COMPARATIVE STUDY OF THE PHYTOCHEMICAL CONSTITUENTS ANTIOXIDANTS PROPERTIES, TOTAL FLAVONOIDS CONTENT AND TOTAL PHENOLIC CONTENT OF LEAVES OF PAWPAW (*Carica papaya*) BAY (*Laurus nobilis L*) AND UTAZI (*Gongronema latifolium*)

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AUGUST, 2021.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES (BIOCHEMISTRY OPTION)

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FORTHE AWARD OF BACHELOR OF SCIENCE (BSC) HONS DEGREE IN BIOCHEMISTRY, FACULTY OF SCIENCE

BENSON IDAHOSA UNIVERSITY, BENIN CITY, EDO STATE, NIGERIA.

AUGUST, 2021.

CERTIFICATION

This certify that this research was carried out by OMORAGBON IGHEJESU,with Matriculation number BAS/BCH/170297, in the Department of Biological Sciences (Biochemistry option), Faculty of Science Benson Idahosa university, Benin city, Nigeria.

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DEDICATION

This report is dedicated to God Almighty for His Grace, sustainability and enablement to complete my project work at Benson Idahosa University.

ACKNOWLEDGEMENTS

I am most grateful to God for knowledge, strength, guidance, and provision he made available for the completion of this research work.

I am forever thankful to my supervisor, Mr. M. E. Egharevba for his timely encouragements, kindness, support and patience throughout the period of this project.

I acknowledge the management of this great institution, Benson Idahosa University, the honorable dean of the Faculty of Science Prof. F. O. J. Oboh, and the Head of Department Dr. Mrs. Tinu Okugbo. I appreciate the Head of Biochemistry unit, Chairman of project committee Mrs. A.I. Okungbowa. I would like to thank and appreciate my lecturers, and the entire staff of Biochemistry Department, Faculty of Sciences for their contribution to my academics. I would like to thank Mr. Derek Ahamioje for his assistance through my project.

Also I appreciate my friends; Onyenise Stanley, Dr Victor, Alabi glory, Adeshina-Ojobaro glory, Ediale faith for their support, motivation, encouragement and prayers.

Also my gratitude goes to my parents Mr. and Mrs. Omoragbon for their support spiritually and financially, and to my only siblingPraise, one could not have wished for a better sibling.

PLAGIARISM ATTESTATION

I certify that this report is my own work, based on my personal research and that I have acknowledged all materials and sources used in its preparation, whether they be books, articles, report, lecture notes, and any other kind of document, electronics or personal communication, I also certify that the report has not previously been submitted for assessment elsewhere, and that I have not copied in part or whole or otherwise plagiarized the work of other students and/or person.

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ABSTRACT

Medicinal plants have been used to treat diseases all across the world for thousands of years. The methanolic extract of powdered leaves of *Carica papaya, Laurus nobilis, and Gongronema latifolium* were subjected to phytochemical screening and antioxidants activity assays. The presence of alkaloids, flavonoids, steroids, and tannins in methanol extract was revealed by phytochemical screening. The leaf extract of *Carica papaya* showed the presence of the flavonoids, cardiac glycosides, alkaloids, tannins, phenols, steroids, terpernoids, saponnis, but quinones were absent. *Laurus nobilis* was found to contain flavonoids, tannins, saponins, cardiac glycosides, steroids, alkaloids, phenols, quinones but terpernoids were absent. *Gongronema latifolium* was found to contain tannins, saponins, terpernoids, alkaloids, quinones, but steroids, phenols, cardiac glycosides, phenols, steroids, flavonoids were absent. The EC50 value of the DPPH radical scavenging activities shows that Bay leaf (0.586± 0.049µg/ml) has the highest radical scavenging activity followed by Pawpaw leaf (0.685± 0.012µg/ml), and Utazi leaf (0.896± 0.104µg/ml). The Phosphomolybdate assay shows that Utazi (0.619±0.012 µg/ml) >Pawpaw (0.752± 0.166µg/ml) and Bay (0.816± 0.094 µg/ml). Hydrogen peroxide: Pawpaw leaf>Utazi leaf >Bay leaf respectively. Reducing power for Pawpaw leaf >Bay leaf >Utazi leaf which had the lowest value. The total flavonoid content shows that Bay leaf (0.053± 0.010mg/gQE) has the highest total flavonoids content followed by Pawpaw leaf (0.233±0.012mg/gQE) and Utazi leaf (0.051± 0.008mg/gQE). The total phenolic content shows that Bay leaf0.145± 0.021mg/gGAE, have the highest total phenolic content followed by Pawpaw leaf 0.055±0.015mg/gGAE, and Utazi leaf 0.041±0.003mg/gGAE. These results show a non-statistical significant difference (p>0.05) in antioxidants activities of Pawpaw leaf, Bay leaf, Utazi leafs and standard antioxidant activity. It also shows that leaves of utazi, pawpaw and bay possess significant phytochemicals and antioxidant activity.

INTRODUCTION

1.1. BACKGROUND OF THE STUDY

The usage of therapeutic plants benefits both individuals and communities. These plants' therapeutic efficacy is attributable to a variety of chemical components that have a distinct physiological effect on the human body. The most important bioactive elements in plants include alkaloids, tannins, flavonoids, and phenolic compounds (Nunes *et al.,*2012). Many of these indigenous medicinal plants are also used in cooking and as spices. Medicinal plants create chemicals that are naturally toxic to bacteria and are low-cost, renewable sources of

pharmacologically active substances (Basile *et al*., 1999). Plants continue to serve an important role in health care, despite tremendous advances in modern medicine during the last few decades. Medicinal plants, on the other hand, have attracted a lot of attention due to their long history of use in folk medicine as well as their potential for disease prevention, particularly in developing countries. A wide variety of medicinal plants have been researched for their antioxidant properties. Natural antioxidants are particularly effective in avoiding oxidative stress-related damage, whether in the form of raw extracts or chemical components. The human body's intrinsic antioxidative process is responsible for a variety of biological functions, including antimutagenic, anticarcinogenic, and anti-aging responses. Antioxidants stabilize or deactivate free radicals, preventing them from damaging biological cells. Natural antioxidants have recently inspired a spike in interest for use in food, cosmetics, and pharmaceutical products, owing to their complex nature in terms of activity diversity and amplitude, as well as their great potential for redressing imbalance (Ivanoi'c *et al*. 2010).The use of medicinal plants with high antioxidant content has been proposed as a possible therapy option for liver injury.

Paw paw is the fruit of the *Carica papaya* plant, which belongs to the Carica genus, according to Eno*et al.* (2000). It is native to the tropics of America and was first grown in Mexico (Everette, 2003). Carotene, vitamin C, vitamin B, flavonoids, folate, and pantothenic acids, as well as minerals including potassium and magnesium. Two biologically active components of papaya, chymopapain and papain, are used to treat arthritis and intestinal problems (Ezugwu, 2008).

L. Laurus nobilis (Family Lauraceae) is a Mediterranean and European evergreen tree with a wide range. It is also known as bay, sweet bay, true laurel, or Roman laurel. It's used in traditional medicine to treat stomachic and carminative disorders, as well as to treat gastric problems. (Gledhil, 2009).*L. nobilis* leaf extracts have been examined for their wound healing,

cytotoxic, and trypanocidal properties. Laurel has attracted interest time and time again because to the pharmacological and physiological benefits connected with numerous compounds contained in the plant. In prior phytochemical research, sesquiterpene lactones, alkaloids, glycosylated flavonoids, monoterpene, and germacrane alcohols were extracted from L. nobilis leaves and fruits(Hasheen, 2007).

Alkaloids, phenols, flavonoids, cyanides, tannins, and saponins were found in the phytochemical contents of G. latifolium (Utazi leaf) extracts. These elements are prevalent in medicinal plants, but their concentrations vary (Baur *et al.,* 2008). Phytochemicals such as tannins, phenols, flavonoids, and saponins are found in many plants.

1.2. Justification of the Study

Most of the common medicinal plant consumed by Nigerians contain phytochemical constituents and antioxidant properties and excess of this phytochemical constituents and antioxidant in the plant consumed into the body strongly associated with increased risk of liver disease(Dias *et al*2014).Medicinal plants have high contents of natural phenolics, flavonoids, and phytochemicals with significant antioxidant activity which could be recommended as useful value added functional ingredients for food industry. Hence, quest and search for these chemicals in plants. It is with this backdrop that the present line of inquiring was designed to conduct a comparative study on the phytochemical constituents, antioxidant properties and total phenolic contents of Pawpaw leaf (*carica papaya),* Bay leaf *(Laurus nobilis L)*, Utazi *(Gongronema latifolium).*

1.3 Aim of the study

The aim of this study was to compare the phytochemical constituents, antioxidant properties, total phenolic content and total flavonoid content of leaves of *Pawpaw, Bay, and Utazi.*

1.4 Objectives of the study

i. To carry out phytochemical screening of*, Pawpaw leaves, Bay leaves, and Utazi leaves.*
ii. Determine the antioxidant properties of *Pawpaw, Bay, and Utazi leaves* by determining their radical scavenging ability.
iii. Determine the total phenolic content of *Pawpaw, Bay, and Utazi leaves.*
iv. Determine the total flavonoid content of *Pawpaw, Bay, and Utazi leaves.*

CHAPTER TWO

LITERATURE REVIEW

2.0 Phytochemicals

Phytochemicals are bioactive molecules found in nature that have been shown to have health effects. Fruits and vegetables, in particular, owe their color, flavor, and scent to them. Many chronic diseases, such as cancer and diabetes, have been demonstrated to be prevented by bioactive chemicals (Brume, 2008). A phytochemical can function in a number of different ways.

It can function as an antioxidant and protect cells from free radical damage for example, polyphenols and carotenoids (Topcagic, 2009).

Plants are found to be rich in phytochemical, Pawpaw leaf (*Carica papaya),* Bay leaf *(Laurus nobilis L) and* Utazi *(Gongronema latifolium)* are amongst the plants that are rich in nutrients and contain phytochemicals such as saponins, terpenoids, flavonoids, and cardiac glycosides (Shahidi*et al*. , 2004).

2.1. Biological functions of phytochemicals

Phytochemicals are found in plant-based foods such as fruits, vegetables, legumes, and grains. Some phytochemicals function as antioxidants or hormone mimics. A diet rich in fruits, vegetables, and whole grains has been shown to reduce the incidence of some cancers and illnesses (Wannes *et al*., 2010).

2.2. Importance of phytochemical in plants

Phytochemicals are naturally found in plants and have biological relevance because they help plants protect themselves against pathogenic microorganisms by displaying antimicrobial activity through inhibition or killing mechanisms (Ugochukwu *et al.,*2003).

2.3. Mechanism of action of phytochemicals

Phytochemicals come in a variety of forms, each with its own set of properties, these include:Antioxidant - Most phytochemicals contain antioxidant activity, which protects our cells

from oxidative damage and reduces the risk of some malignancies. Phytochemicals with antioxidant properties include alyl sulfides (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), and polyphenols (tea, grapes) (Ezugwu *et al.,* 2008). Isoflavones, which are found in soy, are estrogen-like compounds that can help with menopausal symptoms and osteoporosis (Hayat*et al*., 2010).Indoles, found in cabbages, activate enzymes that reduce estrogen's efficacy, potentially lowering the risk of breast cancer. (Barger *et al.,* 2009).Phytochemicals that interfere with enzymes include protease inhibitors (found in soy and beans) and terpenes (citrus fruits and cherries). DNA replication is disrupted. - Saponins contained in beans prevent cancer cells from multiplying by interfering with cell DNA replication (Kim *et al.,* 2003). Capsaicin, a compound found in spicy peppers, defends DNA from carcinogens (Maqsood *et al.,* 2010). Antibacterial characteristics - Allicin, a phytochemical found in garlic, has antibacterial effects (Zengin *et al.,* 2011).Physical action – Some phytochemicals connect to cell walls physically, preventing infections from adhering to human cell walls. The anti-adhesion capabilities of cranberries are due to proanthocyanidins (Gulcin, 2012).Consumption of cranberries reduces the incidence of urinary tract infections and enhances oral health.Phytochemical-rich foods are already a part of our regular diet. Except for some refined foods like sugar or alcohol, most meals contain phytochemicals. Phytochemicals can be found in a variety of foods, including whole grains, vegetables, beans, fruits, and herbs. Eating more fruit (blueberries, cranberries, cherries, apple) and veggies is the easiest approach to get more phytochemicals (cauliflower, cabbage, carrots, broccoli).Every day, you should consume at least five to nine servings of fruits and vegetables. Minerals, vitamins, and fibre are abundant in fruits and vegetables, which are also low in saturated fat.Many foods naturally contain

phytochemicals, but bioengineering is predicted to result in the development of new plants with larger quantities. This would make including adequate phytochemicals into our diets much easier.

2.4. Antioxidants

Antioxidants are a class of chemicals that can interact with free radicals and prevent them from causing damage. Food's antioxidant capacity can be utilized to determine its health advantages. (Prior and Wu 2013, For example Flavonoids, phenolic acids, vitamin C, vitamin E, and tannins are antioxidants with anti-carcinogenic, anti-atherosclerotic, and anti-aging characteristics. They also improve the quality and worth of food items by altering microbial equilibrium, which helps to maintain gut health (Jayaprakasha *et al.,* 2012).

The antioxidant effects of phenolic compounds are connected to their ability to scavenge free radicals, halt radical chain reactions, and chelate metals (Nayak *et al.,* 2015). Plant extracts' overall antioxidant capacity is determined by their chemical composition and antioxidant content.By suppressing lipid peroxidation and guarding against oxidative damage, antioxidants are utilized as food additives to aid foods decompose and extend their shelf lives (Kumaran and Karunakaran 2006).As a result, natural antioxidants are required to replace synthetic derivatives in foods and medicines. Antioxidant activity refers to a bioactive compound's ability to maintain cell structure and function by effectively clearing free radicals, inhibiting lipid peroxidation reactions, and preventing other oxidative damage(Bravo, 2008).Pawpaw leaf (*Carica papaya),* Bay leaf (*Laurus nobilis L*) and Utazi (*Gongronema latifolium*), have been studied because they contain numerous biologically active compounds including natural antioxidants (Kumaran *et al*., 2006). Furthermore, antioxidants may react differently to different radical or oxidant sources. As a result of the numerous reaction features, processes, and phase localizations that are frequently

involved, no one assay can fully reflect all of the radical sources and antioxidants present in a mixed or complex system (Prior and Wu 2013).Plants can be a source of bioactive compounds with health benefits because they contain many important molecules.

2.5. Classification of antioxidants

Antioxidants can be divided into three categories based on their chemical structure:

1. Natural or primary antioxidants: These are antioxidants that participate in the chain-breaking process by reacting with lipid radicals and converting them to more stable components. The majority of the antioxidants in this group have a phenolic structure. These antioxidants are cofactors of antioxidant enzymes, therefore their absence may impact macromolecule metabolism, particularly glucose metabolism.

2. Secondary or synthetic antioxidants: These antioxidants are pheniloc molecules that interrupt chain reactions by collecting free radicals. Butylated hydroxyl anisole (BHA), This group includes like butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), propyl gallate (PG), metal chelating agent (EDTA), and nordihydroguaretic acid (NDGA).

3. Tertiary antioxidants, such as methionine sulfoxide reductase and DNA repair enzymes, are involved in the repair of biomolecules damaged by free radicals (Nagar *et al.,* 2017).

Antioxidants are divided into three categories based on their line of defense mechanism:

a. Antioxidants such as superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), and minerals such as Se, Cu, Zn, and others that may be included in the first line of defense.

b. The second line antioxidants include glutathione (GSH), flavonoids, albumin, vitamin C, vitamin E, carotenoids, and others and

c. Damaged DNA repair enzymes such as transferases, methionine sulphoxide reductase, and others, as well as damaged proteins, oxidized lipids, and peroxides, are all part of the third line antioxidants (Irshad and Chaudhuri, 2002).

2.6. Mechanism of action of antioxidants

Antioxidants are assumed to work in one of two ways. The first is a chain-breaking mechanism in which the system's free radical is given an electron by the main antioxidant. A quenching chain-initiating catalyst is used to eliminate ROS/ reactive nitrogen species initiators (secondary antioxidants) in the second technique. On biological systems, other antioxidant strategies including metal ion chelation, electron donation, co-antioxidants, or gene expression modification are beneficial (Lobo *et al*., 2010).

2.7. Biological roles of antioxidant

By avoiding oxidation, antioxidants remove the presence of free radicals and prevent their proliferation. Because oxidative stress is a key component of many human diseases, antioxidants can help to prevent disease by reducing oxidation in living systems.

2.8. Role of Antioxidants in human body

In order to protect the body's cells and organs from free radicals, (ROS), a highly combined and complicated system has been evolved in human body, which involve a diversity of components, originated both endogenously and exogenously that function interactively and synergistically to reduce the effect of free radicals. These are

1. Antioxidants include carotenoids, ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), and other low molecular weight molecules such as glutathione and lipoid acid.

2. Enzymatic antioxidants, such as glutathione peroxidase, superoxide dismutase, and glutathione reductase, which catalyze free radical quenching processes.

3. Metal-binding proteins, such as lactoferrin, ferritin, albumin, and ceruloplasmin, that seize free iron and copper ions and catalyze oxidative processes.

4. A variety of antioxidant phytonutrients found in a wide range of plant foods (Sunil, 2014).

2.9. Phenolic Compounds

Fruits include phenolic chemicals that are widely found in plants (citrus). The redox characteristics of phenolic compounds are primarily responsible for their antioxidant effects (Hertog, 2003). Metal chelators, reducing agents, hydrogen donors, singlet oxygen quenchers, and singlet oxygen quenchers are all roles they play (Kaviarasan *et al.,* 2007). Natural phenolics in the diet can lower the risk of coronary heart disease and cancer mortality, resulting in a longer life expectancy. They have antiviral and anti-inflammatory properties as well (Ebrahimzadeh *et al.,* 2008). Phenolic compounds are a complex collection of chemical substances with a wide range of chemical structures and biological activity, encompassing about 8000 distinct compounds that are an important element of human and animal diets. They play a crucial role in plant signaling and defense mechanisms. Pathogenic organisms and predators cause stress, which these substances help to alleviate. These chemicals serve a variety of functions in plants, including serving as precursors to more complex compounds, as well as intervening in plant development regulation and control processes and serving as a protective medium (Ugochukwu *et al.,* 2003).By preventing the oxidation of low-density lipoproteins, phenolic substances can act as hydrogen donors or bind metal ions like iron and copper (LDL).These phenolic component properties have been linked to a lower risk of neurological disorders such cardiovascular disease, gastrointestinal malignancies, colon, breast, and ovarian cancers, as well as leukemia

(Kaurinovic *et al*., 2010). Phenolic compounds have anti-allergenic and vasorelaxant properties; they also prevent the oxidation of LDL in vitro and diminish or inhibit free radicals by transferring a hydrogen atom from their hydroxyl group (Eno *et al.,* 2000). . The technique of determining the quantity of phenolic content in samples is known as total phenolic content activity. Plant-derived phenolic compounds have redox characteristics that allow them to serve as antioxidants.

To evaluate TPC in leaf extracts, the Folin–Ciocalteu test was chosen. The procedure of determining the quantity of phenolic content in samples is known as TPC activity.

The Folin-Ciocalteu method is a reducing capacity and phenolic concentration assay based on electron transfer.

2.9.2. Flavonoids

Flavonoids are a type of polyphenolic compound that has health benefits. They are a vast group of secondary metabolites found in plants that have been shown to have chemotaxonomic value. (Horowitz and Gentili, 2007). They're called methylated aglycones, glycosides, and derivatives. Flavonoids have an aglycone structure at its core. In either a -pyrone or its dihydroderivative, a six-member ring is condensed with the benzene chain. In the category of flavonoids, the position of the benzenoid substituent distinguishes flavonoids from is oflavonoids. At the 3-position, flavonols differ from flavanones by having a hydroxyl group and a double bond between C2 and C3.Flavonoids are frequently hydroxylated at positions 3, 5, 7, 2, 3′, 4′, and 5′.Flavonoids have a fifteen-carbon skeleton that is made up of two benzene rings joined by a heterocyclic pyranechain. The pattern of substitution of the A and B rings varies amongst compounds, while various classes of flavonoids differ in the degree of oxidation and the substitution pattern of the

C ring. Flavones, flavonols, flavanols, and anthocyanins are among them. Flavonoids are polyphenolic chemicals found in a wide range of plant diets (Calabro *et al.,* 2004).Several epidemiological studies have found evidence that chronic flavonoid-rich diets are linked to a lower risk of several chronic diseases, such as cardiovascular, neurological, and cancer (Calabro*et al.,*2004). Flavonoids are a wide group of polyphenolic chemicals having a benzo—pyrone structure that are found throughout plants. They are made through the phenyl propanoid pathway. Secondary phenolic metabolites, such as flavonoids, are thought to be responsible for a wide range of pharmacological effects, according to research.Flavonoids are phenolic hydroxylated compounds that are known to be generated by plants in response to microbial infection.

Flavonoids' chemical makeup is dictated by their structural group, degree of hydroxylation, various substitutions and conjugations, and degree of polymerization.

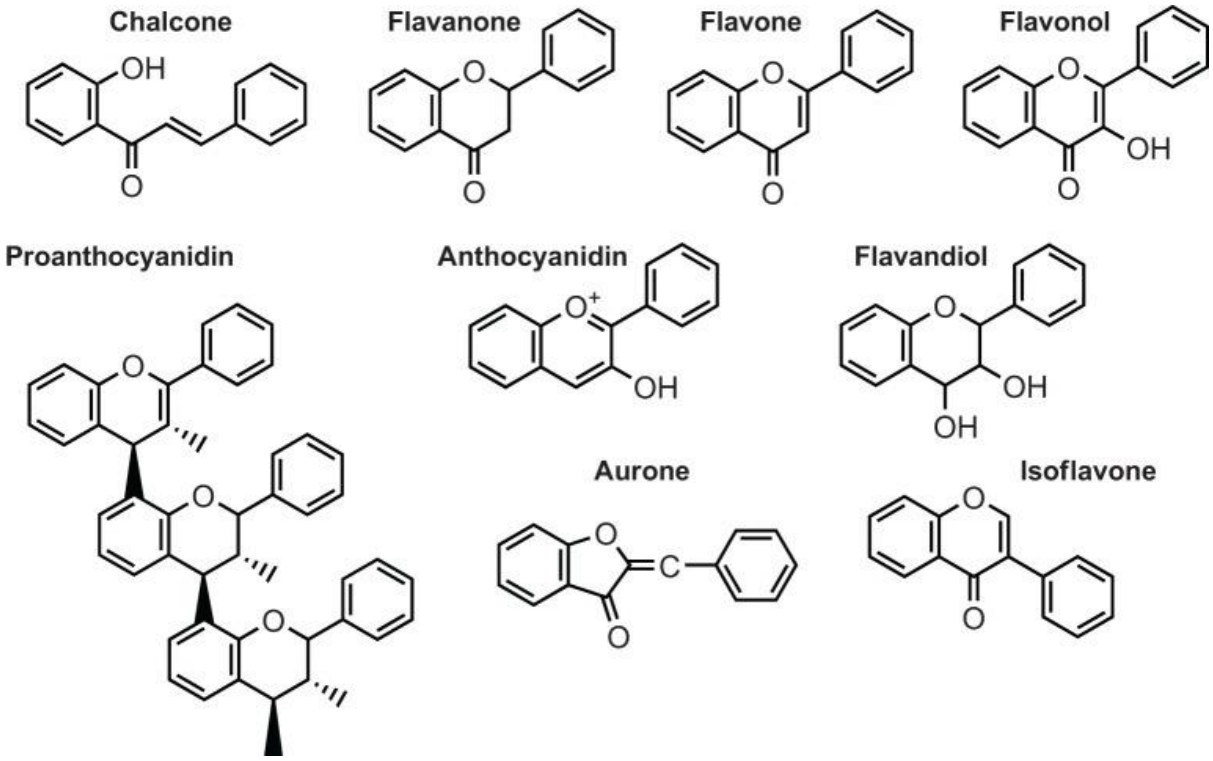


Figure 1: Structures of the main classes of flavonoids (Ferreyra *et al*., 2012).

2.9.3. Mechanism of action of flavonoids

Antioxidants can be found in almost any group of flavonoids. Flavones and catechins have been found to be the most effective flavonoids for protecting the body from reactive oxygen species. (Zengin *et al.,* 2012). Free radicals and reactive oxygen species continue to threaten body cells and tissues, since they are generated during regular oxygen metabolism or activated by external damage. The processes and sequence of events that free radicals use to disrupt cellular functioning aren't entirely known, but lipid peroxidation appears to be one of the most important, resulting in cell membrane destruction. When cells are damaged, their net charge shifts, raising osmotic pressure and promoting swelling and cell death.. Free radicals can attract a range of inflammatory mediators, resulting in an inflammatory response that is more widespread and tissue damage. Living organisms have evolved a number of effective defense systems against reactive oxygen species.The body's antioxidant defense mechanisms include enzymes like superoxide dismutase, catalase, and glutathione peroxidase, as well as non-enzymic equivalents such glutathione, ascorbic acid, and -tocopherol. The increased synthesis during damage of reactive oxygen species leads in endogenous scavenging chemicals being consumed and depleted. The endogenous scavenging chemicals may have an additional effect on flavonoids. As a high-quality way of understanding flavonoids' biological activity, the theoretical order of affinity between flavonoids and amino acid residues has crucial repercussions in theoretical predictions of flavonoid-protein interactions (Ferreyra *et al*., 2012).

2.9.4. Flavonoids as Antioxidants

Flavonoids have been shown to have antioxidant and prooxidant properties in both animal and *in vitro* studies. Flavonoids can scavenge free radicals due to their ability to provide hydrogen atoms due to the high reactivity of the hydroxyl group, resulting in less reactive and stable free radicals. Several flavonoids have been discovered to directly scavenge superoxide, while others have been discovered to directly scavenge peroxynitrite, a radical-derived highly reactive oxygen. Flavonoid antioxidant effects are dependent on the structural arrangement of functional groups, as well as the total number and configuration of hydroxyl groups, according to *in vitro* investigations (Ferreyra *et al*., 2012). The B ring hydroxyl group arrangement is the most critical factor of flavonoid ROS scavenging. The hydroxyl group in rings A and C, on the other hand, has little or no effect on superoxide scavenging. The presence of catechol (orthodihydroxy) in the B ring, which is responsible for electron delocalization, the presence of hydroxy groups at positives 5 (A ring) and 3 (C ring), which is responsible for providing the oxo group with hydrogen bond, and the presence of a 2,3-double bond in conjunction with a 4-ring are all structural properties of flavonoids that help them fight free radicals.Flavonoids interact with antioxidant enzymes like glutathione's phosphate system and nicotinamide adenine dinucleotide to scavenge free radicals in an indirect way.This is accomplished via their ability to stimulate detoxification enzymes including NADPH, quinone oxidoreductase, and S-transferase glutathione, which are critical for oxidative stress protection.and electrophilic toxicants. Flavonoids with a hydroxy-functional group at position C-3 (such as quercetin) are more effective at activating antioxidant mechanisms and inducing the electrophile-responsive feature. The ability of flavonoids to chelate iron and copper has been demonstrated, removing one of the reasons of free radical generation. Quercetin was identified to protect cells from oxidative damage after several oxidizing substances (such as acrolein) altered the release of iron in its

redox state in the erythrocyte membrane (Ferreyra *et al*., 2012). According to a review, the 3-hydroxyl and 4-oxo groups in the C ring, the catechol moiety in the B ring, and the 4-oxo and 5-hydroxyl groups in the A and C rings are the binding sites for trace metal in the flavonoid structure. The catechol moiety in the B ring is the main contributor of metal chelation due to its ability to chelate cupric cation. Flavonoids can stop superoxide-generating enzymes such xanthine oxidases and protein kinase C from working. Flavonoids have also been demonstrated to be potent inhibitors of the enzymes cyclooxygenase, microsomal succin-oxidase, lipoxygenase, and NADH oxidase (Gocer 2011). Based on the molecular structure of flavonoids, lutein was revealed to be the most potent inhibitor of xanthine oxidase, a member of the flavone subclass, resulting in a reduction in oxidative cell injury.Flavonoids have antioxidant properties that include raising uric acid levels, reducing nitric oxide oxidative stress, lowering -tocopheryl radicals, and modulating low molecular antioxidant prooxidant features. Flavonoids found in edible plants and fruits have been shown to protect against oxidative stress by inhibiting the generation of intracellular ROS. It's worth noting that recent scientific study has shifted its focus to the use of natural food supplements to prevent many of these diseases (Ezugwu, 2008).

2.9.5. Pawpaw (*Carica papaya*)

Paw paw is the fruits of the plant Carica papaya belonging to the genus Carica. It is native to the tropics of America and was first cultivated in Mexico (Everette, 2003); Eno *et al*. 2000).

Scientific Classification

Kingdom: *Plantae*

Order: *Brassicales*

Family: *Caricaceae*

Genus: *Carica*

Species: *C.Papaya*

Binomial name (Gledhil, 2009).

Carica papaya is an evergreen shrub that thrives in mild shade to full sun. In hot weather, the plant prefers a lot of water and fertilizer. Blooms resemble Plumeria flowers but are much smaller, waxy, and appear in the axils of the leaves, ripening into fruit that ripens when it feels soft and the skin has turned amber or orange in color. It's been utilized in the treatment of urinary tract infections in the past (Aliyu, 2006).

The enzyme papain is found in the fruits, stems, and leaves of *Carica papaya* (Akah *et al*.., 2007). Wrapping meat with a papaya leaf before cooking tenderizes it. It contains biologically active chemicals that help digestion, such as chymopain and papain. (Barger *et al*., 2009).Papain is a proteolytic enzyme that aids in protein digestion. It has also been used orally to treat less serious digestive issues such as bloating and persistent indigestion since it promotes digestion in general (Baur *et al.,* 2008).Papain is also used to treat arthritis and worms in the intestine. Papain's phytochemicals may boost the immune system and encourage the release of natural compounds that fight cancerous cells (Cordell, 2008).

Carica papaya is a big, tree-like plant with a single stem reaching 5 to 10 m [16 to 33 ft] tall with spirally arranged leaves limited to the top of the trunk, according to Hasheen (2007). Where leaves and fruit were borne, the bottom truck is visibly damaged. The leaves are enormous, measuring 50-70 [20-28 inches] in diameter and have seven lobes that are deeply and palmately

lobed. Every portion of the *Carica papaya* is economically valuable, and its applications range from nutritious to therapeutic. The fruits are widely consumed and processed into juice and wine, while the leaves are prepared as a vegetable (Grayson, 2001). The seeds are useful in the treatment of sickle cell illness and poisoning (Chaudhry *et al.,* 2006).The leaf tea or extract has a reputation for being a tumor killer. The fresh green tea is antibacterial, whilst the brown dried pawpaw leaves are a tonic and blood purifier (Ezugwu, 2008).It is consequently utilized in the treatment of digestion and other disorders such as chronic indigestion, overweight, obesity, high blood pressure, and heart weakness due to its antioxidant and fiber content (Everette, 2003).

2.9.6. Bay Leaf (*Laurus nobilis L*)

Laurus nobilis L, generally known as Bay or laurel leaves, is one of the most well-known plants in the Lauraceae family. Bay is one of the most commonly used spices in the kitchen for flavoring meats, fish, and soups. It is a native plant of the Southern Mediterranean region, growing in warm climates, but it is also grown as an ornamental plant in Europe and the United States**.** In addition, it is commercially grown in, Algeria, Morocco, Portugal, Spain, Italy, France, Turkey and Mexico.

Scientific Classification

Kingdom: *Plantae*

Clade:	*Tracheophytes*
Clade:	*Angiosperms*
Clade:	*Magnoliids*
Order:	*Laurales*
Family:	*Lauraceae*
Genus:	*Laurus*
Species:	*L. nobilis*

Binomial name *Laurus nobilis L* (Dias *et al.,* 2014).

The leaves of *Laurusnobilis L.* (laurel) are commonly used as a spice in cuisine. Many countries' folk medicine employs the plant's infusion in stomachic and carminative medicines, as well as in the treatment of gastrointestinal illnesses. (Ozcan *et al.,* 2010). The total phenolic content of the infusion and methanol extract was also determined using the Folin-Ciocalteu reagent (Kaurinovic *et al.,* 2010).Traditionally, dry bay leaves and their infusions have been used to treat digestive disorders such epigastric discomfort, flatulence, bloating, and eructation. Bay plant leaves and fruits have been used as astringents, diaphoretics, stimulants, emetic, emmenagogues, abortifacients, and insect repellents. Its essential oil is also used in cosmetic items such as soaps, lotions, and fragrances because it is an aromatic plant. Flavonols (kaempferol, myricetin, and quercetin), flavones (apigenin and luteolin), glycosylated flavonoids, sesquiterpene lactones, monoterpene and germacrane alcohols, and glycosylated flavonoids, sesquiterpene lactones,

monoterpene lactones, monoterpene lactones, monoterpene lactones, monoterpen (Dias *et al.,* 2014). Plants that are naturally therapeutic, such as L. nobilis contain a lot of bioactive chemicals. The biological actions of Bay extracts and essential oil have so been reported, including antibacterial, antifungal, and antioxidant effects. In a prior investigation, the aqueous decoction of bay leaf was found to have a 53.4% bactericidal activity against 176 bacterial isolates from 12 different genera. (Fiorini *et al.,* 1998).

The methanolic extract of seed oil demonstrated antioxidant properties in both the 2, 2-diphenyl-lpicrylhydrazyl (DPPH) free radical scavenging and the -carotene/linoleic acid test systems. (Morebise *et al.,* 2002).Another study used the DPPH assay to examine the antioxidant capacity of ethanolic and aqueous extracts of Hypericumperforatum, Ocimumbasilicum, and L. nobilis leaves. The ethanolic extracts of *L. nobilis* scavenged more DPPH radicals than the aqueous extracts. Bay leaves are commonly used in traditional Libyan meals (Wannes *et al*., 2010).

2.9.7 Utazi leaf *(Gongronema latifolium)*

Scientific classification

Kingdom:	*Plantae*
Clade:	*Tracheophytes*
Clade:	*Angiosperms*
Clade:	*Eudicots*
Clade:	*Asterids*
Order:	*Gentianales*

Family: *Apocynaceae*

Subfamily: *Asclepiadiodeae*

Tribe: *Marsdenieae*

Genus *Gongronema (Endl.). Decne*

Binomial name (Ugochukwu *et al.,* 2003).

The non-woody herbaceous plant *Gongronema latifolium* belongs to the Asclepiadaceae family. It can be found all over the world in tropical and subtropical climates, particularly in Africa and South America, with a moderate presence in Northern and South-Eastern Asia (Morebise *et al.,* 2002).*G. latifolium* is known as "Utazi" and "Arokeke" in South-Eastern and South-Western Nigeria, respectively (Agbo and Obi, 2006). *G. latifolium* can be used in a variety of ways in the kitchen, but it is most commonly employed as a spice and vegetable in traditional folk medicine. It can be eaten raw, cooked, or dried (Ugochukwu *et al*., 2003).According to some reports, it includes essential oils, saponins, and pregnanes (Morebise *et al.,* 2002). It possesses anti-inflammatory effects, according to (Morebise *et al.* 2002). It's commonly found in herbal prescriptions or preparations given by herbalists for the treatment and/or management of pains, infertility, hypertension, and ulcers, among other ailments.

Plate 2.1: Pawpaw leaf (*Carica papaya leaves*) (Gledhil, 2009).

Plate 2.2: Bay leaves (*Laurus nobilis L*) (Kaurinovic *et al.,*(2010).

Plate 2.3: Image of Utazi leaves (*Gongronema latifolium*) (Agbo and Obi 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Reagents / chemicals

Aluminum chloride, ferricchloride, Folin-Ciocalteu; sulphuric acid; 2,20-diphenyl-1-picrylhydrazyl (DPPH); Gallic acid; ammonium molybdate; sodium sulphate; sulphosalicylic acid; thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were purchased from Sigma Co. (St. Louis, MO, USA).Hydrogen peroxide, Phosphate buffer, L-ascorbic acid, potassium mercuric iodine; (Mayer's reagent and Hager's reagent), sodium phosphate, iron(iii)chloride, sodium hydroxide, glacial acetic acid; 2-deoxyribose; sodium carbonate, sodium hydroxide, sodium nitrite, and hydrogen peroxide were obtained from Wako Co. (Osaka, Japan). HCL; Bezene and all solvents n-hexane (99.8%); chloroform (99.8%); Methanol; and n-butanol (99.8%)

used were of analytical grade and purchased from Merck Co. (Darmstadt, Germany). Distilled deionized water was prepared by Ultrapure TM water purification system (Lotun Co., Ltd., Taipei, Taiwan),Olive oil; glacial acetic acid; ammonia solution; potassium mercuric iodide; potassium bismuth; phosphate buffer; EDTA.

3.2. Equipment and Apparatus

Spectrophotometer, rotary evaporator, willy millmachine, test tubes and test tube racks, beakers (600ml), measuring cylinder, micro pipette, weighing balance, foil paper, conical flask, water bathe, oven, spatula, filter paper, dropper, whatman filter paper, 100ml volumetric flask. Grinding machines, centrifuge, and extraction thimble.

3.3. Collection and Identification of Plant Materials

3.3.1 Plant Collection

Fresh healthy/disease free, mature, plant leaves of Carica papaya, Bay leaf, and Utazi leaf were collected from uncultivated farmlands located at Benin City, Edo State, Nigeria on April 2021.

3.3.2 Extract Preparation

The fresh, whole plant was collected and shade dried to obtain dry sample which was later coarsely powdered in a Willy Mill to 60-mesh size and used for solvent extraction. For sample preparation, 290 g of dried pawpaw sample, 264 g of dried utazisample,171g of dried bay sample were extracted twice (2000 ml for each) with 95% methanol at 25°C for 48 h and concentrated using a rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan) under reduced pressure at 40°C to yield the leaf extract.

3.4 Qualitative Phytochemical Screening of Leaf Extracts

Phytochemical screening was conducted to qualitatively determine the presence or absence of the following secondary metabolites, Alkaloids, Saponins, Cardiac glycosides, Tannins, Flavonoids using the method outlined by (Enabulele and Ehiagbonare, 2011).

Preparation of Stock Solution

The stock solution was prepared by adding 50 mg of extract of *Carica papaya leaf, Bay leaf* and *Utazi leaf* respectively into 50 mL of methanol. The mixture was allowed to stand overnight before use, and desired working concentrations were made by appropriate dilutions.

Test for alkaloids

0.4 g of extract was stirred with 8 ml of 1% HCl and the mixture was warmed and filtered. 2 ml of filtrate was treated separately with (a) with few drops of potassium mercuric iodide (Mayer's reagent) and (b) potassium bismuth (Dragendroff's reagent). Turbidity or precipitationwith either of these reagents was taken as evidence for existence of alkaloids.

Test for saponins

The ability of saponins to produce emulsion with oil was used for the screening test. 20 mg of extractwas boiled in 20 ml of distilled water in a water bath for five min and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation. 3 drops of olive oil were mixed with froth, shaken vigorously and observed for emulsion development.

Test for terpenoids

Presence of terpenoids in the extractwas carried out by taking 5 ml (1 mg/ml) of a Carica papaya, Bay leaf, and Utazi leaf and mixed with 2 ml of chloroform, followed by 3 ml of concentrated H_2SO_4. A reddish brown colouration of the interface confirmed the presence of terpenoids.

Test for anthraquinones

200 mg ofextractwas boiled with 6 ml of 1% HCl and filtered. The filtrate was shaken with 5 ml of benzene, filtered and 2 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, violet or red colour in the ammoniacal phase indicated the presence of free hydroxyl anthraquinones.

Cardiac glycosides determination

5 ml (10 mg/ml in methanol) of extraction a test tube was mixed with 2 ml of glacial acetic acid having one drop of $FeCl_3$ solution. To the mixture obtained 1 ml of concentrated sulphuric acid was added to form a layer. The presence of brown ring of the interface indicated deoxy sugar characteristic of cardiac glycosides.

Test for Phenols

Drops of 10% aqueous $FeCl_3$ solution were added to 5 mL of extract in a test tube. Formation of blue or green colour indicated the presence of phenols.

Test for Steroids

Into a test tube, 1 mL of the extract was added and mixed with 2 mL of acetic acid and 2 mL of concentrated H_2SO_4. Change of colour from violet to blue-green colouration was positive for steroids.

Test for Quinones

Concentrated sulphuric acid (1 mL) was added to 1 mL of each of the plant extract. Formation of red colour indicated the presence of quinones.

Test for flavonoids

50 mg of extract (filtrate) was suspended in 100 ml of distilled water was pipetted into a test tubeto get the filtrate. 5 ml of dilute ammonia solution was added to 10 ml of filtrate followed by few drops of concentrated H_2SO_4. Presence of flavonoids was confirmed by yellow colouration.

Test for tannins

50 mg of extract (filtrate) was boiled in 20 ml of distilled water and filtered in a test tube and then heated for five minutes to boil. A few drops of 0.1% $FeCl_3$ was added in filtrate and observed for colour change; brownish green or a blue-black colouration was taken as evidence for the presence of tannins.

3.5. Antioxidant Activity Assay

All antioxidant assay were carried out according to Saeed *et al.* (2012).

1, 1-diphenyl- 2- picrylhdrazyl (DPPH) Radical - Scavenging Activity

The free radical scavenging activity of the extractwas measured in vitro by 2,20- diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier. The assay is based on the ability of the antioxidant compounds to reduce DPPH by donation of hydrogen resulting in colour change from deep violet to golden yellow. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517nm.

Procedure

An aliquot (3mL) of 0.1 mM DPPH solution in ethanol was added to 100 µl of various concentrations (10 - 500 mg/mL) of the extracts.

The reaction tubes were shaken and incubated for 15 min at room temperature in the dark;

absorbance read at 517 nm.

All tests were performed in triplicate. A blank containing 24 mg DPPH and 100mL methanol was prepared and treated as the test samples and stored at 20°C.

The Percentage DPPH scavenging activities of the extracts and standards were determined using the equation:

$$\%\ Scavenging\ activity = 100 - \left[\frac{(Abs - Ab)}{Abc} \times 100\right]$$

Where:

Abs = Absorbance of sample (extracts or reference standard)

Ab = Absorbance of blank

Abc = Absorbance of negative control

Results were expressed as inhibitory concentration, IC_{50} (concentration of extract or standard required to scavenge 50% of DPPH radicals), which were determined from a linear regression curve of concentration versus % scavenging activity.

Phosphomolybdate assay (total antioxidant capacity)

The total antioxidant capacity of the extract was determined by phosphomolybdate method using ascorbic acid as a standard.

Procedure

An aliquot of 0.1 ml of sample solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 765 nm against a blank. A typical blank contained 1 ml of the reagent solution and the appropriate volume of the solvent and incubated under the same conditions. Ascorbic acid was used as standard. The antioxidant capacity was estimated using following formula:

Antioxidant effect (%)

$$\%\ Scavenging\ activity = \left[\frac{Abs\ control - Abs\ sample}{Abs\ control}\right] \times 100$$

Where;

Abs sample = Absorbance of sample (extracts or reference standard)

Abs control = Absorbance of control/ blank

Hydrogen peroxide scavenging activity

TheHydrogen peroxide scavenging activity of extract was determined.

Procedure

Hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 ml) of different extracts was transferred into the test tubes and their volumes were made up to 0.4 ml with 50 mM phosphate buffer (pH 7.4) After addition of 0.6 ml hydrogen peroxide solution, tubes were vortexed and absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank. The percentage hydrogen peroxide scavenging activity of the extracts and standards were calculated using the equation:

$$\%\ Scavenging\ activity = \left[\frac{Abs\ control - Abs\ sample}{Abs\ control}\right] \times 100$$

Where;

Abs sample = Absorbance of sample (extracts or reference standard)

Abs control = Absorbance of control/ blank

Reducing power

The reducing power was based on Fe (III) to Fe (II) transformation in the presence of the extract. The Fe (II) can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

Procedure

Various concentrations of the extract each of *Carica papaya, Laurus nobilis and Gongronema latifolium* (2 ml) were mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of

potassium ferricyanide (10 mg/ml). The mixture was incubated at 50°C for 20 min followed by addition of 2 ml of trichloroacetic acid (100 mg/l). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. A volume of 2 ml from each of the mixture earlier mentioned was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates a higher reducing power. Vitamin C served as the reference control.

3.5.1. Determination of Total Phenolic Content(TPC)

The total phenolic content was determined by the spectrophotometric method. In brief, a 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 10 ml of a 7% sodium carbonate solution was added to the mixture followed by the addition of 13 ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample.

3.5.2 Determination of Total flavonoid content (TFC)

Total flavonoid content was determined following a method by Park *et al* (2008). In a 10 ml test tube, 0.3 ml of extracts, 3.4 ml of 30% methanol, 0.15 ml of NaNO2 (0.5 M) and 0.15 ml of $AlCl_3.6H_2O$ (0.3 M) were mixed. After 5 min, 1 ml of NaOH (1 M) was added. The solution was mixed well and the absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavonoids was made using rutin standard solution (0 to 100 mg/l) under the same

procedure as earlier described. The total flavonoids were expressed as milligrams of rutin equivalents per g of dried extract.

3.6 Statistical Analysis

The data obtained in this study were subjected to statistical analysis using SPSS (V2.6). Results were recorded as mean ± standard error of mean. The difference was considered statistically significant when $P<0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1. Qualitative phytochemical screening of methanol extract of *Carica papaya, Laurus nobilis and Gongronema latifolium.*

The phytochemicals present in the methanolic extract of *Carica papaya, Laurus nobilis and Gongronema latifolium* are shown in Table 4.1. Screening of the methanolic extract of *Carica*

papaya showed the presence of the flavonoids, cardiac glycosides, alkaloids, tannins, phenols, steroids, terpernoidsand saponnis, quinones were absent. *Laurusnobilis* was found to contain flavonoids, tannins, saponins, cardiac glycosides, steroids, alkaloids, phenols, quinones and terpernoidswere absent. *Gongronema latifolium* was found to contain tannins, saponins,terpernoids,alkaloids, quinones, steroids,phenols and cardiac glycosides, phenols,steroids, flavonoids.

Table 4.1. Phytochemicals screening of *Carica papaya, Laurus nobilis and Gongronema latifolium leaves*.

Phytochemical	Pawpaw leaf	Bay leaf	Utazi leaf
Flavonoids	+	+	_
Tannins	+	+	+

Cardiac glycoside	+	+	_
Saponins	+	+	+
Steroids	+	+	_
Phenols	+	+	_
Quinones	_	+	+
Anthraquinones	_	_	_
Terpernoids	+	_	+
Alkaloids	+	+	+

Key "+" =Present " - " = absent

4.2. DPPH Radical Scavenging Activity of *Carica papaya, Laurus nobilis and Gongronema latifolium* Leaves

Figure 4.1. shows DPPH radical scavenging activity of Pawpaw leaf Bay leaf and Utazi leaf Radical scavenging values increased with increasing concentration of the extracts.

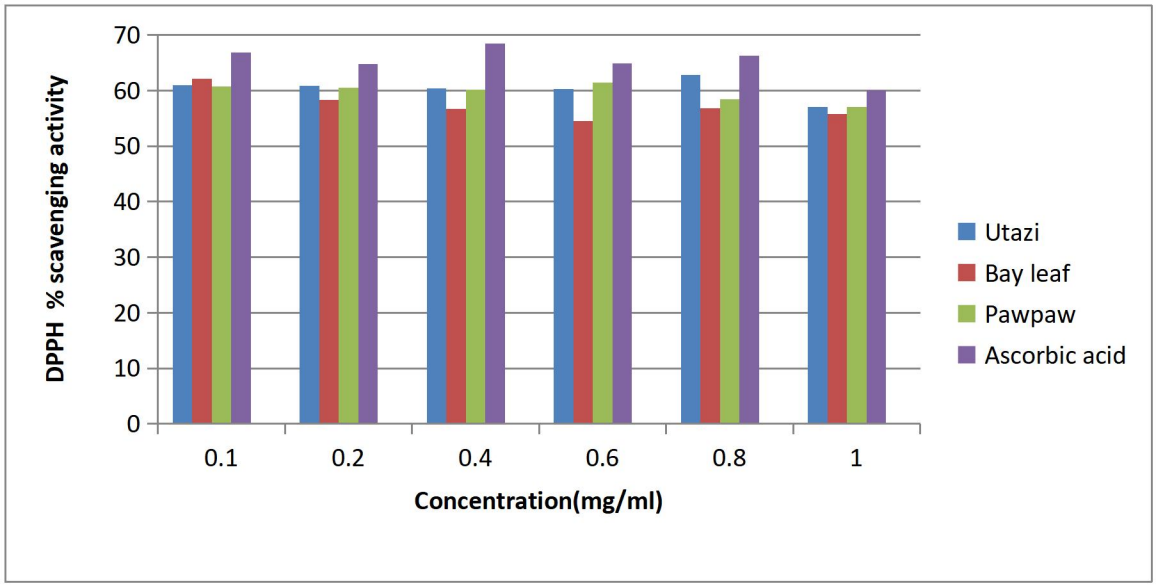


Figure 4.1: DPPH radical scavenging activity of the methanolic extracts of *Carica papaya, Laurus nobilis and Gongronema latifolium*

EC_{50} valuesof DPPH radical scavenging for Pawpaw leaf, Bay leaf and Utazi leaf

Plants extract	**Concentration(mg/ml)**
Pawpaw	0.685± 0.012
Bay	0.586± 0.049
Utazi	0.896± 0.104
Ascorbic acid	0.963± 0.079

Each value in the table is represented at Mean ± SEM (n=3).

4.3. Hydrogenperoxide radical scavenging activity of *Carica papaya, Laurus nobilis and Gongronema latifolium* Leaves

Figure 4.2 shows the scavenging activity ofHydrogen peroxide in Pawpaw leaf, Bay leaf and Utazi leaf.

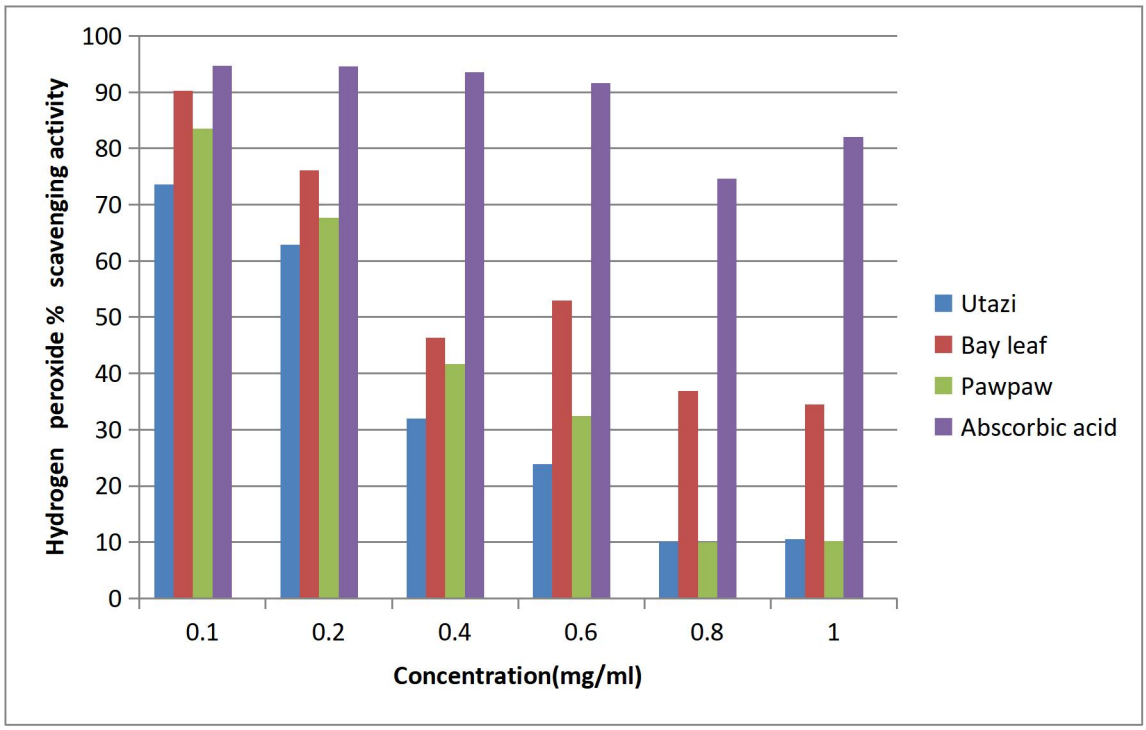


Figure 4.2: Hydrogen peroxide scavenging Activity of methanolic extracts of *Carica papaya, Laurus nobilis and Gongronema latifolium* Leaves

EC50 values (mg/ml) of radical scavenging for Pawpaw leaf, Bay leaf and Utazi leaf.

Plants extract	**Concentration (mg/ml)**
Pawpaw	0.391± 0.116
Bay	0.672± 0.090

Utazi	0.547± 0.024
Ascorbic acid	0.529± 0.016

Each value in the table is represented at Mean ± SEM (n=3).

4.4. Total antioxidant capacity of leaves of *Carica papaya, Laurus nobilis and Gongronema latifolium.*

The phosphomolybdate method is quantitative, since the total antioxidant capacity (TAC) is expressed as ascorbic acid equivalents. The antioxidant capacity of various solvent fractions of The extract was found to decrease in this order: Pawpaw >Utazi> Bay extract. All results showed antioxidant activity in dose dependent manner at concentration 0.1 to 1.0µg/ml.

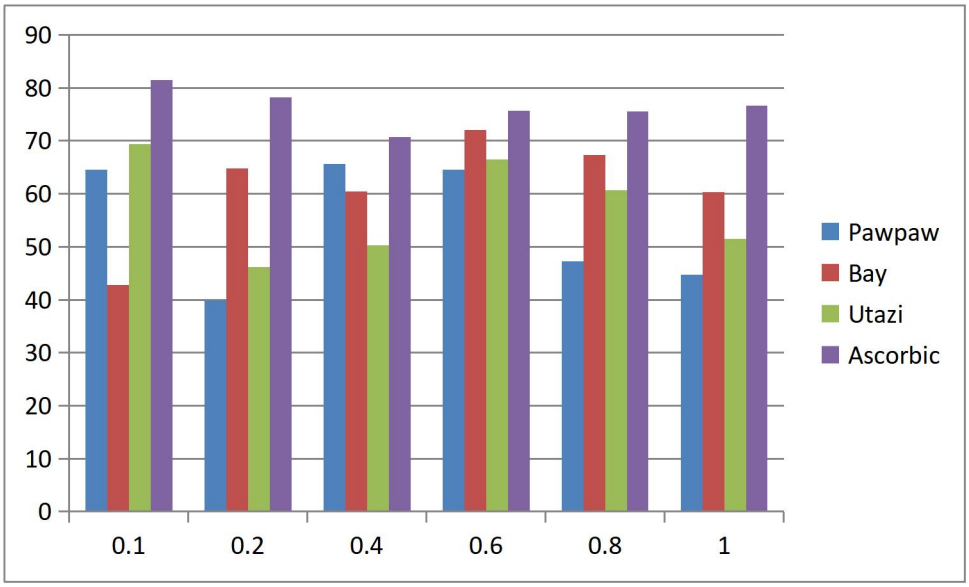


Figure 4.3: Total antioxidant capacity of comparison of *Carica papaya* (Pawpaw), *Laurus nobilis* (Bay), and *Gongronema latifolium* (Utazi) leafs.

4.5. EC_{50} values (mg/ml) of Radical scavenging activity for Pawpaw leaf, Bay leaf and Utazi leaf.

Plants extract	Concentration(mg/ml)
Pawpaw	0.752± 0.166
Bay	0.816± 0.094
Utazi	0.619± 0.012
Ascorbic acid	0.945± 0.004

Each value in the table is represented at Mean ± SEM (n=3).

4.6. Reducing power of methanolic extracts of *Carica papaya, Laurus nobilis and Gongronema latifolium*

Figure 4.3 shows the reducing power activity of Pawpaw leaf, Bay leaf and Utazi leaf. Reducing power increased with increasing concentration of the extracts.

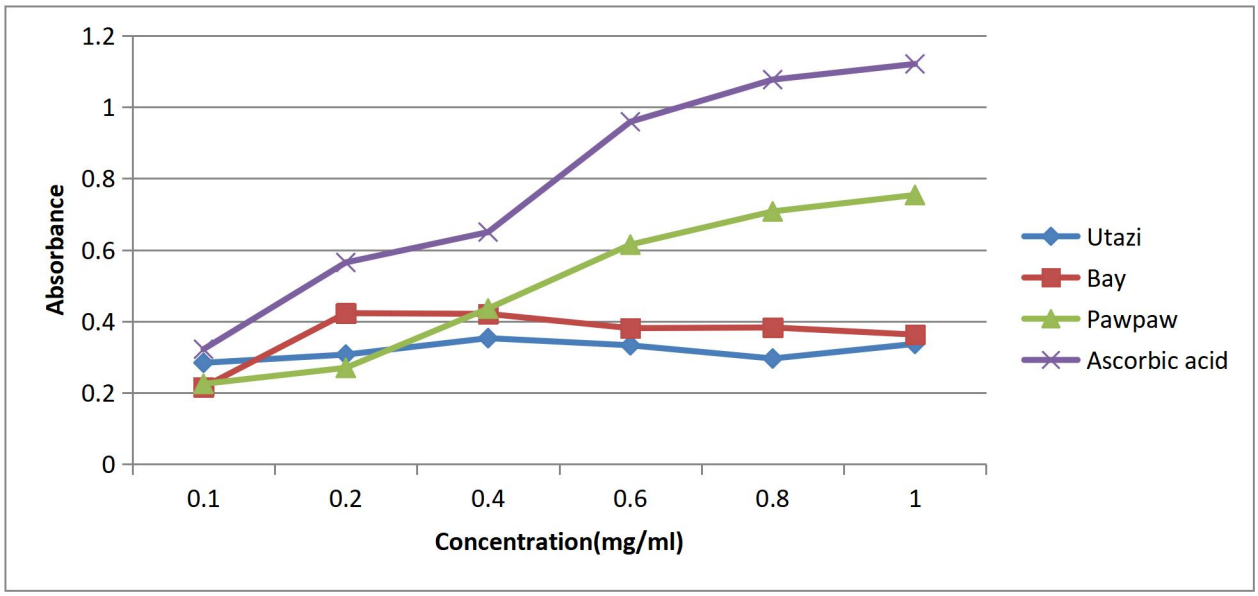


Figure 4.4: Reducing power comparison of *Carica papaya* (Pawpaw), *Laurus nobilis* (Bay), and *Gongronema latifolium* (Utazi) leafs.

4.7. Total phenolic content of *Carica papaya, Laurus nobilis and Gongronema latifolium*

Total phenolic content was estimated by using Folin Ciocalteu reagent. Total phenolic content of the different fractions of three plants samples was solvent dependent and expressed as milligrams of garlic acid equivalents (GAE) equivalent. Table 4.4 summarizes that total phenolic compounds in plants samples varied widely, ranging from 0.055±0.015 and 0.145± 0.021mg/g expressed as gallic acid equivalents (GAE). Bay leaf exhibited the highest total phenolic content.

Table 4.4: shows the TPC of Pawpaw leaf, Bay leaf and Utazi leaf.

Table 4.3 Total phenolic content (TPC) of *Carica papaya, Laurus nobilis and Gongronema latifolium*.

Sample	Total phenols (mgGAE/g)
Paw paw leaf	0.056±0.027
Utazi leaf	0.041±0.005
Bay leaf	0.145±0.037

Mean±SEM(n = 3)

4.8. Total Flavonoid content of *Carica papaya, Laurus nobilis and Gongronema latifolium*

The content of flavonoid expressed as quercetin equivalents, varied from 0.051± 0.008 to 0.233±0.012 mg quercetin equivalent/g extract (Table 4.5). Bay leaf showed the highest amount of flavonoid contents followed by Utazi and Pawpaw leaf.

Table 4.4: shows the TFC of Pawpaw leaf, Bay leaf and Utazi leaf.

Sample	Total flavonoids (mgQE/g)
Paw paw	0.233±0.022
Utazi	0.052±0.014
Bay leaf	0.054±0.018

Mean±SEM (n = 3)

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Phytochemical

Phytochemicals are natural occurring bioactive compounds known for their health benefits due to the presence of these phytochemicals the plant tend to possess certain therapeutic properties such as anti-inflammatory, antitumor, antimicrobial, anti-analgesic and antibacterial activities. The result shows that *Carica papaya* showed the presence of the flavonoids, cardiac glycosides, alkaloids, tannins, phenols, steroids, terpernoids and saponnis, quinones were absent. *Laurus nobilis* was found to contain flavonoids, tannins, saponins, cardiac glycosides, steroids, alkaloids, phenols, quinones and terpernoids were absent. *Gongronema latifolium* was found to contain tannins, saponins, terpernoids, alkaloids, quinones, steroids, phenols and cardiac glycosides, phenols,steroids, flavonoids were absent.

The presence of flavonoids indicates that the plants could possess antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor properties and also antioxidant properties (Ferreyra *et al.*, 2012). The presence of tannins showed that both samples may be useful as astringents against diarrhea, it can be used as diuretics against stomach and duodenal tumors, and it also possess anti-inflammatory and antiseptic properties (Hayat *et al.*, 2010). Saponins shows the presence of antimicrobial effect that prevents the plants from insect infestation. The presence of alkaloids showed the plants may have pharmacological activities such as antihypertensive effect, anticancer action and antimalarial activity. It also showed that the plant had high protection and survival rate against micro-organism such as anti-fungal and anti-bacteria activities (Saeed *et al.*, 2012). The presence of cardiac glycoside shows that plant has the ability to strengthen the

heartbeat of a weak heart muscle. The presence of tannin, flavonoids in pawpaw leaf was in accordance with the work carried out by (Barger *et al.,*2009).

Antioxidants

Polyphenolic substances (phenolic acids and flavonoids), which are abundant in plants and host antioxidant phytophenolics, are primarily responsible for the antioxidant activity of fruits. Phenolic content can be used as an indicator of antioxidant capacity and as a preliminary screen for any product that is to serve as a natural source of antioxidants in functional foods (Viuda-Martos *et al.,* 2011). Table 4.3 revealed that methanolic extract of Pawpaw leaf, Bay leaf and Utazi leaf were able to scavenge DPPH. From the EC_{50}values, Bay leaf had the highest Dpph radical scavenging activity with 0.586± 0.049 mg/mL, followed by Pawpaw leaf with 0.685± 0.012, then Utazi leaf with EC_{50}value of 0.896± 0.104mg/mL. The EC_{50}value of Paw paw leaf was comparable in that of Surajit *et al. (*2015).

The EC_{50} value of total antioxidant capacity for Utazi (0.619± 0.012µg/ml) is higher than Pawpaw (0.752± 0.166mg/ml) and Bay (0.816± 0.094mg/ml). Strong total antioxidant capacity of pawpaw leaf statistically similar to ascorbic acid indicates strong antioxidants in this extracts and these could be attributable to the presence of phenolic compounds.The total antioxidant capacity of Utazi leaf was similar to that obtained by Edith *et al.* (2015) in their study.

Hydrogen peroxide radical scavenging activity in figure 4.2, shows that Bay leaf has the highest radical scavenging activity followed by Paw paw and Utazi leaf. This means that bay leaf is as potent as ascorbic acid in Hydrogen peroxide radical scavenging activity. The Hydrogen peroxide radical scavenging activity of bay leaf was in accordance with Elmastas *et al.* (2006).

Reducing power in figure 4.3.increased with increasing concentration of Paw paw, Bay and Utazi extracts.The reducing power of bay leaf was in accordance with Elmastas *et al.,* 2006.

Data in table 4.3 illustrated that total phenolics (TPC) amount varied greatly and ranged in Bay leaf with a high concentration of 0.145± 0.021 mg/gGAE, Pawpaw leaf 0.055±0.015 mg/gGAE and Utazi leaf 0.041±0.003 mg/gGAE. Thelowest concentration was found in Utazi leaf with 0.041±0.003 mg/gGAE. The total phenolic and flavonoid content of Pawpaw leaf was in accordance with the work carried out by Surajit *et al.,* (2018).

Plants are rich source of natural flavonoids (El–Seedi *et al*., 2012). Table 4.4 shows the total flavonoids content (TFC) of the analyzed plant samples. TFC of the tested plant samples extracted with methanol were generally characterized by the presence of a significant amount of phenolic chemicals. The highest amount of total flavonoids content was found in Pawpaw leaf with (0.233±0.012mg/gQE) Bay leaf (0.053±0.010mg/gQE) and Utazi leaf (0.051± 0.008mg/gQE). Considering flavonoids, Pawpaw leaf gave the highest concentrations in table 4.7.The total phenolic and flavonoid content of bay leafwas not in accordance with the work carried out by Amal *et al.,* 2018, probably because ethanolic extracts was used in this study[]'.

5.2 Conclusion

The methanol extract of *Carica papaya, Laurus nobilis, and Gongronema latifolium* have stocks of potentially beneficial biological products, according to phytochemical contents.Therefore this study justifies that free radical scavenging activity and total phenolic and flavonoids content showed that *Carica papaya, Laurus nobilis, and Gongronema latifolium* can be a potent source of natural antioxidant, from this study *Laurus nobilis* has shown the highest antioxidant potent than *Carica papaya* and *Gongronema latifolium.*

REFERENCES

Agbo, C. U. and Obi, I. U. (2006). Macropropagation technique for different phytological ages of *Gongronema latifolium* Benthcuttings. *Afr. J. Biotech*.13: 12541258.

Akah, P. A., Enwerem, N. M and Gamaniel, K. K. (2007). Preliminary studies on Purgative Effect of *Carica papaya* root Extract, *Journal of fitoterapia*, 12 (6), 327331.

Amal,T., Fatima, E.K., Imane, A., Yassine, E.A., Badiaa, L. and Abdelefattah, A. (2018). Antioxidants activity and total phenolic and total flavonoids content variations of leaf extracts of *Laurus nobilis* L. *J. of Res In Chem. And Pharmaceutical Sci.,* 11:540-543

Barger, G. O., Finar L. and Hormick, C. A. (2009). Papaya Extract, Macmillan Publisher, New York, p. 711.

Basile, A., Giordano, S., Lopez-saez, J. A. and Cobianchi, R. C. (1999). Antibacterial activity of pure flavonoids isolated from mosses. *Phytochemistry*.52:1479- 1482.

Baur, X. M., Sourer, W. P. and Weiss, W. O. (2008), Effects of natural extract of carica papaya on digestibility, performance traits and nitrogen balance of broiler chicks, *Australian Journal of Basic and Applied sciences, 5(*20), 250-262.

Bravo, J,A.,Urbain, A.,Marston, L., SintraGrilo, O., Purev, B., Purevsuren, D,. Batsuren, M., Reist, A., Carrupt, P. A. and Hostettmann, K. (2008). Xanthones from *Gentianellaamarella ssp.acuta* with Acetylcholinesterase and Monoamine Oxidase Inhibitory Activities.187: 507-516

Brume, M. E. (2008). Role of tocopherols in the protection of biological systems Against oxidative damage. *Journal of Photochemistry and Photobiology B:Biology8:211.*

Calabro, M.L, Galtieri, V., Cutroneo, P., Tommasini, S, Ficarra, P. and Ficarra, R. (2004). Study of theExtraction procedure by experimental design and validation of a LC method for determination of Flavonoids in Citrus bergamia juice. *J. Pharm and Biomed Anal.35 :349-363.*

Chaudhry, N.M.and Tariq, P. (2006). Bactericidal activity of black pepper, bay leaf, aniseed and coriander against oral isolates. *Pak J Pharm Sci.* 19: 214-218.

Cordel, I. G. A. (2008). Recent advances in understanding the antibacterial properties of plant Extract. *International Journal of Antimicrobial Agents,*38 (2) 99-107.

Dias, M.I., Barros L, Dueñas M, Alves R.C. and Oliveira, A. (2014). Nutritional and antioxidant contributions of *Laurus nobilis L*. leaves: Would be more suitable a wild or a cultivated sample?. *Food Chemistry* 156: 339-346.

Djeridane, A., Yousfi, M., Nadjemi B., Boutassouna, D., Stocker, P. and Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem,* 97:654–660.

Ebrahimzadeh, M.A., Pourmoradand F. and Bekhradnia, A. R. (2008). Ironchelating activity, phenol and Flavonoid content of some medicinal plants from Iran. *Afr. J. Biotechnol.,* 7:3188-3192.

Edith, A. and Ugiagbe, M.O., (2015). Antioxidant potentials of *Gongronema latifolium* leaf extracts. *Afr. J. Biotech.* 27(2): 85-88.

Elmastas, M., Gulcin, I. and Aboul-Enein, H.Y. (2006). Antioxidants activity of whole plants extracts of *Laurus nobilis* L. Leaves. *Journal of the Iranian Chemical Society* 258-266.

Enabulele, S.A. and Ehiagbonare, J. E. (2011). Antimicrobial, nutritional and phytochemical Properties of Perinari excels seeds *International Journal of Pharma and Bio Sciences* 2(3):459-470.

Eno, A. E., Owo O. I, Itam E. H. and Konya. R. S. (2000). Blood pressure Depression by the fruit juice of *Carica papaya* [*L*] in renal and DOCA-induced Hypertension in the Rat, *Journal of Phytotherapy Research,*9 (4); 235-239.

Everette, B. M. (2003). Carpaine and Alkaloid of *carica papaya*, *Journal of Chemistry and Pharmacology,* 22 (5) pp. 281-298.

Ezugwu, E .C (2008), Phytochemical constituents of some Nigerian medicinal plants. Emeka publisher, Nsukka, pp. 121-160.f *Chemistry and Pharmacology*, Vol. 22, No. 5, pp. 281-298.

Ferreyra, M.L.F., Rius, S.P. and Casati, P. (2012). Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science.*3(222):222.

Fiorini, C., David, B., Fourasté, I. and Vercauteren, J. (1998). Acylatedkaempferol glycosides from Laurusnobilis leaves. *Phytochemistry*47: 821-824.

Grayson, M .O. (2001). Effect of papaya tannin on fermentation quality, proteolysis and protein rumen degradability of alfalfa silage, Biochemistry *Journal of Technology*, 8(2), 322-368.

Gledhil, D .N. (2009). Carica papaya, *Journal of west African Tress.* 10(3)45.

Gocer, H. and Gulcin, I. (2011). Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties*. Int J Food Sci Nutr*, 62:821–825.

Gulcin, I. (2012). Antioxidant activity of food constituents: an overview. *Arch Toxicol*. 86:345–391.

Hasheen, F. M . (2007). Antibacterial activity of *carica papaya* extract. *Oxford University press, New York*, pp. 15-25.

Halliwell, B. (1981). Gutteridge JMC: Formation of thiobarbituric acid reactive substances from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. *FEBS Lett* 128:347–352.

Harman, D. (1998). Free radical theory of aging. *Current status*. *Amster-dam: Elsevier*; p.3–7.

Hayat, K., Zhang, X., Farooq, U., Abbas, S., Xia, S. andJia, C. (2010). Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. *Food Chem.,* 123(2): 423-429**.**

Hertog, P.C., Hollman, A. and Katan, M. B. (2003). Content of potentially anticarcinogenic flavonoids of28 vegetables and 9 fruits commonly consumed in the Netherlands, *Journal of Agricultural andFood Chemistry,*40(12) pp.2379–2383.

Hill, A. F. (1952). Economic Botany.A textbook of useful plants and plant products.2nd edn. *McGarw-Hill Book Company Inc, New York.*

Hill, R. A. (1985). Terpenoids. In Thomson RH, (ed). *Chemistry of Natural Products, Blackie Academic and Professional.London.*106-134.

Horowit, R.M. and Gentili, A. (2007). The citrus flavonoids.In : Sinclair WB editor. The orange Its Biochemistry and physiology, CA, *University of California, Division of Agricultural Science, Los Angeles, pp.334-372.*

Irshad, M. and Chaudhuri, P. S. (2002). Oxidant-Antioxidant System : Role and Significance in Human Body, Indian *Journal of Experimental Biology,* 40 (11) 1233-1239.

Ivanoi'c, J.,Misin, D.,Ristic M, Pesic, O. and Zizovic, I. (2010). Supercritical CO2 extract and essential oil of bay (Laurusnobilis L.)-chemical composition and antibacterial activity.*Journal of the Serbian Chemical Society* 75: 395-404.

Jayaprakasha, G.K., Wilson, C.and Patil, B.S. (2006). Phenolics and carotenoids contribute to antioxidant activity in navel orange. Oral session 25 presented at 103rd Annual International Conference of the American Society for Horticultural Science. *New Orleans, LA, USA.*92:321-324.

Karoui, I. J. and Marzouk, B. (2013). Characterization of Bioactive Compounds in Tunisian Bitter Orange (*Citrus aurantium L.*)Peel and Juice and Determination of Their Antioxidant Activities. *Bio Med. Res. Int.,* 20: 21-26.

Kaurinovic, B., Popovic, M. and Vlaisavljevic, S. (2010). In vitro and in vivo effects of *Laurus nobilis L.* leaf extracts. Molecules 15: 3378-3390.

Kaviarasan, S., Naik, G.H.,Gangabhagirathi, R., Anuradha, C.V. and Priyadarsini, K. I. (2007). *In Vitro* Studies on Antiradical and Antioxidant Activities of Fenugreek (*Trigonellafoenumgraecum)* Seeds. *Food Chemistry,1*03,31-37.

Kim, D. O. and Jeong, S. W. (2003). Lee CY: Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem* 81:321–326.

Kumaran, A. and Karunakaran, R. J. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus. *Food Chem.,* 97(1): 109-114.

Kumarasamy, Y., Byres, M., Cox, P. J., Jaspars, M. and Nahar, L. (2007). Screening seeds of some Scottish plants for free radical scavenging activity. *Phytotherapy Research* 21: 615-621.

Lobo, V, Patil, A, Phatak, A. and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*. 4(8):118-26.

Morebise, O., Fafunso, M. A., Makinde, J. M., Olajide, O. A. and Awe, E. O., (2002).Anti-inflamatory property of the leaves of *Gongronema latifolium. Phytotherapeutic Res.,* 16: 75-77.

Moulehi, I., Bourgou, S., Ourghemmi, I. and Tounsi, M. S. (2012).Variety and ripening impact on phenolic composition and antioxidant activity of mandarin (*Citrus reticulate Blanco*) and bitter orange (*Citrus aurantium* L.) seeds extracts. *Ind. Crops Prod.,* 39: 74-80.

Nager, R.G., Monaghan, P. and Houston, D. C. (2017). Role of tocopherols in the protection of biological Systems against oxidative damage. *Journal of Photochemistry and Photobiology B:Biology* 8:211.

Nayak, B., Dahmoune, F., Moussi, K., Remini, H., Madani, K., Dairi, S., Aoun, O. and Khodir, M.(2015). Comparison of microwave, ultrasound and accelerated assisted solvent extraction for recovery of polyphenols from *Citrus sinensis* peels. *Food Chem.,* 187: 507-516.

Nunes, P. X., Silva, S. F., Guedes, R. J. and Almeida, S.(2012). Biological oxidations and antioxidant activity of natural products, Phytochemicals as nutraceuticals Global Approaches to Their Role in Nutrition and Health.

Nuutila, A. M., Puupponen-Pimia, R., Aarni, M. and Oksman-Caldentey, K. M. (2003). Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem.,* 81: 485-493.

Oluyemi, K. A., Okwuonu, U.C., Baxter D. G. and Oyesola T.O. (2007). Toxic effects of methanolic exract of *Aspiliaafricana* leaf on the estrous cycle and uterine tissues of Wistarrats.*Int J Morphol* 25:609–614.

Ozcan, B, Esen M, Sangun, M. K., Coleri, A. and Caliskan, M. (2010). Effective antibacterial and antioxidant properties of methanolic extract of *Laurus nobilis* seed oil. *Journal of Environmental Biology* 31: 637-641.

Pellegrini, N.,Serafini, M.,Colombi, B., Del Rio, D., Sara, S., Bianchi, M. and Brighenti, F. (2003).Total Antioxidant Capacity of Plant Foods, Beverages and Oils Consumed in Italy Assessed by Three Different In Vitro Assays. *J. Nut.,* 133(9): 2812-2819.

Prior, R. L., and Wu, X. (2013). Diet antioxidant capacity: Relationships to oxidative stress and health. *Am. J. Biomed. Sci.*, 5: 126-139**.**

Saeed,N., Khan, R., and Shabbir, M.(2012). Antioxidants activity, total phenolic and total flavonoid content of whole plants extracts *Torilis Leptophylla L. BMC Complementary and alternative medicine. 12:221.*

Schneider, C., Rotscheidt, K. and Breitmaier, E. (1993).Four new pregnane glycosides from *Gongronema latifolium* (Asckepiadaceae).*Liebigs Annalen Der Chemie,* 10: 1057-1062.

Shahidi, G.E. and Naczk, K.O. (2004). Indicators for evaluation flipidoxidation and off-flavor Development In food Developments in Food Science,*40 pp.55-68.*

Sunil, K. (2014). The Importance of antioxidant and their role in pharmaceutical science -A Review.Asian*J. of Res. In Chem.* And Pharmaceutical Sci.,1:27-44.

Surajit, D.M., Lalmawizuala, R., Vabeiryureilai, M., Nachimuthu, S.K., and Esther.L. (2015). An investigation of the antioxidant property of *Carica papaya* leaf extracts *Journal of Botanical Sciences 4(2) 853-858.*

Topcagic, A., Keskin-Sasic I., Tahirovic I., Klepo, L., Salihovic, M., Ibragic, S., Toromanovic, J., Ajanovic, A.,Velispahic ,E.(2012).Total Phenolic Content and Antioxidant Capacity of FruitJuices.*Glas.hem.tehnol.BosneHerceg,39,25-28.*

Ugochukwu, N. H., Babady, N. E., Cobourne, M. and Gasset, S. R. (2003).The effect of *Gongronema latifolium* leaf extract on serum lipid profile and oxidative stress of hepatocytes of diabetic rats.*J. Biosci*., 28: 1-5.

Vongtau, H. O, Abbah, J, Chindo, B. A., Mosugu, O, Salawu, A.O., Kwanashie, H.O. and Gamaniel, K. S. (2005). Central inhibitory effects of the methanol extract of Neorautanenia mitis root in rats and mice. *J Pharm Biol*, 43:113–120.

Wannes, W. A., Mhamdi B., Sriti J, Jemia M. B., Ouchikh O, Hamdaoui G, Kchouk M.E. and Marzouk, B. (2010). Antioxidant activities of the essential oil and methanol extracts from myrtle (*Myrtuscommunis var. italica L.*) leaf, stem and flower. *Food ChemToxicol,* 48:1362–1370.

Zengin, G., Cakmak Y. S., Guler, G. O. and Aktumsek, A. (2011). Antioxidant properties of methanolic extract and fatty acid composition of *Centaureaurvillei* DC.subsp. hayekiana Wagenitz. Rec Nat Prod 5:123–132.

APPENDIX

Total flavonoids Statistics of Pawpaw leaf

		Concentration(10)	20	40	60	80	100
N	Valid	3	3	3	3	3	3
	Missing	0	0	0	0	0	0
Mean		0.14133	0.12000	0.15767	0.13600	0.1960	0.2333
Std. Error of Mean		0.064605	0.029023	0.035862	0.022723	0.03195	0.01278
Std. Deviation		0.111899	0.050269	0.062115	0.039357	0.05534	0.02214
Variance		0.013	0.003	0.004	0.002	0.003	0.000

Total flavonoids Statisticsof Bay Leaf

		10	20	40	60	80	100
N	Valid	3	3	3	3	3	3
	Missing	0	0	0	0	0	0
Mean		.0337	.0127	.0403	.0493	.0540	.0537
Std. Error of Mean		.01705	.00145	.01789	.02088	.02409	.01017
Std. Deviation		.02954	.00252	.03099	.03617	.04173	.01762
Variance		.001	.000	.001	.001	.002	.000

Total flavonoids Statistics of Utazi leaf

		10	20	40	60	80	100
N	Valid	3	3	3	3	3	3
	Missing	0	0	0	0	0	0
Mean		.06333	.07033	.04800	.02400	.06067	.05167
Std. Error of Mean		.016697	.010349	.011358	.003786	.007688	.008253
Std. Deviation		.028919	.017926	.019672	.006557	.013317	.014295
Variance		.001	.000	.000	.000	.000	.000

Total flavonoids Statistics of Standard (Quercetin)

		10	20	40	60	80	100
N	Valid	3	3	3	3	3	3
	Missing	0	0	0	0	0	0
Mean		.0610	.1570	.2317	.4970	.3807	.4300
Std. Error of Mean		.02875	.02150	.01084	.16289	.02196	.01664
Std. Deviation		.04979	.03724	.01877	.28213	.03803	.02883
Variance		.002	.001	.000	.080	.001	.001

Total phenolic Statistics of standard(Gallic acid)

		v10	v20	v40	v60	v80	v100
N	Valid	3	3	3	3	3	3
	Missing	0	0	0	0	0	0
Mean		.15433	.35200	.39400	.48100	.61000	.65933
Std. Error of Mean		.025654	.033946	.022745	.013650	.008963	.030867
Std. Deviation		.044433	.058796	.039395	.023643	.015524	.053463
Variance		.002	.003	.002	.001	.000	.003

Total phenolic content Statistics of Utazi leaf

		v10	v20	v40	v60	v80	v100
N	Valid	3	3	3	3	3	3
	Missing	0	0	0	0	0	0
Mean		.00967	.01267	.02200	.03867	.02400	.04133
Std. Error of Mean		.002333	.004631	.003000	.005925	.004583	.003180
Std. Deviation		.004041	.008021	.005196	.010263	.007937	.005508
Variance		.000	.000	.000	.000	.000	.000

Total phenolic content Statistics of Pawpaw leaf

		v10	v20	v40	v60	v80	v100
N	Valid	3	3	3	3	3	3
	Missing	0	0	0	0	0	0
Mean		.13433	.09733	.04300	.05300	.06867	.05567
Std. Error of Mean		.025835	.014495	.003786	.011790	.009528	.015344
Std. Deviation		.044747	.025106	.006557	.020421	.016503	.026577
Variance		.002	.001	.000	.000	.000	.001

Total phenolic content Statistics of Bay leaf

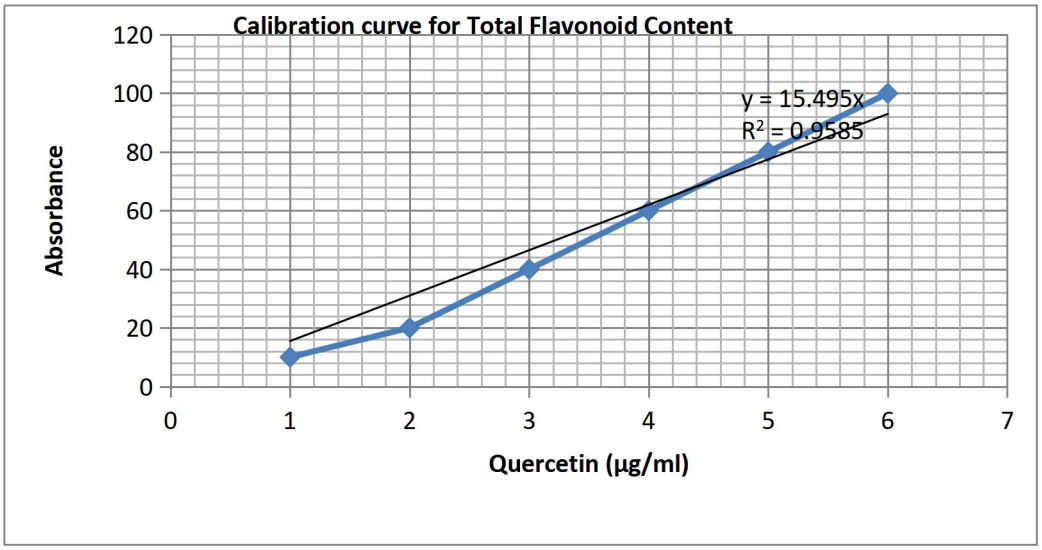
		v10	v20	v40	v60	v80	v100
N	Valid	3	3	3	3	3	3
	Missing	0	0	0	0	0	0
Mean		.03200	.03833	.06067	.07367	.10400	.14533
Std. Error of Mean		.004726	.008090	.008570	.016506	.024987	.021310
Std. Deviation		.008185	.014012	.014844	.028589	.043278	.036910
Variance		.000	.000	.000	.001	.002	.001

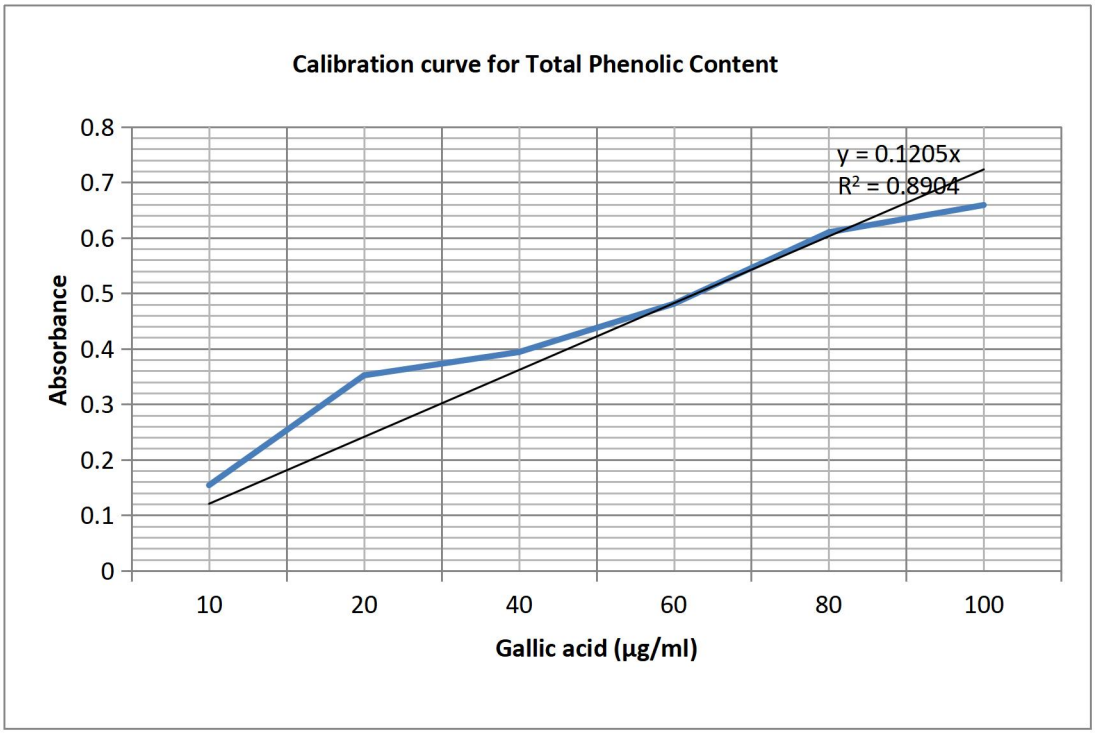
Weight of extract (g/)100ml	**Pawpaw**	**Bay Utazi**	**Standard**
0.1	0.118± 0.013	0.070±0.0060.190±0.022	0.038±0.022
0.2	0.232± 0.027	0.172±0.007 0.267±0.026	0.039±0.001
0.4	0.419± 0.058	0.386±0.065 0.547±0.056	0.046±0.001
0.6	0.486± 0.044	0.338±0.0090.731±0.137	0.060±0.003
0.8	0.647± 0.076	0.454±0.0210.949±0.041	0.182±0.011
1.0	0.792± 0.017	0.471±0.1051.285±0.065	0.129±0.053

Table A3: The Hydrogen peroxide analysis of mean absorbance and standard error of mean of the plants samples.[a]Mean±SD(n = 3), Are significantly different (P< 0.05).

Weight of extract (g/)100ml	Pawpaw	Bay	Utazi	Standard
0.1	0.694±0.038	0.814±0.020	0.517±0.007	0.712±0.205
0.2	0.699±0.028	0.897±0.010	0.563±0.023	0.758±0.176
0.4	0.664±0.052	0.932±0.091	0.636±0.060	0.721±0.171
0.6	0.830±0.023	0.977±0.006	0.726±0.045	0.754±0.191
0.8	0.895±0.007	0.929±0.014	0.799±0.096	0.726±0.175
1.0	0.923±0.034	0.951±0.018	0.924±0.054	0.944±0.162

Table A3: The DPPH analysis of mean absorbance and standard error of mean of the plants samples. [a]Mean±SD(n = 3), Are significantly different (P< 0.05).


Calibration curve for Total Flavonoid Content
y = 15.495x
R² = 0.9585
Absorbance
Quercetin (µg/ml)
0
20
40
60
80
100
120
0
1
2
3
4
5
6
7


Calibration curve for Total Phenolic Content
y = 0.1205x
R² = 0.8904
Absorbance
Gallic acid (µg/ml)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
10
20
40
60
80
100