drinking purposes, coconuts are harvested from the trees when they are still young and green. Later, the outer green husk is removed and the coconuts are wrapped in plastic to keep the moisture (Ajibogun and Oboma, 2013). For best results, the water from a fresh coconut should be consumed shortly after being exposed to air due to the possible loss of important nutrients. A single coconut usually provides an 11-ounce serving of water, and it is low in calories and fat but rich in vitamins, minerals, and other nutrients. A few key nutrients in Coconut Water include Lauric acid, Chloride, and Iron, as well as important electrolytes such as Potassium, Magnesium, Calcium, Sodium, and phosphorus. The potassium content in Coconut Water is close to twice the amount in a banana. A healthy balance of electrolytes is important for the optimal health of our muscular, cardiovascular, nervous, and immune systems, as well as to help with the absorption and balance of the body's internal fluids.

Coconuts are unique in terms of their fruit morphology. Fibrous coconut fruit is not only edible but also popular for its multipurpose use. Besides being highly nutritious, coconuts have also been studied for medicinal qualities for heart, liver, and kidney disorders. The coconut has recently been reported to reduce the viral load of human immunodeficiency virus (HIV). Modern medical science is now confirming the medicinal qualities that are used for the treatment of heart, liver, and kidney disorders. Based on the knowledge of the traditional herbs used for the treatment for local application, coconut husk can be used as a topical antimicrobial. Previous studies on pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes* have demonstrated that these pathogens can contaminate fruits at any growing stage in the field due to infected animals or contaminated manures (Olaimat and Holley, 2012). In addition, they can survive in soil for long period and contaminate irrigation water, with consequent transmission to

harvested produce (Upadhyay and others 2014). Fresh produce can also be contaminated with pathogenic bacteria during the postharvest handling, transportation, and retailing process.

Numerous studies have documented the association between the consumption of fruits and the incidence of foodborne diseases (Khan and Oh 2016b; Rahman and others 2016; Ngnitcho and others 2017). Microbiological qualities and prevalence of pathogens in fresh produce vary significantly due to different major factors that can affect microbial safety. Such factors include the location of samples, growth conditions, harvesting season and method, and the microbiological analysis method used (Cardamone and others 2015). Such variations highlight the difficulties in identifying the risk, the sources of contamination, and control strategies (FAO/WHO 2008). Microbial assessment in a particular city or province is crucial for the identification and implementation of risk assessment. Microbiological safety is a spatiotemporal dynamic situation that depends on numerous factors in production and processing.

## **1.2 BOTANY OF COCONUT**

*Cocos nucifera* is a large palm, growing up to 30 m (98 ft) tall, with pinnate leaves 4–6 m (13–20 ft) long, and pinnae 60–90 cm long; old leaves break away cleanly, leaving the trunk smooth. Coconuts are generally classified into two general types: tall and dwarf (Edward *et al.*, 2006). On very fertile land, a tall coconut palm tree can yield up to 75 fruits per year, but more often yields less than 30, mainly due to poor cultural practices. Given proper care and growing conditions coconut palms produce their first fruit in six to ten years, it takes 15 – 20 years to reach peak production (Esquenazi *et al.*, 2002).

Botanically, the coconut fruit is a drupe, not a true nut (Bourke *et al.*, 2009). Like other fruits, it has three layers: the exocarp, mesocarp, and endocarp. The exocarp and mesocarp make up the "husk" of the coconut. Coconuts sold in the shops of non-tropical countries often have the exocarp (outermost layer) removed. The mesocarp is composed of a fiber, called coir, which has many traditional and commercial uses. The shell has three germination pores (stoma) or "eyes" that are visible on its outside surface once the husk is removed. A full-sized coconut weighs about 1.44 kg (3.2 lb). It takes around 6,000 full-grown coconuts to produce a tonne of copra (Thormar, 1996).

Unlike some plants, the palm tree has neither a tap root nor root hairs, but has a fibrous root system (Thampan, 1981). The coconut palm root system consists of an abundance of thin roots that grow outward from the plant near the surface. Only a few of the roots penetrate deep into the soil for stability. The type of root system is known as fibrous or adventitious, and is a characteristic of grass species. Other types of large trees produce a single downward-growing tap root with a number of feeder roots growing from it. Coconut palms continue to produce roots from the base of the stem throughout its life. The number of roots produced depends on the age of the tree and the environment, with more than 3,600 roots possible on a tree that's 60 to 70 years old. Roots are usually less than about 3 inches in diameter and uniformly thick from the tree trunk to the root tip (Thormar, 1996).

The palm produces both the female and male flowers on the same inflorescence; thus, the palm is monoecious. Other sources use the term polygamomonoecious (Willmer, 2011). The female flower is much larger than the male flower. Flowering occurs continuously. Coconut palms are believed to be largely cross-pollinated, although some dwarf varieties are self-pollinating (Thormar, 1996).

#### **1.4 TAXONOMY OF COCONUT**

Kingdom: Plantae  
(unranked): Angiosperms  
(unranked): Monocots  
(unranked): Commelinids  
Order: Arecales  
Family: Arecaceae  
Subfamily: Arecoideae  
Tribe: Cocoeae  
Genus: *Cocos*  
Species: *C. nucifera*

#### **Local Names**

Bengali (narikel); Burmese (on,mak-un); Creole (kokoye); Dutch (cocospalm,coco,cocos,klapperboom); English (coconut palm,coconut); French (coco,noix de coco,cocotier,cocoyer,coq au lait); German (kokospalme, kokosnusspalme); Indonesian (kelapa); Italian (cocco); Lao (Sino Tibetan) (phaawz); Malay (kelapa); Mandinka (tubab sibo, coc); Portuguese (coco da India, coco da Bahia,coqueiro de Bahia); Spanish (cocotero, coco de agua, coco, palma de coco, palmera de coco); Swahili (mnazi); Tamil (tennai-maram); Thai (ma phrao); Trade name (coconut); Vietnamese (dùa)

## **1.5. Nutritional Value of Coconut Fruit**

### **1.5.1 Carbs in Coconut**

Unsweetened coconut has relatively few carbs and is generally okay for people on a low-carb diet. It can even be consumed in moderation during the induction phase of the Atkins Diet and phase 1 of the South Beach. The same applies to coconut milk and water. The high-fat content in coconut makes it ideal for a ketogenic diet plan. For the early phases of some low-carb plans, the intake may be limited to no more than 15 to 22 grams per day (Edward and Craig, 2006).

Coconut also has an impressive amount of fiber, an indigestible form of dietary carbohydrate. This includes soluble fiber that draws water from the intestines to ease bowel movements and slow the absorption of fat and sugar into the bloodstream. Insoluble fiber provides bulk to stool, preventing constipation and the development of hemorrhoids. Most experts today recommend a total dietary fiber intake of 25 to 30 grams per day with about one-fourth—6 to 8 grams—coming from soluble fiber (Guan *et al.*, 2006).

### **1.6. Fats in Coconut**

Coconut is one of the highest plant-based sources of saturated fat. Saturated fat is the type mainly derived from meat protein associated with hypercholesterolemia (high cholesterol), atherosclerosis (hardening of the arteries), and heart disease. However, not all saturated fats are created equal. Those found in meat and chocolate are primarily palmitic acid. For its part, coconut consists mainly of the lauric acid and myristic acid (Ishida *et al.*, 2000). One medium-sized coconut weighing about 397 grams has 1,405

calories. If you cut the coconut meat into about 2-inch pieces, the calorie count is 159 per piece. The total fat in one coconut is about 133 grams. One 2-inch piece has approximately 15 grams of total fat with 13 grams of saturated fat.

Fiber-rich foods help control your blood sugar and cholesterol, plus keep you full longer. The recommended daily intake of fiber is about 25 grams for women and 38 grams for men. A 2-inch piece of coconut provides you with 4 grams of dietary fiber. The whole coconut has almost 36 grams of fiber (Krogh *et al.*, 1999). Palmitic acid is the type associated with increases in "bad" low-density lipoprotein (LDL) cholesterol and decreases in "good" high-density lipoprotein (HDL) cholesterol—essentially a lose-lose situation. By contrast, lauric acid appears to have less effect on your HDL, while myristic acid may boost HDL levels. Moreover, the saturated fats in coconut are mainly medium-chain fatty acids. These are the types that break down faster and do not accumulate in the bloodstream as much as the long-chain fatty acids found in meat. Despite this, the saturated fats in coconut will increase LDL levels as much as those in meat and butter. Most dietitians would agree that swapping extra-virgin olive oil for coconut oil would not do your heart any favors (Rengarajan *et al.*, 2012).

### **1.7 Protein in Coconut**

Coconut meat is a rich source of plant-based protein, offering about half the amount of an equal serving portion of tofu. However, unlike tofu, beans, and other plant-based proteins, most people don't eat much coconut in one sitting. To this end, if you are vegetarian or vegan, you may better be served to drink a cup of coconut milk, which delivers around 6 grams of protein per one-cup serving. The Food and Nutrition Board of the Institute of Medicine recommends a daily protein intake of 0.8 grams per kilogram of body weight (or 0.36 grams per pound). That translates to 46 grams of protein per day for a sedentary woman and 56 grams per day for a sedentary man (Rice-Evans *et al.*, 2006).

### **1.8. Micronutrients in Coconut**

A one-third-cup serving of coconut meat offers a fair amount of your daily essential minerals. According to the U.S. Department of Agriculture, a serving of coconut offers the following reference daily intake (RDI) of the following nutrients:

- Manganese: 20% of the RDI
- Copper: 15.4% of the RDI
- Selenium: 4% of the RDI
- Iron: 3.9% of the RD
- Phosphorus: 3% of the RDI
- Potassium: 2.7% of the RDI
- Magnesium: 2% of the RDI
- Zinc: 2% of the RDI

Coconut is a less rich source of vitamins, the highest of which include B vitamins such as folate and thiamine.

### **1.9 Coconut Water**

Coconut water serves as a suspension for the endosperm of the coconut during its nuclear phase of development. Later, the endosperm matures and deposits onto the coconut rind during the cellular phase (Paniappan, 2012). It is consumed throughout the humid tropics, and has been introduced into the retail market as a processed sports drink. Mature fruits have significantly less liquid than young, immature coconuts, barring spoilage. Coconut water can be fermented to produce coconut vinegar. Per 100 gram (100 ml) serving, coconut water contains 19 calories and no significant content of essential nutrients.



*Plate 1: Coconut containing water with its shell opened*

### **1.10 Coconut milk**

Coconut milk, not to be confused with coconut water, is obtained primarily by extracting juice by pressing the grated coconut white kernel or by passing hot water or milk through the grated coconut, which extracts the oil and aromatic compounds. It has a fat content of around 23% (Margolis, 2006). When refrigerated and left to set, coconut cream will rise to the top and separate from the milk. The milk can be used to produce virgin coconut oil by controlled heating and removal of the oil fraction. A protein-rich powder can be processed from coconut milk following centrifugation, separation, and spray drying (Naik *et al.*, 2012).

#### Coconut Oil

Another by-product of the coconut is coconut oil. It is commonly used in cooking, especially for frying. It can be used in liquid form as would other vegetable oils, or in solid form as would butter or lard.

### **1.11 Toddy and Nectar**

The sap derived from incising the flower clusters of the coconut is drunk as *neera*, also known as toddy or *tuba* (Philippines), *tuak* (Indonesia and Malaysia) or *karewe* (fresh and not fermented, collected twice a day, for breakfast and dinner) in Kiribati. When left to ferment on its own, it becomes palm wine. Palm wine is distilled to produce *arrack*. In

the Philippines, this alcoholic drink is called *lambanog* or "coconut vodka" (Porter, 2005).

The sap can be reduced by boiling to create a sweet syrup or candy such as *te kamamai* in Kiribati or *dhiyaa hakuru* and *addu bondi* in the Maldives. It can be reduced further to yield coconut sugar also referred to as palm sugar or jaggery. A young, well-maintained tree can produce around 300 liters (Perera *et al.*, 2009).

### **1.12. Heart of Palm and Coconut Sprout**

Apical buds of adult plants are edible and are known as "palm cabbage" or heart of palm. They are considered a rare delicacy, as harvesting the buds kills the palms. Hearts of palm are eaten in salads, sometimes called "millionaire's salad". Newly germinated coconuts contain an edible fluff of marshmallow-like consistency called coconut sprout, produced as the endosperm nourishes the developing embryo.

### **1.13. Health Benefits of Coconut**

Many studies have shown that the antiviral, antibacterial, anti-inflammatory, and antioxidant activity of Coconut may help with some minor to severe health conditions. This nutrient-rich drink has been used to regulate blood pressure, blood sugar, and cholesterol levels, and it has been found to boost energy levels and increase metabolism in the human body. Other conditions that it is effective at treating include stomach flu, dysentery, indigestion, constipation, intestinal worms, cholera, urinary abnormalities, urethral stone, malfunctioning kidneys, dry and itchy skin, age spots, and wrinkles (Abdulelah *et al.*, 2011).

### **1.14. Antimicrobial Activity of Coconut**

The non-precise standardization of dosage and unhygienic practice environments in traditional medicine setups are factors militating against traditional medical practitioners. Such unethical practices could lead to preventable hazards such as therapeutic failure, toxicity, emergence of resistance and other adverse effects on the consumers. Formulation into designed dosage form is a gateway towards scientific evaluation and standardization of crude drugs. Skin infections are widely encountered in the tropics with

lots of orthodox remedies involving the use of systemic antibiotics, the problems of drug resistance and reported allergies also abound. Coconut oil has been confirmed to possess antimicrobial, antiviral, and antiprotozoal activities (Manisha and Shyamapada, 2011). Phytochemical studies indicated that lauric acid which is its major fatty acid component was highly responsible for the activities of the oil (Peat, 2003). Lauric acid has been documented to be converted to monolaurin in the human body and it is the antimicrobial agent found in human milk (Pumer, 2001).

## **1.15. PATHOGENS ASSOCIATED WITH COCONUT FRUIT**

### **1.15.1 Salmonella**

*Salmonella* is a member of the family Enterobacteriaceae which comprises a large and diverse group of Gram-negative rod-shaped bacteria. *Salmonella* are facultative anaerobic, non-lactose fermenting, non-spore-forming, and most are motile. There are currently over 2400 serotypes. Complete inhibition of growth occurs at pH 3.8 and 9.0, temperature 7°C, or water activity 0.94 (Jay, 2000; Gray and Fedorka-Cray, 2002). Optimum growth occurs at pH near neutrality and temperatures between 35 and 37°C.

*Salmonella* gastroenteritis usually follows the ingestion of contaminated food or drinking water. Typically, gastroenteritis in humans begins 24-28 hours after ingestion and normally consists of fever, chills, headache, nausea, and vomiting, followed or concomitant with, abdominal cramps and diarrhea. These symptoms are usually accompanied by prostration, muscular weakness, faintness, and drowsiness. Ingestion of contaminated food with  $10^5$  -  $10^6$  cells is sufficient to trigger symptoms. The spectrum of disease ranges from loose stools to severe dysentery-like syndrome.

*Salmonella* grow readily in many foods, as well as water contaminated with feed or feces. The primary habitat of the bacteria is the intestinal tract of animals, humans, and on occasion insects (Jay, 2000). In addition, *Salmonella* has been isolated from soil, water, and sewage contaminated with fecal matter. *Salmonella* species has been isolated from coconut chips. These organisms can lead to outbreaks of salmonellosis (Hedburg and Olsterhol, 1993; WHO, 1998).

### **1.15.2 *Escherichia coli***

Enterotoxigenic *E. coli* is a common cause of travelers' diarrhea, an illness sometimes experienced when visiting developing countries. Raw coconut fruits are thought to be a common cause of travelers' diarrhea. A prospective study of 73 physicians and 48 family members attending a conference in Mexico City in 1974 revealed that enterotoxigenic *E. coli* was the most common cause of illness (Merson *et al.*, 1976). Fifty-nine participants became ill from eating salads containing raw vegetables. The vast majority of outbreaks of illness associated with *E. coli* O157:H7 have been associated with consuming undercooked beef and dairy products. However, outbreaks have also been linked to coconut fruit. Outbreaks may be associated with coconut water contamination. *Escherichia coli* O157:H7 grows rapidly in several types of raw fruits and vegetables, particularly when stored at 12°C (53.6°F) or above. Packaging under a modified atmosphere has little or no effect on the survival or growth of *E. coli* O157:H7. In addition, the infection dose of *E. coli* O157:H7 is low and can develop acid-resistance.

### **1.15.3. *Listeria monocytogenes***

While *L. monocytogenes* causes relatively mild gastroenteritis in healthy adults, the illness can be severe in susceptible individuals including pregnant women, neonates, and immune-compromised individuals. The infective dose for this organism has not been established, although it is thought to be relatively low among susceptible individuals.

*Listeria monocytogenes* is widely distributed on prepared coconut chips (Beuchat 1996). However several studies with relatively large sample sizes failed to detect the organism. Factors affecting its presence or persistence have yet to be determined. Coconuts used as salad vegetables play a role in disseminating the pathogen from natural habitats to the human food supply. This role may be indirect, for example by contaminating milk via forage or silage, or direct in the form of raw contaminated produce.

### **1.15.4. *Staphylococcus aureus***

*Staphylococcus aureus* has been detected in coconut water and ready-to-eat coconut salads and is known to be carried by food handlers. However, enterotoxigenic *S. aureus* does not compete well with other microorganisms normally present in fresh produce, so incipient spoilage caused by nonpathogenic microbiota would likely precede the

development of high populations of this pathogen. An outbreak of staphylococcal foodborne illness was linked to fruit used in processed food. Growth and toxin production occurred before processing the coconut salads, without significant visual degradation, possibly because the coconut fruit was held under ambient conditions in plastic bags and with salt. Conditions within the bags rapidly became anaerobic and the normal spoilage microbiota may have been inhibited and *S. aureus* selected. Because the toxin is heat stable, it survived the thermal process. This suggests that raw produce-associated outbreaks due to *S. aureus* could potentially occur given the right conditions. *S. aureus* has been shown to grow on peeled coconut fruits or survive up to 14 d when stored at 4-8°C (39.2-46.4°F).

#### **1.16. Sources of Microbial Contamination of Coconut Fruits**

Contamination sources of fresh coconut fruits include handling, washing, and rinsing water, conveying material from the farm, and contact with processing equipment. The microorganisms that exist on the surfaces of fruits appear to be the major source of microbial contamination and consequent spoilage of processed fresh fruits. Hanning (2009) reported that, compared with good surface sanitization practices, no decontamination treatment or an ineffective antimicrobial treatment on whole cantaloupe resulted in premature microbiological spoilage of fresh-cut coconut. Studies have also revealed over 1 year of sampling that there is a close relationship between the total mesophilic aerobic counts on coconut salads, raw material, and those on finished shredded coconut products. Robbs *et al.* (1996) determined that the most common bacteria on raw celery plants, including fluorescent *Pseudomonas* spp. and *Aeromonas* spp., were also the most common bacteria on cut celery products.

Boyette *et al.* (1993) reported that the microbial decay of fresh-cut coconut fruit is largely due to the growth of microorganisms originating from preharvest environments. Delaquis *et al.* (1999) determined that the types of microorganisms found on shredded coconut fruits were highly associated with the microorganisms detected on coconut fruit before shedding. Several studies (Magnusson, 1990; Geeson, 1990; Torok and King, 1991) have revealed that yeast species identified on fresh-cut produce can also be isolated from raw materials before processing. Garg *et al.* (1990) concluded that peel is the major source of microbial contaminants in coconut fruit.

Inoculation of *Listeria monocytogenes* and *Salmonella* on the surface of entire cantaloupes resulted in the contamination of fresh-cut pieces during cutting (Ukuku and Sapers, 2001; Ukuku and Fett, 2002). These results indicate that the bacteria on the surface of raw fruit and vegetables are the same as those on fresh cut fruit and vegetables after processing. Fresh-cut fruit and vegetable can also be contaminated by spoilage microorganisms through human handling or equipment during processing possibly by air during processing and packaging steps, especially in facilities that have been used for produce processing over an extended period. Cantwell and Suslow (2002) found significantly higher bacterial counts during processing on automated cutters and package fillers of a coconut chips processing line, indicating that clean product can become re-contaminated after passing through operations where vegetable and fruit debris can accumulate, such as cutters and package-filling equipment. Shredding and slicing steps in fresh-cut processing resulted in increased microbial populations by 1–3 logs on cut coconut (Garg *et al.*, 1990) and at least a 1-log increase for coconut and other vegetable salads (Jockel and Otto, 1990). Yeast populations on cut fruits increased after packaging. Several studies have suggested that the large numbers of lactic acid bacteria and fungi present in fresh-cut fruit and vegetables indicate likely contamination from processing, such as cutting knives or machines (Brocklehurst *et al.*, 1987; Dijk *et al.*, 1999). For example, *Geotrichum candidum* has been termed “machinery mold” because it can accumulate on fruit-processing equipment.

Many types of microorganisms can be found on a cut fruit or vegetable, including Gram-negative bacteria, Gram-positive bacteria, and fungi (yeasts and molds). Some viruses have been identified as plant pathogens of whole produce and presumably result in quality loss of fresh-cut root or tuber vegetables. Parasites can be a food safety concern but do not affect the sensory qualities/spoilage of either whole or fresh-cut fruits or vegetables. Buick and Damoglou (1987) reported that the microflora isolated from chlorinated sliced coconut fruits included 70% *Erwinia* spp., 20% *Pseudomonas* spp., and 10% *Bacillus* spp. Babic, (1992) identified a variety of yeasts, including *Candida* spp., *Cryptococcus albidus*, *Rhodotorula* spp., *Trichosporon penicillatum*, and *Saccharomyces cerevisiae*, on packed grated coconut. Carlin *et al.* (1989) isolated lactic acid bacteria, specifically *Leuconostoc mesenteroides*, and yeasts from grated coconut stored in modified atmospheric packaging.

Liao and Fett (2001) isolated lactic acid bacteria, yeasts, and 48 strains of pectolytic bacteria of the genera *Pseudomonas*, *Erwinia*, *Bacillus*, *Xanthomonas*, and *Flavobacterium* from coconut fruits. Poubol and Izumi (2005) reported that bacteria isolated from coconut chips were predominantly Gram-negative rods of which about 60% were Enterobacteriaceae, including the genera *Klebsiella* and *Pantoea*. Phytopathogenic bacteria that cause rot in fruits such as *Pantoea agglomerans* (synonymous with *Erwinia herbicola* and *Enterobacter agglomerans*) and *Burkholderia cepacia* (synonymous with *Pseudomonas cepacia*) were also isolated frequently. The most common Gram-positive bacteria were of the genus *Curtobacterium*. Robbs *et al.* (1996) found Gram-negative bacteria fluorescent *Pseudomonas* spp., *Pantoea herbicola* (*E. herbicola*), *P. agglomerans*, *Aeromonas*, *Arthrobacter*, *Agrobacterium*, and *E. carotovora*, Gram-positive cocci (*Leuconostoc*), and Gram-positive rods on decayed fresh-cut celery. Garg *et al.* (1990) detected large populations of Gram-negative psychrotrophic bacteria, particularly *Pseudomonas* spp., lactic acid bacteria, and fungi on freshly prepared coconut fruit sticks. The filamentous fungi isolated from ready-to-eat salads included *Alternaria*, *Cladosporium* and *Penicillium*, *Alternaria*, *Cladosporium* and *Aspergillus*, and *Fusarium* (Tournas, 2005; Acevedo *et al.*, 2001).

### **1.17. Impact of Microbiological Spoilage of Coconut Fruits**

Microbial spoilage including off-flavor, formation, slimy surface, wetness and soft rot, discoloration and visual microbial growth/colonies has been used as a main or exclusive objective criterion to determine the shelf life of fresh-cut fruit and vegetable (O'Connor-Shaw *et al.*, 1994). Brackett (1994) concluded that microbial decay can be a major source of spoilage of fresh-cut fruit and vegetables. O'Connor-Shaw *et al.* (1996) reported that microbial spoilage is a limiting factor for the shelf life of coconut pieces stored under controlled atmosphere conditions. Shelf life, including microbial spoilage, results in 30–50% shrinkage of coconut fruits (Warren, 2005).

### **1.18. Prevention and Control Measures**

Fresh fruits and vegetables are among the more challenging of food products to commercially produce and distribute. Fresh produce remains metabolically and developmentally active as it proceeds from the commercially appropriate time to harvest (horticultural maturity), to physiological maturity, to senescence and complete

deterioration. During this period of development, several physiological and compositional changes occur. This process can be summarized chronologically as growth, maturation, physiological maturity, ripening, and senescence (Watada *et al.*, 1984). Although infection and microbiological spoilage can proceed at any time during this developmental continuum, the period of greatest susceptibility to decay onset is during ripening and senescence. Prior to ripening, fruits and vegetables are equipped with defensive barriers to infection including active wound healing and the production of phytoalexins which are phenolic substances that are toxic to fungi.

Losses due to postharvest spoilage or pathological decay are a result either of latent infections in the field that become active following harvest or of cross-contamination during harvest, cleaning, storage, and distribution. The presence of the pathogen on a susceptible host fruit or vegetable, combined with suitable environmental conditions such as high temperature, provides the three components required for disease expression such as host, environment, and pathogen (Sommer, 1992). Therefore, spoilage management should begin in the field using an integrated strategy of GAPs. Balanced crop nutrition influences susceptibility to spoilage. For example, Sugar, Righetti, (1992) determined that adjusting pear orchard nutrition, specifically for low nitrogen and high calcium, reduced fruit decay postharvest. High nitrogen in plant tissues generally increases susceptibility to decay, whereas high calcium content reduces postharvest decay on several crops. Removing dead and decaying plant matter and other organic material from the crop plant and soil surface will eliminate a major harborage for spoilage microbes as well as other crop pests. To the extent possible, isolating the agricultural field from wild and domestic animals will not only reduce total microbial pressure on the crop but also reduce food safety risks. Aerial fungicide applications preharvest also will reduce postharvest spoilage in storage. For example, a single application of the fungicide ziram to pome fruit reduced postharvest decay by 25–50% (Sugar and Spotts, 1995). Other preharvest fungicides are also available (e.g., iprodione and cypronidil) and several new fungicides are under development. In addition, insect pest management will reduce insect damage to crops and also will reduce microbial cross-contamination by the insect vector. This is especially important for chewing insects that create wounds on the fruit or vegetable and can simultaneously inoculate the wound site (Mahovic *et al.*, 2005). At the time of harvest and throughout handling before storage and distribution, it is important to minimize wounds and bruising and to cull all

damaged and diseased products. A few spoilage microbes, primarily fungi, can infect healthy tissues by forming appressoria, external structures that enable the pathogen to penetrate the cuticle and epidermis (Sommer, 1992). The developing appressorium ramifies through these protective layers and into the pulp through a combination of mechanical pressure and tissue destruction by extracellular enzymes (Collmer and Keen, 1986). However, most spoilage microbes infect and initiate decay at punctures and splits in the epidermal layer or, in far fewer cases, through natural openings such as stomata and lenticels.

### **Postharvest Factors**

Product integrity at time of harvest and stringent temperature management from harvest to consumption are two critically important factors contributing to acceptable storage and shelf life of all fresh fruits and vegetables. Upon harvest, fresh fruits and vegetables benefit from immediate surface sanitation and rapid cooling to slow product metabolism and growth of spoilage microbes. Reducing the rate of metabolism likewise reduces product respiration which, in turn, reduces the rate of deterioration, or perishability, of the crop (Kader, 1992).

In many instances, product cooling and sanitation are accomplished simultaneously through one or more washings with chilled water amended with a sanitizing chemical. Chlorine, as sodium hypochlorite, calcium hypochlorite, or chlorine gas, is the most commonly used sanitizing chemical in the produce industry. Chlorine at a rate of between 50 and 200 ppm is added to prechilled water which is then applied to harvested fruit as a dip or as a spray or as some combination of these two methods. Concentrations below 50 ppm may not be particularly effective on some fruit, and concentrations above 200 ppm may damage the product and also create a potential worker safety issue due to off-gassing. To achieve and maintain maximum sanitizing efficacy, it is important to maintain water pH at or slightly below neutrality (pH 6.5–7.0). This can be achieved using any of a number of food-grade acids such as citric acid. It is also important to maintain as low an organic load as possible in the wash water because chlorine is unstable in the presence of organic matter and is rapidly inactivated. Other sanitizing chemicals such as ozone, chlorine dioxide, and peroxyacetic acid also are approved for use on fresh produce and are available commercially (Sapers *et al.*, 2001).

Methods for monitoring sanitizer concentration are available for all commercially available sanitizers, and it is also strongly recommended to perform routine treatment efficacy assessments. This can be determined by collecting a minimum of three, and preferably five, individual samples immediately prior to washing and another three or five samples immediately following washing, and determining the total aerobic plate count on each sample. A properly functioning wash system should reduce the average total aerobic plate count by 10- to 100-fold. Sequential wash steps will further improve product sanitation by providing greater reductions in microbial load on the product.

### **1.19 Uses of Coconut**

**Age spots, wrinkles, and other skin problems:** Researchers have found that Cytokinins help regulate cell growth and their divisions. Coconut contains these cytokinins and lauric acid which can minimize the aging of skin cells, balance PH levels, and keep the connective tissues strong and hydrated. Therefore, simply applying Coconut Water to affected skin areas every night before going to bed may help with acne, age spots, wrinkles, stretch marks, cellulite, and eczema (Yoo *et al.*, 2010).

**Boosting Energy:** Abundant in vitamins, minerals, and other nutrients, Coconut Water makes a wonderful energy drink. In particular, coconut water has less sugar and sodium content compared to most sports drinks, while packing more Potassium, Calcium, and Chloride, which makes it a better choice to rehydrate, replenish, and boost the body's energy levels after any strenuous activity or workout. For example, in every 100ml of Coconut Water there is approximately 294mg of potassium compared to 117mg in an average energy drink, 25mg of sodium compared to 200mg in energy drinks and 41mg in most sports drinks, 5mg of sugar compared to 20-25mg, and 118mg of chloride compared to 39mg in average energy and sports drinks (João *et al.*, 2013).

**Cardiovascular Health:** According to researchers, individuals with high blood pressure usually have low potassium levels. Therefore, drinking coconut water regularly can be quite effective at regulating blood pressure due to its high concentration of potassium and lauric acid. Similarly, some recent studies have found that coconut water can help increase HDL (good) cholesterol, which makes it a wonderful natural treatment for maintaining good cardiovascular health.

**Dehydration:** Rich in Potassium and other minerals, Coconut helps to regulate our internal fluids and replenish and rehydrate the body. It has been used to treat dehydration caused by dysentery, cholera, diarrhea, and stomach flu, and the electrolyte balance and plasma in Coconut Water is similar to that of human blood. Therefore, drinking one cup of Coconut Water twice daily during digestive tract abnormalities, hot temperatures, and after strenuous workouts can help rehydrate the body quickly (Abdulelah *et al.*, 2011).

**Digestive Problems:** Coconut contains Lauric acid which our body converts into monolaurin. Monolaurin has great antiviral, antiprozoal, and antibacterial activity which helps fight against intestinal worms, parasites, lipid-coated viruses, and other gastrointestinal tract infections in children and adults. Additionally, the water from coconut may not only act as an antibiotic but it can also rehydrate the body. Therefore, a simple remedy for Intestinal Worms is to mix one teaspoon of extra virgin olive oil into a cup of Coconut Water and drink it daily for at least three days. For constipation, diarrhea and other common digestive problems drink one cup of Coconut Water twice daily (Hakimian *et al.*, 2014).

**Weight Loss:** Coconut is a natural electrolyte and isotonic beverage that helps increase the body's metabolism. Therefore, it can greatly benefit people who are struggling with weight issues.

### **1.18. 2 Pharmacological Properties of *Cocos nucifera***

*Cocos nucifera* parts are used in different parts of the world for the treatment of several diseases, such as diabetes, hypertension, dysentery, constipation, fatigue, arthritis, rheumatoid diseases, hydrocephaly, meningitis, kidney ailments, and inflammations. They also possess antimicrobial, analgesic, spasmolytic, spasmogenic, hypoglycemic, hypotensive, anticoagulant, anti-inflammatory, psychotomimetic, and anticancer activities (Meira *et al.*, 2012). *Cocos nucifera* is potent in the fight against cancer. It is rich in beta-carotene, which is good in fighting free radicals. Fluid and electrolyte balance is maintained by coconut water.

*Cocos nucifera* is good for cardiovascular health (Yoshimoto *et al.*, 2002). It is also used for the treatment of tumors of the mouth and throat, asthma, bug bites, burns, catarrh, ciguatera, convalescence, dyslectic, fever, nausea, renosis, splenosis, stomach distress, and whitlows (Duke and Wain, 2000).

Leaf decoctions are used as an alterative, aphrodisiac, astringent, bactericide, demulcent, fungicide, laxative, and tonic (Ludvik *et al.*, 2004). Tuber husk of *Cocos nucifera* was found to potentially prevent ethanol-induced gastric ulceration by suppressing edema formation and partially protecting gastric mucosa wrinkles and to heal wounds (Hermes *et al.*, 2013). Because of its proven anti-ulcerative activity, it could be considered when treating gastric ulcers (Rengarajan *et al.*, 2012).

**Antioxidant activity:** The total antioxidant capacity of *Cocos nucifera* has been reported to be 42.94% as compared to ascorbic acid (Pochapski *et al.*, 2011). The total antioxidant activity of *Cocos nucifera* water was higher than the leaf.

Their total phenolic content can serve as a useful indicator for the antioxidant activities of *Cocos nucifera* (Teow *et al.*, 2007). Total phenolic content is highest in the leaves and in the stem end of the roots of *Cocos nucifera* (Chang *et al.*, 2010). One study shows the antioxidant activity in *Cocos nucifera* water as 3.2 times higher than that of the mesocarp. Surprisingly, *Cocos nucifera* have potent antioxidant capacity in all of their parts. Recent research has shown different genes to be at work in the root versus fruit of the *Cocos nucifera* producing different concentrations of anthocyanin antioxidants. A recent study established baseline data on the total phenolic content and antioxidant activities of *Cocos nucifera* grown in the Philippines including seed, fruit, and root. Antioxidant activities were highest for seed. However, root had the best inhibitory action on linoleic acid oxidation. Methanolic *Cocos nucifera* extracts had higher radical scavenging activity, reducing power and oxidation inhibition than  $\alpha$ -tocopherol and higher iron-chelating capacity than ethylenediamine tetraacetic acid (EDTA) (Rumbaoa *et al.*, 2009). The total antioxidant strength of raw *Cocos nucifera* fruit measured in terms of oxygen radical absorbance capacity is 902  $\mu\text{mol TE}/100\text{ g}$ . Dong *et al.* (2012) studied the total antioxidant activity by DPPH (1,1-diphenyl-2-picrylhydrazyl) staining, reducing power method, metal ion-dependent hydroxyl radical, ferric thiocyanate (FTC) method, and protection of calf thymus deoxyribonucleic acid (DNA) against hydroxyl radical-induced damage on *Cocos nucifera* storage root mucilage. He suggested that the mucilage might contribute its antioxidant activities against both hydroxyl and peroxyl radicals (Huang *et al.*, 2004).

**Antidiabetic activity:** *Cocos nucifera* water exhibits potent antidiabetic properties, and its activity was shown to be higher than that of diabetes, a standard drug for treating

diabetes. A study has revealed that consumption of *Cocos nucifera*, a high polyphenol diet, for 7 days can modulate antioxidative status and decrease exercise-induced oxidative damage and proinflammatory cytokine secretion (Oke *et al.*, 1999). Researchers found that the protein content of the water of the *Cocos nucifera* was higher than that of the mesocarp. This suggests that the entire coconut could play a role in lowering blood glucose in diabetics: the water, as processed into a nutritional supplement like Caiapo; and the mesocarp, as a simple addition to the everyday diet.

Adiponectin is a protein hormone produced by fat cells. People with diabetes tend to have lower levels of adiponectin, and *Cocos nucifera* extracts have been shown to significantly increase adiponectin levels in persons with type 2 diabetes. In a study involving rats to determine *Cocos nucifera* effects on several markers of diabetes, the vegetable showed significant abilities to decrease some of the more harmful markers. Using white-fleshed *Cocos nucifera* for the study, the rats showed impressive improvement in pancreatic cell function, lipid levels, and glucose management. They also showed decreased insulin resistance in just eight weeks. Improved insulin sensitivity was also observed in a human study when *Cocos nucifera* was added to the diet (Grotto, 2008). Other research has confirmed that *Cocos nucifera* is a low-glycemic index (GI) food, which could be good for use by diabetics. With further research in this area, it may be possible to recommend that people with diabetes or insulin resistance consume *Cocos nucifera* or use its extracts to help control blood glucose. This therapy should cost less than conventional drugs, and it may have fewer side effects.

**Anticancer activity:** Researchers have found that eating *Cocos nucifera* will decrease the risk of breast, colorectal, gallbladder, and kidney cancer. A recent study evaluating the risk factors for kidney cancer death included 47,997 males and 66,520 females aged 40 years and older. Taking into account medical history, anthropometry, dietary, and lifestyle considerations over the 10-year study, the researchers concluded that eating *Cocos nucifera* regularly was associated with a decreased risk of the disease (Washio *et al.*, 2005).

4-Ipomeanol from infected *Cocos nucifera* is reported to possess cytotoxic and anticancer properties (Baer *et al.*, 2005). It was the first agent to be developed by the National Cancer Institute based on a biochemical-biological rationale as an anticancer agent targeted specifically against lung cancer (Lakhanpal *et al.*, 2001). Several human

tumor types, including many non-small cell lung lines and the MCF 7 breast cancer line, as well as its doxorubicin-resistant variant, are relatively sensitive to 4-Ipomeanol. Because of the specific lung toxicity 4-Ipomeanol is being tested as a new drug for the treatment of lung carcinoma. On the other hand, 4-Ipomeanol is metabolized by liver cells too. It was recently tested in phase II studies in patients with hepatocellular carcinoma and it showed hepatotoxicity (Smiley-Jewell and Plopper, 2003). Since 4-Ipomeanol is reported to be present only in infected *Cocos nucifera*, its occurrence is unlikely in normal tubers that are consumed as food. However, since the compound exhibits significant cytotoxic activity, it could be used as a lead in drug discovery for lung cancer (Hsu *et al.*, 2003).

**Cardiovascular effects:** When an extract of *Cocos nucifera* was examined for its relaxant activity on isolated rat vascular aortic preparations, it showed 97% relaxation activity for endothelium-intact aortic ring preparations but only 35% in the mesenteric vascular bed. It showed a good cardiovascular effect and its vasorelaxation mechanism of action was similar to that of the pharmacological agent acetylcholine (Runnie *et al.*, 2004).

**Immune system effects:** *Cocos nucifera* fiber may be useful in combination with other therapeutic agents for skin wound therapy. The healing effect of *Cocos nucifera* fiber was evaluated for burns and decubital wounds in rats over 19 days. Outcome measures included a reduction in size and differences in wound severity. Rats treated with the *Cocos nucifera* fiber covering had decreased wound areas (Suzuki *et al.*, 2006).

In a mouse model, purified *Cocos nucifera* polysaccharide (PSPP) isolated from the roots acted as a biological response modifier. In a dose-dependent manner, mice treated with PSPP (50, 150, and 250 mg/kg body weight for 7 days) had increased phagocytic function, hemolytic activity, and serum immunoglobulin (IgG) concentration (Zhao *et al.*, 2005).

**Anti-ulcer activity:** The anti-ulcer activity of the fruit of *Cocos nucifera* was studied in cold stress and aspirin-induced gastric ulcers in Wistar rats. Methanolic extracts of *Cocos nucifera* fruits were evaluated in cold stress and aspirin-induced gastric ulcer models using cimetidine and omeprazole respectively as standards for 7 days in the cold stress model and for 1 day in the aspirin-induced gastric ulcer model. Gastroprotective

potential, and status of the antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase), along with glutathione and lipid peroxidation, were studied in both models. The results showed that *Cocos nucifera* fruit possessed gastroprotective activity, as evidenced by its significant inhibition of mean ulcer score and ulcer index, and a marked increase in glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase levels, as well as reduction in lipid peroxidation in a dose-dependent manner (Panda and Sonkamble, 2012).

### **Side Effects of Coconut Water**

Fresh Coconut is one of the best natural drinks on the face of the earth. It doesn't have any known side effects unless somebody is prone to having allergic reactions or has severe nut allergies. It is considered to be safe for children, pregnant, and breastfeeding women. If you are taking potassium supplements it is recommended to regulate your potassium levels since Coconut is high in potassium (Hakimian *et al.*, 2014).

#### **1.20. Aim of Study**

This study aimed to characterize molecularly bacteria associated with coconut water.

##### **1.20.1 Objectives of Study**

Specific objectives include to;

1. Isolate bacteria species from coconut water
2. Determine the molecular characteristics of bacteria isolated from coconut water
3. Determine the antibacterial sensitivity pattern of the bacteria isolates

## **CHAPTER TWO**

### **MATERIALS AND METHODS**

#### **2.1 PLANT MATERIAL**

The coconut fruits used in this study were of the tall green variety. The coconuts fruits were obtained from fruit New Benin market in Edo state, Nigeria. All the coconut fruits were at the best state of maturity, healthy and unbroken.

#### **2.2 MICROBIAL EVALUATION**

##### **2.2.1 Media Preparation**

###### **2.2.1.2 Sterilization of Glasswares**

All glass-wares and slides used in this study were washed in detergent, rinsed in several changes of tap water, and finally distilled water and allowed to dry. They were then sterilized in an electric oven at a temperature of 60°C for 24 hours. Coverslips were flame sterilized just before use. The droppers, pipettes, cotton wool, and plugs were covered with aluminum foil to prevent the entry of condensed water vapour into the media.

###### **2.2.1.3 Preparation of Nutrient Agar**

28 g of nutrient agar powder will be suspended in one litre of distilled water, the mixture will be stirred to fully dissolve all components and heated. The dissolved mixture will be autoclaved at 121°C for 15 minutes. Once the nutrient agar has been autoclaved, it will be allowed to cool but not solidify. Nutrient agar will be poured into each plate on a sterile surface and left until the agar has solidified. The plates were then stored in a refrigerator.

#### **2.3. SAMPLE PREPARATION**

##### **2.3.1 Surface Sterilization of the Coconut Fruits**

The coconut was dehusked in the laboratory by using a cutlass to peel the coconut flesh away from the husk. Then it was washed with sterile water and soaked in 70% sodium hypochlorite solution for 30 minutes for surface sterilization.

### **2.3.2 Isolation of bacterial from Coconut Water**

This was done by carefully identifying the softer germination pore at the endocarp of the coconut, after which it was cleaned with a ball of cotton wool soaked in alcohol to further disinfect the external surfaces. With the aid of a 10 ml sterile syringe needle, the already surface sterilized germination pore was pierced through into the coconut meat, and 5 ml of the coconut water was drawn from each coconut fruit sample using the 5 ml syringe. This process was done in an aseptic condition.

### **2.3.3. Method of inoculation**

The pour plate method of inoculation was used in isolating bacteria associated with the coconut water samples. This was done by dispensing 1ml of each syringe of coconut water samples into the already labeled sterilized Petri dishes in an inoculating chamber, and 9mls of NA was poured into these Petri dishes and allowed to incubate under room temperature.

### **2.3.4 Determination of microbial load**

The microbial load of the samples was determined visibly by counting the colony-forming unit after 24hrs for bacteria

The microbial load/ml will then be determined by the formula stated below

$$\text{Count/ml} = \frac{\text{No of colonies on plate}}{\text{Amount plated}} \times \frac{1}{\text{dilution factor}}$$

## **2.4 CHARACTERIZATION AND IDENTIFICATION OF BACTERIAL ISOLATES**

### **2.4.1 Cultural characteristics**

For the bacterial isolates, cultural characteristics will be observed on Nutrient agar plates. The cultural characteristics include. Size, shape, surface, opacity, texture, elevation, and pigmentation will be determined by visual observation.

#### **2.4.2 Gram staining**

The Gram staining technique was used for differentiation between gram-positive and gram-negative bacterial strains according to Benson (1994). A drop of sterile distilled water was placed on a neat and clean glass slide, and a single isolated colony of 24-hour-old culture was mixed in it. The smear was made by spreading the culture. This smear was air-dried and fixed by rapidly passing the slide three times over the flame. It was then flooded with crystal violet for 1 minute and then washed off with distilled water. Then gram's iodine solution was added to the smear and the glass slide was left for one minute and rinsed with distilled water. This step was followed by the application of a decolorizing agent (ethanol) for 30 seconds. The decolorizing agent was immediately washed with distilled water and the smear was counter-stained with safranin for one minute. The slide was washed with distilled water; air dried and was observed under the microscope.

#### **Gram reaction**

This was carried out to differentiate gram position from gram-negative organisms.

#### **Method:**

A wire loop was sterilized in a Bunsen burner and allowed to cool then a loopful of growth was collected from the agar plate and applied on a clean grease-free slide then a drop of normal saline was added, emulsified and heat fixed by passing over a flame three times. The smear was flooded with crystal violet for 30-60 seconds and then covered with iodine for 30-60 seconds and then washed off; it was decolorized with acetone until no colour ran off the slide and rinsed immediately. The slide was covered with safranin for 1 minute and then washed off with clean water. The slide was kept in a rack to air dry after wiping the back with cotton wool. The stained smear was then examined microscopically under oil immersion at 100 magnification objective lens. Gram-positive bacteria appeared dark purple while gram- negative bacteria appeared pink.

### **2.4.3. Motility test**

A motility test was done by stabbing a slant with the isolated bacteria. If bacteria is not motile, there will only be growth along the stab line.

### **2.4.4 Catalase test**

Three milliliters (3ml) of hydrogen peroxide solution were poured into a sterile test tube. Then a sterile glass rod was used to collect several colonies of the test and inoculate into the hydrogen peroxide solution. It was observed for immediate active bubbling for a positive test.

### **2.4.5. Coagulase test**

A drop of sterile distilled water was placed on each end of a sterile slide. Then a colony of the test organism was emulsified on each spot to make two thick suspensions. A loopful of plasma was added to one of the suspensions and mixed gently. The slide was examined for clumping or dotting of the organisms within 10 seconds. Plasma was not added to the second suspension which serves as control.

### **2.4.6 Oxidase test.**

A piece of filter paper was placed in a clean Petri dish and 2-3 drops of fresh or nascent oxidase reagent was added. A colony of test organisms was collected using a glass rod smeared on the filter paper and observed. Blue-purple color within few a seconds showed a positive test.

### **2.4.7 Urease test**

The test organism was heavily inoculated onto Christensen's urea broth in a bijou bottle using a sterile wire loop and incubated at 35°C- 37°C for 18- 24 hours and examined, thereafter a pink color in the medium showed a positive test.

### **2.4.8. Citrate test**

Simon's citrate agar medium was prepared in a slant bijou bottle, then a sterile wire loop was used to inoculate the test organism onto the slant medium and incubated at 35°C for 48 hours after which it was examined for color formation. A bright blue color in the

medium gave a positive citrate test. *K. pneumonia* and *E. coli* were employed as positive and negative controls respectively.

#### **2.4.9 Vogues -Proskauer Test**

Buffered glucose broth was inoculated with the test organism and incubated at 37 °C for 3 days. Three milliliters (3ml) of naphthol was then added followed by 3ml of sodium hydroxide solution, mixed well, and allowed to stand for 1 hour at room temperature. The formation of a pink color in the medium within 1 hour indicates a positive result. *Klebsiella pneumonia* and *Escherichia coli* were used as positive and negative controls respectively.

#### **2.4.10. Indole test**

A sterile wire loop was used to inoculate a colony of test organisms into 2ml peptone water containing tryptophan. The tube was stopped and incubated at 37°C for 24 hours. Kovac's reagent was added to the medium. Observation of red coloration on the surface layer within 10 minutes showed a positive result.

#### **2.4.11. Carbohydrate fermentation test**

The four sugar solutions were prepared and poured into test tubes well stopped with a Durham tube for gas collection. The sugar was autoclaved after which a loopful of test organisms was introduced into the sugar solution (Buchana, and Caibbons. 1994). A change in color from pink to yellow shows fermentation and the collection of gas bubbles in the Durham tube shows gas production which is a positive test. A control was set up without the organism inoculated.

#### **2.4.12. Methyl red test**

Glucose phosphate peptone water was used for inoculation of test organisms and incubated for 48 hours at 37°C after which a few drops of methyl red solution was added to the culture and read immediately. The formation of red color immediately showed a positive test.

## **2.5 DETERMINATION OF THE ANTIBACTERIAL ACTIVITY OF THE COCONUT WATER**

### **2.5.1 Preparation of the Bacteria Broth Solution**

28g of nutrient broth powder was dispensed into 1000 ml of distilled water. The solution was then dispensed into a smaller flask of 50mls corked with cotton wrapped in aluminum foil paper. The flask containing the broth was then sterilized by autoclaving at 121°C for 15 minutes.

### **2.5.2 Collection of the Coconut Water**

This was done by carefully washing the back of the coconut with detergent, sponge, and running water. After which, the coconut was put into a container containing ... amount of water with 3.5% sodium hypochlorite solution, Tween20 was also added. The coconut fruit was then soaked in the solution for 20 minutes. After 20 minutes, the coconut fruits were rinsed with sterile water and the endosperm of the coconut was cleaned with ethanol. With a sterile scapula, one eye of the coconut was opened and the sterile syringe was inserted, 5mls of the coconut water was drawn. The process was done in an aseptic condition.

### **2.5.3 Antibacterial Activity of Coconut Water Using the Agar Well Diffusion Method**

The antibacterial activity screening of coconut water was carried out using the agar well diffusion method. The inoculum containing each bacterial culture to be tested was spread on a nutrient agar plate with a sterile swab moistened with the bacterial suspension. Subsequently, 4 wells with the aid of the sterile cork borer of 6mm diameter were punched into the agar medium and filled with

## CHAPTER THREE

### RESULTS

This chapter presents the findings of the study on the antibacterial activity of coconut water against selected gastrointestinal bacteria. The results include morphological characteristics, biochemical properties, and antibacterial effects of coconut water from different sources: Oluku Market, NIFOR, and Iguosa Market. Tables 1 to 5 illustrate these findings in detail.

Table 1 highlights the morphological characteristics of the bacterial isolates, including colony shape, elongation, margin, and size. These variations provide insights into bacterial diversity in coconut water and help identify possible contaminants.

Table 2 presents the biochemical properties of the bacterial isolates, including Gram reaction, cell shape, catalase, oxidase, citrate utilization, sugar fermentation, gas production, motility, and urease activity. These biochemical characteristics aid in the identification and classification of the bacteria found in coconut water, providing a deeper understanding of their metabolic properties.

Tables 3, 4, and 5 show the antibacterial effects of coconut water samples collected from Oluku Market, NIFOR, and Iguosa Market, respectively. The findings indicate that matured coconut water did not exhibit any antibacterial activity, as no inhibition zones were observed. However, young coconut water displayed varying degrees of inhibition against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Listeria monocytogenes*. The effectiveness of young coconut water differed across locations, suggesting possible variations in its bioactive components

#### **Table 1: Morphology of Bacteria Present in Coconut Water**

Table 1 presents the morphology of bacteria isolated from coconut water, highlighting key characteristics such as colony shape, elongation, margin, and size. The bacterial colonies exhibited diverse morphological traits, which provide insights into their identification and classification.

The colony shape varied among the isolates, with some appearing circular, while others displayed an irregular or filamentous structure. In terms of elongation, the bacterial cells were predominantly short rods (bacilli) and spherical (cocci), with some demonstrating elongated or curved forms. The margins of the colonies ranged from smooth and entire to undulating, lobate, or serrated, indicating variations in bacterial species.

Regarding size, the bacterial colonies differed in diameter, with some being small (<1 mm), while others were medium (1–3 mm) or large (>3 mm). The variations in colony morphology suggest the presence of multiple bacterial species, some of which may have adapted to the coconut water environment. These findings provide a foundational understanding of the bacterial diversity present in coconut water, which is essential for determining their potential health implications and antimicrobial susceptibility.

**.Table 1: Morphology of Bacteria Present in Coconut Water**

<b>Samples</b>	<b>Colony shape</b>	<b>Elevation</b>	<b>Margin</b>	<b>Size</b>
<b>A1</b>	Circular	Convex	Entire	Large
<b>A2</b>	Irregular	Convex	Lobate	Large
<b>A3</b>	Circular	Flat	Entire	Small
<b>B1</b>	Circular	Convex	Entire	Large
<b>B2</b>	Circular	Convex	Entire	Small
<b>B3</b>	Irregular	Convex	Undulate	Large
<b>C1</b>	Circular	Convex	Entire	Large
<b>C2</b>	Irregular	Raised	Lobate	Large
<b>C3</b>	Circular	Convex	Entire	Small

## **Table 2: Biochemical Properties of Bacteria Isolated from Oluku Market, NIFOR, and Iguosa Market**

Table 2 presents the biochemical characteristics of bacterial isolates obtained from coconut water samples across the three locations. The analysis includes Gram staining, cell morphology, and various biochemical tests used for bacterial identification.

The results show that *Bacillus* sp., *Listeria* sp., and *Staphylococcus* sp. are Gram-positive (+Ve), while *Escherichia* sp. is Gram-negative (-Ve). In terms of cell morphology, *Bacillus* sp. and *Listeria* sp. were observed as rod-shaped, while *Staphylococcus* sp. and *Escherichia* sp. were cocci-shaped.

For biochemical reactions, all bacterial isolates tested positive for catalase (C) activity, indicating their ability to break down hydrogen peroxide into water and oxygen. Oxidase (O) activity was negative for all species except *Staphylococcus* sp., which tested positive. Citrate (Cit.) utilization was observed in all isolates except for *Bacillus* sp.

Sugar fermentation tests revealed variations in the ability of the isolates to metabolize different carbohydrates. Glucose (G) and lactose (L) fermentation were positive in *Listeria* sp., *Staphylococcus* sp., and *Escherichia* sp., while *Bacillus* sp. did not ferment either sugar. Sucrose (S) fermentation was observed in *Listeria* sp. and *Staphylococcus* sp., but absent in *Bacillus* sp. and *Escherichia* sp.

Gas production was detected only in *Escherichia* sp., while hydrogen sulfide (H<sub>2</sub>S) production was absent in all isolates. Motility tests showed that *Bacillus* sp., *Listeria* sp., and *Escherichia* sp. were motile, whereas *Staphylococcus* sp. was non-motile. Urease activity was negative for all isolates except *Staphylococcus* sp., which tested positive, indicating its ability to hydrolyze urea into ammonia.

These biochemical characteristics provide insights into the metabolic and enzymatic activities of the bacterial isolates found in coconut water. Their presence suggests potential contamination and highlights the need for further investigation into their health implications and antimicrobial susceptibility.

**Table 2: Biochemical Properties of the Bacteria Isolated from Oluku Market, NIFOR and Iguosa Market**

<b>Characteristics</b>												
Gram	Cell	C	O	Cit.	G	L	S	Gas	H <sub>2</sub> S	Motility	Urea	
Bacteria			stain	type								prod
isolated												
+Ve	Rod	+	-	+	-	-	-	-	-	+	-	
<i>Bacillus sp.</i>												
+Ve	Rod	+	-	+	-	+	+	-	-	+	-	
<i>Listeria sp.</i>												
+Ve	Cocci	+	-	+	+	+	+	-	-	-	+	
<i>Staphylococcus sp.</i>												
-Ve	Cocci	+		-	+	+	+	+	-	+	-	
<i>Escherichia sp.</i>												

Table 3 presents the antibacterial activity of coconut water samples from Oluku Market, measured by the diameter of zones of inhibition (mm) against selected gastrointestinal bacteria. The antibacterial effect was tested using both matured (M) and young (Y) coconut water at a volume of 0.25 mL.

The results indicate that matured coconut water (M) showed no inhibition against any of the tested bacterial isolates, as no measurable zone of inhibition was recorded. In contrast, young coconut water (Y) exhibited antibacterial activity against all tested bacteria, with varying degrees of inhibition.

Among the bacterial isolates, *Staphylococcus aureus* was the most susceptible, showing the highest zone of inhibition at 20 mm. *Escherichia coli* followed closely with a 19 mm inhibition zone, while *Bacillus cereus* exhibited a 16 mm zone of inhibition. The lowest antibacterial effect was observed against *Listeria monocytogenes*, which had a 10 mm zone of inhibition.

These findings suggest that young coconut water possesses antibacterial properties against common gastrointestinal bacteria, whereas matured coconut water lacks significant inhibitory effects. The observed antibacterial activity could be attributed to the bioactive compounds present in young coconut water, such as lauric acid and other antimicrobial peptides. These results support the potential use of young coconut water as a natural antibacterial agent for combating gastrointestinal infections. Further studies are recommended to explore its effectiveness in clinical applications and its possible use as an alternative to conventional antibiotics.

**Table 3. Diameter of Zones of Inhibition of Sample A (Coconut water from Oluku Market Coconut samples) against some gastrointestinal bacteria.**

<b>Diameter of Zones of Inhibition (mm)</b>		
<b>Volume (mL)</b>	<b>0.25</b>	<b>0.25</b>
<b>Isolates</b>	<b>M</b>	<b>Y</b>
<i>Staphylococcus aureus</i>	-	20
<i>Bacillus cereus</i>	-	16
<i>Escherichia coli</i>	-	19
<i>Listeria monocytogenes</i>	-	10

Key:

M – Matured coconut water

Y – Young coconut water

#### **Table 4: Antibacterial Activity of Coconut Water from NIFOR Against Gastrointestinal Bacteria**

Table 4 presents the antibacterial effects of coconut water samples obtained from NIFOR, measured by the diameter of zones of inhibition (mm) against selected gastrointestinal bacteria. The study examined the effects of matured (M) and young (Y) coconut water, using a volume of 0.25 mL.

The results indicate that matured coconut water (M) did not exhibit any antibacterial activity against any of the tested bacterial isolates, as no inhibition zones were observed. However, young coconut water (Y) demonstrated varying degrees of antibacterial activity, though less pronounced than in the Oluku Market samples.

Among the bacterial isolates tested, *Staphylococcus aureus* exhibited the highest susceptibility, with a 14 mm zone of inhibition. *Bacillus cereus* showed a smaller inhibition zone of 5 mm, indicating limited antibacterial effects. Notably, *Escherichia coli* and *Listeria monocytogenes* were completely resistant to young coconut water, as no inhibition zones were observed.

These findings suggest that while young coconut water from NIFOR possesses some antibacterial properties, its effectiveness varies among bacterial species. The weaker inhibition compared to the Oluku Market samples may be due to differences in the composition of bioactive compounds in coconut water from different sources. Further research is recommended to analyze the specific antimicrobial compounds present and their potential applications in combating bacterial infections.

**Table 4. Diameter of Zones of Inhibition of Sample B (Coconut water from NIFOR coconut samples) against some gastrointestinal bacteria.**

<b>Diameter of Zones of Inhibition (mm)</b>	<b>0.25</b>	<b>0.25</b>
<b>Isolates</b>	<b>M</b>	<b>Y</b>
<i>Staphylococcus aureus</i>	-	14
<i>Bacillus cereus</i>	-	5
<i>Escherichia coli</i>	-	-
<i>Listeria monocytogenes</i>	-	-

### **Table 5: Antibacterial Activity of Coconut Water from Iguosa Market Against Gastrointestinal Bacteria**

Table 5 presents the antibacterial effects of coconut water samples obtained from Iguosa Market, measured by the diameter of zones of inhibition (mm) against selected gastrointestinal bacteria. The study analyzed the impact of matured (M) and young (Y) coconut water, using a volume of 0.25 mL.

The results indicate that matured coconut water (M) did not exhibit any antibacterial activity against the tested bacterial isolates, as no inhibition zones were recorded. However, young coconut water (Y) showed antibacterial effects against all four bacterial species, though the inhibition zones were smaller compared to samples from Oluku Market and NIFOR.

Among the bacterial isolates, *Escherichia coli* showed the highest susceptibility to young coconut water, with a 10 mm zone of inhibition. *Bacillus cereus* exhibited a slightly lower inhibition zone of 9 mm, while *Staphylococcus aureus* and *Listeria monocytogenes* showed 7 mm and 6 mm inhibition zones, respectively.

These findings suggest that young coconut water from Iguosa Market possesses mild antibacterial properties, with varying levels of effectiveness against different bacterial species. The relatively smaller inhibition zones compared to samples from Oluku Market and NIFOR indicate potential differences in the concentration of bioactive antimicrobial compounds present in coconut water from different sources. Further studies are recommended to determine the factors influencing these variations and to explore the potential of young coconut water as a natural antibacterial agent against gastrointestinal pathogens.

**Table 5. Diameter of Zones of Inhibition of Sample C (Coconut water from Iguosa Market coconut samples) against some gastrointestinal bacteria.**

<b>Diameter of Zones of Inhibition (mm)</b>		
<b>Volume (mL)</b>	<b>0.25</b>	<b>0.25</b>
<b>Isolates</b>	<b>M</b>	<b>Y</b>
<i>Staphylococcus aureus</i>	-	7
<i>Bacillus cereus</i>	-	9
<i>Escherichia coli</i>	-	10
<i>Listeria monocytogenes</i>	-	6

## CHAPTER FOUR

### DISCUSSION

#### 4.1 Discussion

The findings from this study suggest that young coconut water exhibits antibacterial activity against selected gastrointestinal bacteria, whereas matured coconut water does not show any inhibition (Kader, 1992). This indicates that the bioactive compounds responsible for antibacterial properties are either more abundant or more effective in young coconut water than in matured coconut water (Brocklehurst *et al.*, 1987).

The morphological and biochemical analysis of bacterial isolates (Tables 1 and 2) provided essential information on their diversity and metabolic characteristics. The presence of both Gram-positive and Gram-negative bacteria in coconut water highlights the potential risk of contamination, raising concerns about the microbial safety of coconut water available in local markets (Hamilton *et al.*, 2006). The biochemical tests, including sugar fermentation and enzyme activities, were useful in further classifying and understanding the metabolic functions of these bacteria (João *et al.*, 2013).

The antibacterial activity results varied across the three sample locations. Young coconut water from Oluku Market showed the highest inhibition, with *Staphylococcus aureus* (20 mm) and *Escherichia coli* (19 mm) exhibiting the most significant susceptibility (Pumer *et al.*, 2001). In contrast, young coconut water from NIFOR exhibited much lower inhibition levels, with *Escherichia coli* and *Listeria monocytogenes* showing no inhibition at all (João *et al.*, 2013; Khan *et al.*, 2016). Samples from Iguosa Market displayed moderate antibacterial activity, with *Escherichia coli* showing the highest inhibition (10 mm) (Ukuku *et al.*, 2002). These variations may be due to environmental factors, coconut variety, or microbial load at each market location (Frank-Peterside *et al.*, 2006).

The antibacterial activity of young coconut water can be attributed to its lauric acid content and other antimicrobial compounds, which are known to disrupt bacterial cell membranes, leading to cell death [Mantena *et al.*, 2003]. The differences in inhibition

levels across locations suggest that additional factors, such as storage conditions, coconut maturity, and handling methods, may influence its potency

## **4.2 Conclusion**

This study provides valuable insights into the antibacterial potential of coconut water, particularly young coconut water, against common gastrointestinal bacteria (Taiwo *et al.*, 2011). The findings suggest that young coconut water may serve as a natural antimicrobial agent, potentially beneficial for managing gastrointestinal infections (Oke and Adefisan *et al.*, 1999). However, further research is needed to standardize its application, determine its exact mechanisms of action, and evaluate its clinical effectiveness (Ajibogun *et al.*, 2013).

Future studies should explore the chemical composition of young coconut water, assess its stability over time, and investigate its effectiveness against a wider range of bacterial pathogens (Hakimian *et al.*, 2014). Additionally, understanding the optimal conditions for preserving its antibacterial properties could enhance its potential as a natural remedy for bacterial infections. By further investigating the antibacterial properties of coconut water, this study contributes to the growing interest in natural antimicrobial agents, which may help in addressing antibiotic resistance and promoting alternative treatments for bacterial infections (You, *et al.*, 2010).

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