ANTI-SALMONELLA ACTIVITY OF LEAVES EXTRACTS OF *Carica papaya*, *Citrus aurantifolia*, *Mangifera indica* and *Psidium guajava*

BY

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DECLARATION

I hereby declare that this work is the product of my research efforts undertaken under the supervision of Dr. Muhammad Yusha'u and has not be presented anywhere for the award of a degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this thesis/dissertationand the subsequent write-upby Abdulrazak Mohammed Hussain with registration number SPS/13/MMB/00035 were carried out under my supervision.

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DEDICATION

This thesis is dedicated to my parents and siblings.

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CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

ABSTRACT

This research is aimed at determining the antibacterial activity of leaves extracts of*Psidium guajava*, *Mangifera indica*, *Carica papaya* and *Citrus aurantifolia* against some bacteria.Dried leaves of *Psidium guajava, Mangifera indica, Carica papaya* and *Citrus aurantifolia* were obtained from Bayero University, Kano old campus and were extracted using water, ethanol and chloroform solvents via percolation method. The leaves extracts were subjected to phytochemical screening in order to detect the presence of secondary metabolites, toxicity studies, thin layer chromatography and GC-MS analysis. The extracts were further tested for antibacterial activity against clinical isolates of *Salmonellatyphi*, *S.paratyphi*A and *S. paratyphi*B, using agar well diffusion method. The results of phytochemical screening revealed the presence of these secondary metabolites;alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, phenols, and xanthoproteins while that of the antibacterial activity showed that chloroform extracts of *C. aurantifolia* produced highest zone of inhibition (24.50mm) against *S. paratyphi* A, then chloroform extracts of *C. papaya* against *S. paratyphi* B (16.80mm) with the least been all the extracts of *C. papaya* against *S. paratyphi* A (0.00). The seven different TLC fractions showed wide distinctions in terms of their chemical composition. The major similarities observed in the bioactive GC-MS fractions was in the case of oleic acid and palmitic acids which were the major compounds present in all the fractions followed by stearic acid which was present in four out of the seven fractions. However, from the result of the toxicity study all the extracts appeared toxic with values less than 100µg/ml except for chloroform extracts of *C. papaya* which presents LC_{5O} value of 11,651.01µg/ml.

CHAPTER ONE

1.0 INTRODUCTION

1.1Background

Demand for most befitting and affordable alternatives in the face of increasing antibacterial drug resistance has led researchers into exploring the use of plant extracts in the treatment of infections (*Akujobi et al.,2001).* In Brazil alone, about 80,000 species of higher plants were described which offer tremendous prospects for discovering new compounds with therapeutic property (Nakaruma*et al.,* 1999). Alternative herbal medicine has been used to treat various infections for decades. Natural plants contain phyto-constituents having similar chemical properties as of synthetic antibiotics. In northern Nigeria, combination of leaves extract of *Mangifera indica*, *Psidium guajava*, *Citrus aurantifolia,* and *Carica papaya* in the treatment of typhoid fever infection could be traced to the first inhabitants of the region and has been proven effective; though not scientifically, as a "therapy of sorts" in the treatment of typhoid fever infection. Bacteria could be killed by the rupture of cell walls and membranes and by the irregular disruption of the intracellular matrix when treated with plant extracts (Kim and Fung, 2004).

Typhoid fever is a serious infection and failure of its treatment results from multi-drug resistant (MDR) bugs of *Salmonella typhi*. Due to multiple and repeated issues with antibiotics efficacy, it became essential to evaluate biological properties of plants extracts (Hannan *et al.,* 2013).

The antimicrobial properties of plants have been investigated by a number of studies and researches worldwide among which many of them have been used as therapeutic alternatives because of their antimicrobial properties (Adriana *et al.,* 2007). Plants have many antimicrobial

properties associated with secondary metabolites such as alkaloids, phenolic compounds, etc. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections (Vijayan et al., 2004; Dilhuydy, 2003).

Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics (Shiota *et al.,* 2004 and Abu-shanab 2004). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies (Zy *etal.*, 2005; Rojas *et al.,* 2006). Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes and researches should be carried out to analyze scientifically those constituents or phytochemical compounds responsible for antimicrobial properties in our traditional medication.

1.2 Statement of Research Problem

The use of medicinal plants has always been part of human culture and is wide spread in Africa. In some countries, like Ghana, government encourages the use of indigenous forms of medicine rather than expensive imported drugs. Also in Nigeria, a large percentage of the populace depends on herbal medicines because the commercially available orthodox medicines are becoming increasingly expensive and out of reach (Lawal *et al.,* 2012).

1.3 Justification

While a number of synthetic and natural antibacterial agents are available for controlling bacterial infections, increased resistance calls for new antibacterial drugs, one source of which

are traditional medicinal plants (Green, 2004. Many medicinal plants around the world contain many compounds with antibacterial activity (Marjorie, 1999). Moreover, to many communities in the developing countries, antibacterial pharmaceuticals are not accessible to the majority of the people who need them. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies (Zy *et al.,* 2005; Rojas *et al.,* 2006).

These issues and lots more calls for the research to prove scientifically the antimicrobial activity or otherwise of herbal remedies including leaves extracts of *Mangifera indica*, *Psidium guajava*, *Citrus aurantifolia*and *Carica papaya* used in the treatment of typhoid fever, in northern Nigeria. This is simply because the practice has been in place since prehistoric times in the region and the herbs are used by typhoid fever patients especially the economically disadvantaged group (the poor).

1.4 Hypothesis

H_1 Leaves of*Carica papaya, Citrus aurantifolia*,*Mangifera indica* and *Psidium guajava* used in folk medicine in northern Nigeria have antibacterial activity

H_2 Leaves of *Carica papaya, Citrus aurantifolia*,*Mangifera indica* and *Psidium guajava* used in traditional medicine in northern Nigeria are safe for consumption.

1.5 Aim of the Study

The aim of this research is to determine the antibacterial property of leaves extract of Guava (*Psidium guajava*), lemon (*Citrus aurantifolia*), mango (*Mangifera indica*) and pawpaw (*Carica papaya*) against clinical isolates of *Salmonella typhi and Salmonella paratyphi*.

1.6 Objectives of the Study

1. To detect the phytochemical componentsof the plants using various solvents (chloroform, ethanol and water)
2. To determine the antibacterial property of the plant leaves
3. To separate the phytochemical component and determine the most bioactive using sensitivity test
4. To determine toxicity status of the extracts using Brine Shrimp Lethality Assay (BSLA).

1.7 Research Question

i. What type of phytochemicals can be found in the leaves of *Psidium guajava*, *Citrus aurantifolia*,*Mangifera indica* and *Carica papaya and* at what concentration*?*

ii. Do the leaves of the above plants have any antibacterial activity?

iii. What are the active components responsible for the activity?

iv. Are the leaves safe for consumption?

CHAPTER TWO

2.0 LITERATURE REVIEW

2. 1 Phytochemicals

Phytochemicals are certain non-nutritive plant chemicals which have some disease preventive properties. They are not required by the human body for life sustenance, but they offer protection against pathogens (Kokate *et al.,* 2006). There are different ways in which a phytochemical can work. It can act as an antioxidant and protect cells against free radical damage, eg. polyphenols, carotenoids and so on. It can stimulate certain enzymes, thereby reducing risk for breast cancer, eg. terpenes. It may act as an anti-bacterial and hormonal-stimulant component. It may even act as binders which may prevent the adhesion of pathogens to the human cell walls (Phytochemical, 2011). Phytochemicals are already a part of our diet through vegetables and fruits. Citrus fruits are found to be rich in phytoconstituents (Disease education, 2011).

2. 2 Guava (*Psidium guajava*)

The guava (*Psidium guajava*) is a phytotherapic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases. The many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions (Abdelrahim*et al.,* 2002, Scopus*et al.,* 1999a and Scopus*et al.,* 1992). This plant has also been used for the controlling of life-changing conditions such as diabetes, hyp ertension, and obesity (Abdelrahim*et al.,* 2002) and (Sunagawa*et al.,* 2004)

The genus *Psidium* belongs to the family Myrtaceae, which is considered to have originated in tropical South America. Guava crops are grown in tropical and subtropical areas of the world

like Asia, Egypt, Hawaii, Florida, Palestine, and others. The genus *Psidium* comprises approximately 150 species of small trees and shrubs in which only 20 species produce edible fruits and the rest are wild with inferior quality of fruits (Scopus*et al.,* 2011). The most commonly cultivated species of *Psidium* is *P. guajava* L. which is the common guava. Other species are utilized for regulation of vigor, fruit quality improvement and resistance to pest and disease (Scopus*et al.,* 2011). Guava fruit today is considered minor in terms of commercial world trade, but it is widely grown in the tropics, enriching the diet of hundreds of millions of people in those areas of the world.

2.2.1 Phytochemistry

Phytochemicals are nonnutritive chemicals produced by plants for their own protection, but they have been found to protect humans against diseases through recent research. Scientists have identified thousandsofphytochemicals, although only small fractions have been studied closely and each one works differently (Yadav and Agarawala, 2011). Begum *et al.,* (2002) reported the isolation of two triterpenoids: guavanoic acid and guavacoumaric acid from the leaves of guava. Four flavonoids were isolated and identified by Arima and Danno (2002) which were found to inhibit the growth of *Salmonella enteritidis* and *Bacilluscereus*. A study was done to evaluate the spasmolytic activity quercetin" is responsible for spasmolytic activities, which is formed when flavonoids of guava leaves are hydrolyzed by the gastrointestinal fluids.

Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fibre and fatty acids. Guava fruit is higher in vitamin C than citrus fruits (80 mg of vitamin C in 100g of fruit) and contains appreciable amounts of Vitamin A as well. Guava fruits are also a good source of pectin (Sunttornusk, 2002).

The leaves of guava are rich in flavonoids, particularly quercetin. It has demonstrated

antibacterial and anti-diarrheal effects and is able to relax the intestinal smooth muscle and inhibit bowel contractions. Guava has antioxidant properties attributed to polyphenols found in its leaves. The bark of guava tree contains considerable amounts of tannins (11-27%), and hence is used for tanning and dyeing purposes (Begum *et al.,* 2004). Leucocyanidin, luectic acid, ellagic acid and amritoside have been isolated from the stem bark. Five constituents, including one new pentacyclictriterpenoid:guajanoic acid and four known compounds-beta-sitosterol, uvaol, oleanolic acid and ursolic acid, have been recently isolated from the leaves of *P. guajava* (Begum *et al.,* 2004).

2.2.2 Extraction procedure

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, Soxhlet extraction, aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction, supercritical fluid extraction, and phytonic extraction.Maceration extraction is crude extraction; solvents diffuse into solid plant material and solubilize compounds with similar polarity (Green, 2004). Effect of plant material depends on its origin, variations in the extraction technique, the time, temperature of extraction, solvent concentration and polarity, quantity, and secondary metabolite composition of an extract (Sofowora, 1993). Variations in extraction methods are usually found in the length of the extraction period, the solvent used pH, temperature, particle size, and the solvent-to-sample ratio (Ncube *et al.,* 2015).

2.2.3 Antibacterial Activity

Antibacterial screening has been done selectively by many researchers in guava essential oil and solvent extract (Kim and Fung, 2004, Jaiarj *et al.,* 1999, Ibrahim *et al.,* 2011 and Hogue *et al.,* 2007). The mechanism by which they can inhibit the microorganisms can involve different

modes of action. It has been reported that these oils and extracts penetrate the lipid bilayer of the cell membrane, rendering it more permeable, leading to the leakage of vital cell contents (Burt, 2004 and Juven, 1999). Sanches *et al.,* (2005) evaluated the antibacterial activities of *guava* against gram-positive and gram-negative bacteria testing ethanol and water extract of *P. guajava* leaves, stem, bark and root, and aqueous extract against *Staphylococcusaureus* were found to be more active by using ethanol and water extract than with just aqueous extract Kim and Fung, 2004, Karawya *et al.,* 1999). Sacchetti *et al.,* (2005) reported that the oil showed a strong resistance against *Yarrowia lipolytica* which is a pathogenic yeast. Vieira *et al.,* (2001) have also reported the antibacterial effect of guava leaves extracts and found that they inhibited the growth of the *S. aureus*. Gnan and Demello (1999) testing guava leaf extract found good antimicrobial activity against nine different strains of *Staphylococcus aureus*. The antibacterial activity of guava leaf extract was tested against acne developing organisms by Qa'dan *et al.,* (2005) concluding that the leaf extracts may be beneficial in treating acne especially when they are known to have anti-inflammatory activities.

2.2.4 Toxicity status

Recently Elekava *et al.,* (2009) revealed that *Psidium guajava* acclaimed as poor man's apple of the tropics has a long history of traditional use for a wide range of diseases. The brine shrimp *Artemia salina* aquatic (Leach) (Artemiidae) is an invertebrate component of saline aquatic and marine ecosystems used in laboratory bioassay of toxicity and other biological actions through estimation of medium lethal concentrations (LC50 values). The brine shrimp lethality (BSL) bioassay has been shown to be useful and quick invitro test for predicting toxicity of plant extracts and guiding their phytochemical fractionation (Fatope *et al.,* 1993; Parra *et al.,* 2001and Begum *et al.,* 2002a). It has been previously that the crude extracts of the leaves of the plant are

very active using BSL bioassay (Ayo *et al.,* 2007; Oyewale *et al.,* 2004), but the active constituents of the leaves were not isolated. There is paucity of information in the available literature on the chemical compounds isolated from the leaves of *P.guava* (L.) and their extracted hyperoside compounds act towards biological activities especially cytotoxicity Lin-lin*et al.,* 2007.

2.2.5 GCMS analysis

Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structure determination of phytochemicals (Roberts and Xia, 1995).

2.3 Mango (*Mangifera indica*)

The mango is a juicy stone fruit belonging to the genus *Mangifera*, consisting of numerous tropical fruiting trees, cultivated mostly for edible fruit. The majority of these species are found in nature as wild mangoes. They all belong to the flowering plant family Anacardiaceae. The mango is native to South and Southeast Asia, from where it has been distributed worldwide to become one of the most cultivated fruits in the tropics. The highest concentration of *Mangifera* genus is in the western part of Malaysia (Sumatra, Java and Borneo) and in Burma and India (Morton, 1987).

While other *Mangifera* species (e.g. horse mango, *M. foetida*) are also grown on a more localized basis, *Mangifera indica*—the "common mango" or "Indian mango"—is the only mango tree commonly cultivated in many tropical and subtropical regions. It originated in Indian subcontinent (present day India and Pakistan) and Burma (Kostermans and Bompard, 1993).

It is the national fruit of India, Pakistan, and the Philippines, and the national tree of Bangladesh bdnews24, 2013). In several cultures, its fruit and leaves are ritually used as floral decorations at weddings, public celebrations, and religious ceremonies (Suiltan, 2009).

2.3.1 Phytochemistry

Numerous phytochemicals are present in mango leaves, peel and pulp, such as the triterpene, lupeol which is under basic research for its potential biological effects (Chaturvedi *et al.,* 2008). An extract of mango branch bark called Vimang, containing numerous polyphenols (Rodeiro *et al.,* 2006) has been studied in elderly humans (Pardo-Andreu *et al.,* 2006).

Mango peel pigments under study include carotenoids, such as the provitamin A compound, beta-carotene, lutein and alpha-carotene (Berardini *et al.,* 2005, Gouado*et al.,* 2007) and polyphenols, such as quercetin, kaempferol, gallic acid, caffeic acid, catechins and tannins (Mahattanatawee *et al.,* 2006), (Singh *et al.,* 2004). Mango contains a unique xanthonoid called mangiferin (Andreu *et al.,* 2005).

Phytochemical and nutrient content appears to vary across mango cultivars (Rocha *et al.,* 2007). Up to 25 different carotenoids have been isolated from mango pulp, the densest of which was beta-carotene, which accounts for the yellow-orange pigmentation of most mango cultivars (Chen *et al.,* 2004). Mango leaves also have significant polyphenol content, including xanthonoids, mangiferin and gallic acid (Barreto *et al.,* 2008).

The pigment euxanthin, known as Indian yellow, is often thought to be produced from the urine of cattle fed mango leaves; the practice is described as having been outlawed in 1908 due to malnutrition of the cattle and possible urushiol poisoning (Kühn, 2013). This supposed origin of

euxanthin appears to rely on a single, anecdotal source, and Indian legal records do not outlaw such a practice (Finlay, 2003).

2.3.2 Antibacterial activity

The leaves contain the glucoside mangiferine. The bark of the mango tree contains 16–20% tannin (Wauters *et al.,*1995; Dweck, 2001). The leaves have been reported to contain saponins, glycosides, unsaturated sterols, polyphenols, euxanthin acid, mangiferine, mangin, gallic tannins, etc. The ashes of the leaves are used to treat burns, scalds, sores, cough and diarrhoea in South America and other parts of the world (Dweck, 2001; Hirte, 2002). The use of leaf extracts as antiseptics in the treatment of burns, scalds, sores, wounds, abscesses and other infections in humans and animals has been reported in a number of ethnobotanical surveys (Nadan, 2003; Duke, 1997; Bukenya and Kamoga, 2003; Tabuti *et al.,* 2003). This study investigated the antibacterial activity of leaf extracts of M. indica plants against the micro-organisms Escherichia coli [American Type Culture Collection (ATCC) 25922], Pseudomonas aeruginosa (ATCC 27853) and Staphylococcus aureus (ATCC 25923).

2.4 Pawpaw (*Carica papaya*)

Carica papaya (papaya) is a tree-like herbaceous plant, a member of the small family Caricaceae and widely cultivated for its edible fruits. It originates in the lowlands of eastern Central America, from Mexico to Panama, and can be found in all tropical countries and many sub-tropical regions of the world. Parts of the plant are used in tropical diets as a fruit or vegetable; it is sometime used as a therapeutic remedy for several of its medicinal properties. Papaya fruit is thought to contain some immuno-stimulating and anti-oxidant agents (Aruoma *et al.,* 2006; Mehdipour *et al.,* 2006). Immature fruits and roots are used for their abortifacient activity (Cherian, 2000; Sarma and Mahanta, 2000); the seeds are now being used as a potential post-testicular anti-fertility drug (Lohiya *et al.,*1992); the pulp is used by African hospitals for treating wounds and burns (Starley *et al.,* 1999); the latex and the seeds are used in the care of gastrointestinal nematode infections and they have shown anthelmintic activity (Stepek *et al.,* 2004); and the seeds and immature fruit have shown bacteriostatic activity against the human enteric pathogens (Osato *et al.,* 1993). The leaves are used to relieve the symptoms of asthma and as a vermifuge, in the treatment of gastric problems, fever and amoebic dysentery. Methanolic leaf extract demonstrated vasodilatatory and anti-oxidant effects, both implicated in the reduction of cardiovascular risks (Runnie *et al.,* 2004). The aqueous extract showed beneficial effects for the acceleration of wound healing processes in rats (Mahmood,2009).

2.4.2 Phytochemistry

Carica papaya plants produce natural compounds (annonaceousacetogenins) in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties. It was suggested that a potentially lucrative industry based simply on production of plant biomass could develop for production of anti-cancer drugs, pending Food and Drug Agency approval, and natural

(botanical) pesticides (McLanghlin, 1992). The high level of natural self-defence compounds in the tree makes it highly resistant to insect and disease infestation (Peter, 1991). *Carica papaya L*. leaf tea or extract has a reputation as a tumour-destroying agent. (Walter, 2008) The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present. Aside from its value as a remedy in dyspepsia and kindred ailments, it has been utilized for the clarification of beer. The juice has been in use on meat to make it tender, (Wilson, 1994). The seed is used for intestinal worms when chewed. The root is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases. The unripe fruit is used as a remedy for ulcer and impotence, (Elizabeth, 1994). Fresh, green pawpaw leaf is an antiseptic, whilst the brown, dried pawpaw leaf is the best as a tonic and blood purifier (Atta and Bonsu,1999). Chewing the seeds of ripe pawpaw fruit also helps to clear nasal congestion, (Elizabeth,1994). The green unripe pawpaw has a therapeutic value due to its antiseptic quality. It cleans the intestines from bacteria, more so that (only a healthy intestine is able to absorb vitamin and minerals, especially vitamin B12). The tea, prepared with the green papaya leaf, promotes digestion and aids the in treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Mantok,2005).

2.5 Lime (*Citrus aurantifolia*)

Citrus aurantifolia is a member of the Rutaceae family. It is an ever green tree which can reach up to 5m high. The leaves are medium sized measuring 6-9cm long, ovate, bluntly pointed at tips, rounded to cunate at the base. The flowers are white, solitary or in a short recemes, small and fragrant. The fruits are yellow when ripe, globose measuring 4-5cm in a diameter with thinner rind and very sour (Khan*et al.,*2010).

2.5.1 Plant part used

Fruits, leaves, flowers, bark and roots (Khan *et al.,* 2010, Khare, 2007, Kunow, 2003).

2.5.2 Antimicrobial activity

Citrus aurantifolia root extract was amongst those found to be effective in inhibiting the growth of *S. aureus*, *K. pneumonia*, *P. mirabilis*, *P. aeruginosa*, B*eta-haemolytic streptococci*, *E. coli*, and *N. gonorrheae*. The fruit on the other hand was found to inhibit facultative unaerobic bacteria i.e. *S. aureus* ATCC 25213, *S. aureus*, *S. paratyphi*, *S. flexnerii*, *S. faecalis*, *Citrobacter spp*, *Serratia spp*, *K. pneumoniae*, *P. aeruginosa*, *E. coli* ATCC 25922, and *E. coli*. *Citrus aurantifolia* proved to have antimycobacterial activity especially against isoniazid resistant strain (Ebane *et al.,* 1991, Albinu *et al.,* 2006, Camacho-Corona *et al.,* 2008 and Rahman *et al.,* 2011).

2.5.3 Cytotoxic Activity

Two studies done on the anticancer activity of concentrated lime juice found that it could effectively inhibit the growth of human lymphoblastoid B cell line (RPMI-8866) and human pancreatic cancer cells (Panc-28 cells). In the case of RPMI 8866 cells it was concluded that is activity is attributed to the presence of biologically active macromolecules, while in Panc-28 cells the apoptosis was inducted by the presence of flavonoids and limonoids (Gharagozloo*et al.,* 2002).

2.5.4 Toxicities

Expressed lime oil contains certain constituted coumarins which are known to cause phototoxicity in humans. It has also been found to promote tumour formation on the skin and forestomach epithelium of mice caused by 9, 10-dimethyl (-1, 2-benxanthracene) and benzo-[a]-pyrene, repectively. The ditilled lime oil is devoid of these toxic activities (Khan, 2010).

2.5.5 Antioxidant activity

The essential oil of *Citrus aurantifolia* has significant antioxidant activity, the highest among 3 citrus species tested. The juice and peel extracts also showed antioxidant activity in a dose dependent manner (Tundis *et al.,* 2012, Boshtam *et al.,* 2011).

2.5.6 Traditional use

Citrus aurantifolia is considered antiscorbutic, stomachic, appetizer, refrigerant, and febrifuge. It is bitter, sour and cooling and dissipates blocked energy, expectorant, mucolytic, diuretic, diaphoretic, and digestive. In traditional Malay medicine, the juice fruit of C. aurantifolia is much revered as a solvent for many concoctions. It forms the medium for dissolving active principles from plants parts into the medicine (Globinmed, 2015).

C. aurantifolia is widespread in tropical and subtropical regions around the World such as North America (Florida, Texas, California, Mexico, *etc*.), India, Egypt, and Central America (Morton, 1987). Lime essential oils are not only used as flavoring agents in beverages, manufactured foods, and pharmaceutical forms, but also as ingredients in perfumes (Morton, 1987). Additionally, *C. aurantifolia* is used in traditional medicine as an antiseptic, anthelmintic, mosquito bite repellent, for stomach ailments, tonic, antiscorbutic, astringent, diuretic, headache, arthritis, digestive and appetite stimulant, and for colds, coughs and sore throats (Morton, 1987, Apraj *et al.,* 2011). Previous investigations of *C. aurantifolia* have reported flavonoids,

coumarins, and terpenoids (Ferger *et al.,* 2000, Johann *et al.,* 2007, Piccinelli *et al.,* 2008 and Jiwajinda, 2000). Peel oil of *C. auranifolia* has been analyzed by GC-MS analysis several times (Afolayan and Asekun, 2008). Lime peel oil has shown antimicrobial activity Jafari *et al.,* (2011) radical scavenging, anti-cholinesterase Tundis *et al.,* (2012), anthelmintic Taur *et al.,* (2009), and anticancer activities (Gharagozloo, 2002). Furthermore, leaves of limeshowed protective effect against osteoporosis Shalaby *et al.*, (2011) and induced platelet aggregation (Piccinelli *et al.,* 2008).

2.6 Salmonella

Salmonella is one of the members of Entrobacteriaceae family. They are gram-negative, oxidase negative, non-spore forming, facultative anaerobic, rod shape and motile by peritrichous flagella. The cell wall of Salmonella compounds the structure of lipids, polysaccharides, protein and lipoproteins. The lipopolysaccharide portion of the cell wall and lipid A is endotoxin. Endotoxin is responsible for the biological effects. The common 3 center monosaccharaides and polysaccharides of endotoxin are also called somatic O antigens. Salmonella has about 60 of O antigens that are nominated by numbers. Furthermore, there are some unlike flagella (H) antigens that they are recognized by numbers and letters. Based on these somatic antigens, Salmonella may be divided into groups which are using specific antisera. By using somatic and flagella agglutination reactions almost 2500 (2483 in the year 2000 REF) serovars have been recognized. The Kauffman-White classification shows that majority of the Salmonella involved in human disease belong to groups A, B, C, C2, D and E. Salmonella Typhi hold a capsular antigen called the virulence antigen which envelops the cell wall. Salmonella grows readily on blood agar, MacConkey agar or Eosin-Methylene blue. Bismuth Sulfate agar or desoxycholate

agar should be used for the identification of Salmonella and it (Salmonella) ferments glucose and mannose but not lactose or sucrose (Hawkey, 1998).

2.6.1 Epidemiology of Salmonella

Salmonella can stay alive for long times in the environment even if no important growth occurs. Infections in wild fauna like as rodents are usually secondary to the illness of farm animals although it can continue the infection cycles. The control of Salmonella is required to decrease the number of organisms that are discharged into the environment. Water and soil are also a part of the epidemiological cycle and can transfer bacteria to vegetables and herbs. The most high risk food categories which cause salmonellosis are raw or undercooked meat, eggs and products containing raw eggs, unpasteurized milk, and juices (Plym and Wierup, 2006).

2.6.2 Sources of infection

Dramatic increasing in *S.* Enteritidis isolates from outbreaks and sporadic salmonelloses cases in people has started an international debate on sources and spreading pathways of this microorganism. Although poultry meat represents the main infection source of *S.* Enteritidis, epidemiologic data suggest that eggs are the source of infection of great importance (Rabsch, 2007). Salmonellae survive very easily in eggs if there are some irregularities in preparing, cooking and storing the food made of these ingredients, and the scale of infection spreading is amplified in case of centralized food preparation.. The international poultry meat trading, particularly contributes to spreading of infections caused by *S.* Enteritidis due to the fact that it allows introducing of virulence clones into new geographical area.

The presence of *S.* Enteritidis in class A eggs is linked with infection of chickens, in the ovaries and oviducts of which bacteria can be found responsible for contamination of eggs before eggshell is formed. Chickens could be infected by contaminated food of animal origin, while the

contributing factor is stress, caused by irregular intake of food and water. *S.* Enteritidis could be spread by vertical transmission as well as through the contact with rodents, insects, wild birds, domestic animals, people, and waste material. Often, *S.* Enteritidis could be isolated from contaminated environment of infected flocks. This type of salmonella has been evidenced in Canada in 1%, and in 2.7% of environmental samples (Poppe, 1991). Identification of infected poultry is a special issue due to difficulties in salmonella identification by cultivation of cloacal swab, and determination of serum antibodies in birds is followed by technical difficulties in particular.

2.6.3 Virulence factors

Salmonellae could cause different types of diseases, starting with acute enterocolitis to the typhoid fever. Once salmonella enters the ileum, it interacts with the mucosa above the mesenterial lymph node. Bacterium runs through the intestinal epithelium inside the vacuole by transcytosis. In order for microorganism to get inside the host cell, the increase of ribonucleic acid (RNA) synthesis starts as well as creation of bacterial outer membrane proteins essential for the invasion. As a part of a global regulatory network, these proteins are created through the induction in epithelial cells probably, and they are essential for intracellular bacteria survival. During the contact between salmonella and the top surfaces of epithelial cells, it causes the loss of apical, epithelial microvilli and a smaller disruption in intercellular connections partially due to bacterial cell wall lipopolysaccharide (Ohl, 2001).

In some cases, salmonella could pass through the basal membrane of the intestinal tract mucosa. Once salmonella has reached monocytomacrophagic system, it survives, multiplies, and disseminates throughout other tissues, causing typhus fever. Intracellular survival and multiplying is possible due to salmonella ability to use purine and aromatic compounds, while

the presence of the iron siderophor (enterochelin) does not seem to be necessary for the process (Ohl, 2001).

There are numerous salmonella virulence factors as adhesins, toxins, virulence plasmids, cell wall lipopolysaccharides.

2.6.3.1 Adhesins

The exact role of adherence factors (fimbriae type 1 and mannose-resistant hemagglutinin) and flagella still remains unknown. Flagella are not necessary in colonization of the gastrointestinal tract, but they are essential for growth and survival in the host spleen and liver. Flagella either prevent macrophages to destroy microorganisms or contribute to the intracellular multiplication in macrophages (Ohl, 2001).

2.6.3.2 Toxins

It is considered that salmonella have the ability to produce at least three types of toxic substances. A thermolabile enterotoxin is one of these, and it binds to gangliosides, increases the level of intracellular cyclic adenosine monophosphate

(cAMP), and intensifies the liquid secretion. The second one is cytotoxin, a non – lipopolysaccharidic component of the outer membrane, which inhibits the protein synthesis in eukaryotes leading to the elongation of tissue culture cells – CHO (Chinese Hamster Ovary) cells. Endotoxin, lipid A, a component of the cell wall lipopolysaccharide, activates macrophages and lymphocytes, and consequently triggers a series of biological effects: fever, leukocytosis, lowering of blood pressure (Ohl, 2001).

2.7 Transmission

Salmonella is mostly transferred to animals, food and environment (water, crops) by fecal shedding. Faucal or intestinal contagion of carcasses is the main resource of human foodborne

infections. It is the exception when pathogen is directly transmitted into the food product, such as S. Enteritidis into eggs and sometimes other serovars into milk. Humans excrete the bacteria as animals do. Excreted bacteria infect other animals on the farm and can transmit to rodents and other wild fauna live near humans or domestic animals (WHO, 2007).

2.8 Prevention of infection

Preventive measures based on elimination of broken eggs or washing of eggshell, are not sufficient in cases where eggs are contaminated before the formation of eggshell which wraps yolk and albumen. Also, a measure like prohibition of egg consumption from symptomatically infected hens is not completely efficient, because infection in hens could proceed asymptomatically (Rodrigue, 1990).

Efficient preventive measures would be the change in poultry meat preparation, investigation of flocks related with outbreaks and their extermination and eggs pasteurization. Additionally, it is important to store eggs in the refrigerator as well as to avoid pooling the eggs prior to meal preparation. Note that persons particularly susceptible to *S.* Enteritidis infections (children, elderly, pregnant women, immunocompromised, as well as HIV-positive individuals) should avoid the consumption of raw eggs and insufficiently thermally treated meals containing eggs. For these risk subgroups, it is strongly recommended to consume pasteurized eggs and pasteurized eggs products.

2.9 Control of Salmonella

In 1980, World Health Organization (WHO) formulated three steps of control of Salmonella which still involve valid planned approaches to risk mitigation:

- First step is the Food-Producing Animal Regulations (pre-harvest control).

- Second step is the hygiene improvements during the slaughtering and additional processing of the meat (harvest control).
- Third step is the final preparation of food by training and education of the food processing industries and consumers about good hygiene practices (post-harvest control).

Since any serovar is a possible risk to human health, prevention of food-borne salmonellosis should be directed at all serovars of Salmonella. Though, a Salmonella reduction plan which is limited to a few elected serovars could also have a protective effect on most other serovars because of similar way of transmission. When a plan is implemented, a surveillance program will be required to notify the frequency of zoonotic serovars. Without interventions in the early stages of food or animal production, there is a chance of wide spread infection that could result in epidemic proportions in a short time. Networking between producers is a useful way to prevent respiratory and enteric infections in pig production, and establish appropriate methods for limiting the risk of Salmonella (Mathew *et al.,* 2007).

One way of controlling Salmonella is by using antibiotic in animals for four purposes:

1) For healing the sick animals

2) For Metaphylaxis , treatment and prevent disease

3) Prophylactic, prevention in times of risk such as transportation or weaning

4) Growth encouragement to the progress supply and product

Using of antibiotic in human and animals lead to resistance and monitoring program is necessary also (Mathew *et al.,* 2007).

2.10 Treatment of Salmonella

Fluoroquinolones are usually regarded as the first line treatment of salmonellosis in adults. They are fairly inexpensive, well tolerated, good oral absorption and effective on the majority of

Salmonella strains. Third-generation cephalosporin is used for children with severe infections. Chloramphenicol, Ampicillin and Trimethoprim-Sulfamethoxazole are infrequently used as alternatives but have either more side-effects. The MDR Salmonella strains with resistance to fluoroquinolone and Third-generation cephalosporin can still be treated with other antibiotics but they are generally more dangerous and expensive (WHO, 2009).

Control of antibiotic resistant Salmonella is most efficiently through the reduction the consumption antibiotic. Control of Animal feed, husbandry, hygiene in abattoir routinely, sanitation at all stages and food services are ways to minimize the need for antibiotic treatment(WHO, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Sample Collection

The plant materials (leaves) were collected from Bayero University, Old campus, Kano, Nigeria. Their identity was comfirmed by a botanist, in the Department of Plant Biology, Bayero university, Kano andvoucher specimens were deposited in the Institute's herbarium. The leaves after collection were air-dried at room temperature for 3 weeks. Dry leaves were then pounded in a porcelain mortar to a fine powder for ease of extraction of active compounds (Aliyu, 2006). The voucher numbers are: BUKHAN 0336 *for Psidium guajava,* BUKHAN 0113 for *Citrus aurantifolia,* BUKHAN 0348 for *Mangifera indica* and BUKHAN 0012 for *Carica papaya.*

3.2 Plants Extraction

Fifty grams (50g) each of the dried powder of the plants leaves was weighed into 3 different bottles of 1 liter capacity and sequentially extracted with 250ml each for Ethanol and chloroform and 500ml for aqueous by percolation method for one week, during which the bottles were undergoing shaking at regular intervals. The extracts were filtered using Whatman No. 1 filter paper. Each of the resulting filtrate was then concentrated by complete evaporation of solvent at room temperature except for aqueous extract which was evaporated in a water bath at 45^{o}C. The filtratewas carefully labeled, weighedand transferred into sterile air tight bottles after which it was stored in the refrigeratorfor further use (Fatope *et al.,* 1993).

The percentage yield of each extract was calculated from the respective weights of the extracts using the formula:

$$\text{Percentage Yield} = \frac{Mass\ of\ the\ extract}{Total\ mass\ of\ sample\ extracted} X\ 100$$

Other physical parameters such as color, odor and texture of the extract were also recorded.

3.3 Preparation of Crude Extract

Stock solutions of each crude extract was prepared by weighing 0.001g of each extract on a meter balance and dissolved in 1ml 10% Dimethyl sulfoxide (DMSO) to give a concentration of 1000µg/ml. Four different concentrations were prepared from each stock solution viz: 400µg/ml, 200µg/ml, 100µg/ml and 50µg/ml. Each solution was stored in a refrigerator after being collected into clean sterile glass bottle for subsequent use.

3.4 Phytochemical Analyses

Phytochemical analyses (qualitative) for the screening and identification of bioactive chemical constituents in the leaves extract was carried out using standard procedures as follows:

3.4.1 Test for Flavonoids

Four milliliter of the extract and a piece of magnesium ribbon was added followed by drop-wise addition of conc. HCL. A color change from orange to red indicates the presence of flavonoids (Sofowara, 1993).

3.4.2 Test for Tannins

Two milliliter of the extract was diluted with distil water in separate testubes, 2-3 drops of 5% Ferric chloride ($FeCl_3$) solution was added. A green-black coloration indicates the presence of tannins (Ciulci, 1994).

3.4.3 Test for Steroids

In a testube 1 ml of concentrated H_2SO_4 was added to the extract. The solution was mixed with 2ml of water. A red color indicates the presence of steroids (Evans and Trease, 1999; Cannel, 2000).

3.4.4 Test for Glycosides

To 1ml of the extracts 10ml of 50% H_2SO_4 was added in separate testubes and the mixture heated for 15minutes followed by addition of 10ml of Fehling's solution and boiled. A brick red precipitate indicates the presence of Glycosides (Sofowara, 1993).

3.4.5 Test for Alkaloids

To 0.1ml of the extract and fraction in a testube, 2-3 drops of Dragendoff's reagent was added. Absence of orange precipitate indicates absence of alkaloids (Ciulci, 1994).

3.4.6 Test for Saponins

To 2ml of the extract 5ml of distill water was added and shaken vigorously. Formation of foam following shaking indicates the presence of saponins (Brain and Turner, 1975).

3.4.7 Test for Terpenoids

Extract was mixed with 2 mL of chloroform. Then 2 mL of concentrated sulfuric acid was added carefully and shaken gently. A reddish brown coloration of the interphase formation show positive results for the presence of terpenoids (Harborne, 1973).

3.4.8 Test for Phenols

Ferric Chloride Test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

3.4.9 Test for anthraquinones

A few drops ofmagnesium acetate solutions were added to the test solution of the extract. Formation of pink color indicated the presence of anthroquinones.

3.4.10 Test for Xanthoproteins

To the test solution of the extract a few drops of concentrated nitric acid and a few ml of ammonia were added. Appearance of a red precipitate indicated the presence of xanthoproteins.

3.5 Bioassay Studies

3.5.1 Preparation of Media

Mueller Hinton Agar was prepared by dissolved 38g in 1000 liters of distilled water.The media was autoclaved and dispensed into sterile petri-dishes which were allowed to gel. And Nutrient broth was prepared according to manufactures specification.

3.5.2 Test Organisms

Bacteria comprising *Salmonella typhi*, *Salmonella paratyphiA*, and *Salmonella paratyphi B*, were collected to test their sensitivity or otherwise to the extracts while *Salmonella paratyphi C*was not available as at the time when the sample was collected. All isolates were obtained from Department of Microbiology, Aminu Kano Teaching Hospital, Kano, Nigeria.

3.5.3 Identification of Bacteria

The test bacterial identity was confirmed using various biochemical and serological tests as described by Cheesbrough, (2006).

3.5.4 Standardization of Inoculum

Using sterile inoculation wire loop, 2-3 coloniesfrom an overnight culture of the test organism was transferred into a tube of saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National Committee for Clinical Laboratory Standard (NCCLS, 2008).

3.5.5 Antimicrobial Susceptibility Test

The agar well diffusion method as described by Nester *et al.,* (2004) was used for the antimicrobial susceptibility test. A loop full of the standardized inoculum was streaked on the agar plate. Four wells of 6mm each was made in each plate using a sterile cork borer. The wells were filled with 0.1ml of diluted concentrations (400µ/ml, 200µ/ml, 100µ/ml and 50µ/ml) of the extract with the aid of sterile pipettes per well. Likewise, 400µ/ml, 200µ/ml, 100µ/ml and 50µ/ml of the standard antibiotic (amoxicillin) were used in separate plates to serve as positive control. While sterile distilled water was used as negative control on separate plates. The plates were allowed to stand for 15 minutes on a Table to allow free diffusion of the extracts. Diameters of zones of inhibition were measured using transparent plastic ruler after 24 hours of incubation at 37^{o}C (Dahiru *et al.,* 2013).

3.5.6 Determination of Minimum Inhibitory Concentration of the Extract (MIC)

Plants extract that showed activity in the agar well diffusion method were considered for the determination of Minimum Inhibitory Concentration (MIC).The extract was prepared by serial doubling dilution using Dimethyl Sulfoxide (DMSO) to obtain concentrations of 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml and 6.25µg/ml. A stock solution of the extract and that of amoxicillin were serially diluted in testubes containing double strength Nutrient broth. Equal volume of the extract in a nutrient broth (i.e. 2ml each) was dispensed into sterilized testubes. Specifically 0.1 ml of the standardized inoculum was added to each of the test tubes above. Tube containing broth and extracts without inocula serve as a positive control while tubes containing broth and inocula without extract served as negative control. The tubes were incubated at 37^{o}C for 24 hours and observed for the least concentration without turbidity (Fatope, 1994). MIC was recorded as lowest concentration of the extract inhibiting the visible growth of the bacteria. This was carried out by comparing the tubes with the control tubes against a source of light with white background and some contrasting black lines.

3.5.7 Determinationof Minimum Bactericidal Cconcentration (MBC)

MBC was determined by inoculating samples from the MIC tubes that showed no bacterial growth on Mueller Hilton agar plates separately and then incubated at 37^{o}C for 24 hours. After the incubation the plates were observed for presence or absence of growth. The least concentration of the extract that showed no bacterial growth was considered as the MBC.

3.6 Preparative Thin Layer Chromatography

Preparative thin layer chromatography was carried out on both the ethanolic, chloroform, and aqueous extract in order to find the best solvent system that could fractionate the extracts in the main analytical TLC. Each of the extract was subjected to the preparatory TLC using glass slides coated with silica gel as the adsorbent material (stationary phase). The resultant plates were dried and activated by heating in an oven for thirty minutes at 110^{o}C. The thickness of the adsorbent was made to around 0.5 to 2.0mm thickness according to Joseph and Bernard (1991). Various solvent systems ranging from high polar to low polar combinations were tried. Chloroform, Methanol and water ratios 70:20:10, 60:30:10, and chloroform and methanol were tried at ratios, 60:40, 65:35, 70:30, 80:20, 85:15, 90:10, 95:5 and lastly 100 percent chloroform which happened to have given better separation. It was curiously observed that, the more the ratio of chloroform was increased the better the separation as such chloroform was eventually considered. For aqueous extract chloroform and methanol at ratios, 60:40 have given better separations after a lot of trials at, 65:35, 70:30, 75:25, 80:20, and 90:10 alongside several others.

3.7 Analytical Thin Layer Chromatography

The thin layer chromatography plates were manually pre-prepared in the laboratory on a glass 20cm X 20cm sizes to fractionate each of the extract into various components using the respective solvent systems discovered from the preparatory TLC analysis. The samples were spotted on the thin layer plates using fine capillary tubes. Several spots were made separately (about forty). Samples for spotting were prepared by dissolving 2g in 4ml of the solvent used for the initial extraction. After enough samples were spotted the solvent was allowed to evaporate and then the plate was placed under a short wavelength ultraviolet lamp such that a purple spot

on the background of green was clearly visible. This was to ensure that adequate quantity of the material was spotted. The developed chromatograms were tank containing the developing solvent. The tank was covered with a glass. A foolscap sheet was used beneath the glass cover to keep the atmosphere in the beaker saturated with the solvent vapor. The developing solvent was poured into the container to a depth of some millimeters. The spotted plate was then placed in the container, spotted end down; the solvent level was made below the spots. The solvent then slowly rose in the adsorbent by capillary action. When the solvent front has moved to within about 1cm of the top end of the adsorbent the plates were removed from the developing chamber, the position of the solvent front marked, and the solvent allowed to evaporate. The plates were visualized by shining ultraviolet light of 254nm and 365nm wave length.

3.8 Determination of Bio-active Component(s) using Bio-autography

Direct contact bio-autography was used using the TLC fractions of each extract to test their antibacterial activity. To each of the dried TLC fractions 2mls of methanol were added in order to dissolve them. Filter paper disc were soaked to absorb the compounds contained. The discs were removed and allowed to dry and thereafter were tested for antibacterial activity on the test bacteria. The real activity was determined by observing presence or absence of zones of inhibition formed around the disc (Bauer*et al.,* 1966).

3.9 Gas Chromatography Mass Spectrophotometry Analysis

Gas Chromatography Mass Spectrophotometry analysis was carried out on the most bioactive components of the TLC fractions of each among the extract identified during the Contact by Autography Test.

3.10 Brine Shrimp Lethality Assay (BSLA):

Brine shrimp eggs were obtained from Chemistry research laboratory, Bayero University Kano, as a gift by a student friend for the research work. Filtered artificial seawater was prepared by dissolving 38g of sea salt in 1 liter of distilled water for hatching the shrimp eggs. The sea water was put in a small improvised container (hatching chamber) made from a type of sponge case that has cover. It was wrapped (the sponge case) with a black masking tape with a hole from the top for light penetration. Shrimp eggs were added into the dark side of the chamber while the lamp above was meant to attract the hatched shrimp. Two days were allowed for the shrimp to hatch and mature as nauplii (larva). After two days, when the shrimp larvae were ready, 4mL of the artificial seawater was added to each test tube and 10 brine shrimps were introduced into each tube. Thus, there was a total of 30 shrimps per dilution. Then the volume was adjusted with artificial seawater up to 5mL per test tube. The test tubes were left uncovered under the lamp. The number of surviving shrimps were counted and recorded after 24 hours. Using probit analysis, the lethality concentration (LC50) was assessed at 95% confidence intervals.

3.11 Statistical Analysis

Data obtained during the Brine Shrimp Lethality assay were analyzed statistically using IBM SPSS software.

CHAPTER FOUR

4.0 RESULTS

4.1 Physical Properties and Percentage Yield of the Extract

The physical properties and percentage yields of the aqueous, ethanolic and chloroform extracts were shown in Table 4.1. During the extraction the filtrates appeared greenish, reddish brown, yellowish-green and dark green with gummy and thick texture and the odor of the extracts ranges from, odorless, chocolate, minty, lemony and fragrance. The highest percentage yield of the extract was observed in *C. papaya* aqueous extract which was 20.04% of the total sample extracted, followed by that of *M. indica* with 13.34% while chloroform extract of P. gauajava had the least yield of 1.12%.

4.3 Phytochemical Analysis

Phytochemical screening for the bioactive components present in the aqueous, ethanolic and chloroform extracts of the leaves of *P. guajava, C. papaya, M. indica* and *C. aurantifolia* revealed the presence of numerous secondary metabolites including; alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, phenols, and xanthoproteins as shown in (Table 4.2). Ethanolic extracts had the highest number of phytochemical components having nine different compounds in all the extracts while the aqueous and chloroform extracts were found to contain the same number of components having eight different compounds each. Tannins and flavonoids were present in each of the extracts, while anthraquinones and glycosides were absent in all the extracts. Xanthoproteins were detected in the ethanolic extract of *M. indica* only.

4.3 Antibacterial Susceptibility Test

The result of the antibacterial activity of aqueous, ethanolic, chloroform extracts as well as that of the amoxicillin antibiotic at four different concentrations (400µg/ml, 200µg/ml, 100µg/ml and 50µg/ml) against the test bacteria were presented in Table 4.3, 4.4, 4.5and 4.6.

For *P. guajava* extracts against the test bacteria, highest zones of inhibition was observed in *S. paratyphi* B, (17.75mm), followed by *S. paratyphi* A (16mm) and with the least being *S. typhi* (12mm). *S. Paratyphi* Bwas resistant to both ethanolic and chloroform extract. *S. typhi* was found to be resistant to chloroform extract as well. *S. paratyphi* A appeared to be the most sensitive to all the extract regardless of solvent. Therefore the susceptibility pattern of the test bacteria to the *P. guajava* extract is; *S. paratyphi* B>*S.paratyphi* A>*S. typhi*.

For *M. indica* extract, highest zone of inhibition was observed in ethanolic extract with 19.0mm for *S. paratyphi* A, 17mm for *S. paratyphi* B, and 15.75mm for *S. typhi*. This is followed by aqueous extract with 16.5mm for *S.paratyphi* Band 16mm for *S. typhi*. *S. paratyphi* A, was resistant to both aqueous and chloroform extracts of *M. indica*. *S. paratyphi B* was also resistant to chloroform extract. It can therefore, be deduced that, chloroform extract of *M. indica* was the least bioactive. It is pertinent however, to state that, lowest concentration of 50 and 100µ/ml showed little or no activity on the test bacteria. Thus, the bioactivity of the extracts followed the sequence; Ethanolic Extract > Aqueous Extract > Chloroform Extract. While the hierarchy of the susceptibility pattern of the tested bacteria to the extract is: *S. paratyphi* A>*S. paratyphi B* >*S. paratyphi* C.

For *C. papaya* extract, highest bioactivity was observed in chloroform extract with zones of inhibition of 17mm for *S. paratyphi B*, and 16mm for *S. typhi*. This is followed by aqueous

extract with zones of inhibition of 16mm for *S. typhi* and 15mm for *S. paratyphi B*. *S. paratyphi A* was resistant to all the three extracts while *S. paratyphi B* was resistant to ethanolic extract. It is important to note that, all the tested bacteria showed resistance to ethanolic extract. Therefore, the bioactivity pattern of the extract to the tested bacteria followed the sequence: Chloroform Extract >Aqueous Extract >Ethanolic Extract. And the hierarchy of the susceptibility of the bacteria to the extract followed the order: *S. paratyphi B >S. typhi >S. paratyphi A*.

For *C. aurantifolia,* aqueous extract produced the highest zone of inhibition of 24.5mm against *S. paratyphi A*, followed by chloroform extract with zone of inhibition of 19mm against the same bacteria. *S. typhi* was resistant to both aqueous and chloroform extract of the *C. aurantifolia* while been sensitive to ethanolic extract even at lowest concentration. Both *S. paratyphi A* and *S. paratyphi B* showed resistance to the ethanolic extract of *C. aurantifolia*. The sequence of bioactivity of the extract against the bacteria therefore is: aqueous extract > chloroform extract > ethanolic extract. While the hierarchy of the susceptibility of the bacteria to this extract is: *S. paratyphi A >S. paratyphi B >S. typhi*.

4.1Physical Properties of the Extract

Leaves Extract	Solvent	Color	Odor	Texture	Amount Recovered(g)	% yield
Psidium guajava	Chloroform	Dark green	Minty	Gummy	0.56	1.12
✓	Ethanol	Deep green	Minty	Gummy	3.03	6.06
✓	Aqueous	Reddish brown	Chocolate	Sticky	1.5	3.00
Mangifera indica	Chloroform	Dark green	Odorless	Slightly sticky	1.23	2.46
✓	Ethanol	Dirty green	Chemical	Slightly sticky	3.37	6.74
✓	Aqueous	Reddish brown	Odorless	Thick and sticky	6.62	13.24
Carica papaya	Chloroform	Dark green	Odorless	Thick and oily	1.82	3.64
✓	Ethanol	Dark green	Minty	Slimy	1.18	2.36
✓	Aqueous	Brown	Pungent	gummy	10.02	20.04
Citrus aurantifolia	Chloroform	Dark green	Lemony	Thick and oily	3.09	6.18
✓	Ethanol	Yellowish Green	Fragrant	Slightly slimy	1.35	2.70
✓	Aqueous	Reddish brown	Pungent	Gummy	4.98	9.96

4.2 Phytochemical Constituents of the Plants Extracts

S/N	Phytochemical Constituent	Aqueous Extract				Ethanolic Extract				Chloroform Extract			
		Guava	Mango	Pawpaw	Lemon	Guava	Mango	Pawpaw	Lemon	Guava	Mango	Pawpaw	Lemon
1	Alkaloids	-	+	-	+	-	+	+	+	-	+	+	+
2	Saponins	+	-	+	+	+	-	-	-	+	-	-	+
3	Tannins	+	+	+	+	+	+	+	+	+	+	+	+
4	Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
5	Steroids	-	+	-	+	+	+	+	-	-	-	+	-
6	Glycosides	-	-	-	-	-	-	-	-	-	-	-	-
7	Terpenoids	+	+	+	+	+	-	-	-	+	-	-	-
8	Phenols	+	-	-	+	+	+	-	+	-	+	-	+
9	Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
10	Xanthoproteins	-	-	-	-	-	+	-	-	-	-	-	-

Key: (+) Indicates presence, while (–) indicates absence

Table 4.3: Antibacterial Activity of Various Extracts of the Leaves of*P. guajava*on the Test Organism by Agar-well Diffusion Method

Test Organism	Aqueous Extract (µg/ml)				Ethanolic Extract (µg/ml)				Chloroform Extract (µg/ml)				Amoxicillin (µg/ml)				Activity Index		
	50	100	200	400	50	100	200	400	50	100	200	400	50	100	200	400	Aqu	Eth	Chl
S. Typhi	6	6	13	14	6	6	13	14	6	6	6	6	17.8	18	20.8	24.2	0.26	0.26	0.00
S. Paratyphi A	14	14.3	15	15.6	6	6	6	14.3	13.8	14.5	15.5	16	15	16.5	17	19.8	0.79	0.19	0.81
S. Paratyphi B	15.5	16	17.75	18	6	6	6	6	6	6	6	6	14	15	20	22	0.92	0.00	0.00

P. I. = Aqu = 0.83, Eth = 0.25, Chl = 0.33 **KEY: Aqu = Aqueous, Eth = Ethanol, Chl = Chloroform, P. I. Proportionate Index**

Table 4.4: Antibacterial Activity of Various Extracts of the Leaves of*M. indica*on the Test Organism by Agar-well Diffusion Method

Test Organisms	Aqueous Extracts (µg/ml)				Ethanolic Extract (µg/ml)				Chloroform Extract (µg/ml)				Amoxicillin (µg/ml)				Activity Index		
	50	100	200	400	50	100	200	400	50	100	200	400	50	100	200	400	Aqu	Eth	Chl
S. Typhi	6	6	14.8	16	13	14	14.5	15.8	6	6	14	16	17.8	18	20.8	24.2	0.33	0.56	0.32
S. Paratyphi A	6	6	6	6	6	6	16.3	19	6	6	6	6	15	16.5	17	19.8	0.00	0.52	0.00
S. Paratyphi B	6	13.2	14.8	16.5	6	6	14.3	17	6	6	6	6	14	15	20	22	0.56	0.41	0.00

P. I. = Aqu =0.58, Eth =0.33, Chl = 0.83 **KEY: Aqu = Aqueous, Eth = Ethanol, Chl = Chloroform, P. I. Proportionate Index**

Table 4.5: Antibacterial Activity of Various Extracts of the Leaves of *C. papaya* on Test Organism by Agar-well Diffusion Method

Test Organisms	Aqueous Extract (µg/ml)				Ethanolic Extract (µg/ml)				Chloroform Extract (µg/ml)				Amoxicillin (µg/ml)				Activity Index		
	50	100	200	400	50	100	200	400	50	100	200	400	50	100	200	400	Aqu	Eth	Chl
S. Typhi	6	6	6	16	6	6	6	6	13.5	14.5	15.3	16	17.8	18	20.8	24.2	0.18	0.00	0.62
S. Paratyphi A	6	6	6	6	6	6	6	6	6	6	6	6	15	16.5	19	19.8	0.00	0.00	0.00
S. Paratyphi B	6	6	13.3	15	6	6	6	6	12	12.8	16.8	17	12	15	20	22	0.36	0.00	0.82

P. I. = Aqu =0.25, Eth =0.00, Chl = 0.67 **KEY: Aqu = Aqueous, Eth = Ethanol, Chl = Chloroform, P. I. Proportionate Index**

Table 4.6: Antibacterial Activity of Various Extract of the Leaves of *C. aurantifolia* on the Test Organism by Agar-well Diffusion

Test organisms	Aqueous Extract (µg/ml)				Ethanolic Extract (µg/ml)				Chloroform Extract (µg/ml)				Amoxicillin (µg/ml)				Activity Index		
	50	100	200	400	50	100	200	400	50	100	200	400	50	100	200	400	Aqu	Eth	Chl
S. typhi	6	6	6	6	15.5	16	17	17	6	6	6	6	17.8	18	20.8	24.2	0.00	0.73	0.00
S. paratyphi A	12	12.5	16.75	24.5	6	6	6	6	16	16.3	17.8	19	15	16.5	17	19.8	1.03	0.00	1.02
S. paratyphi B	6	6	16	17.5	6	6	6	6	6	6	6	14	14	15	20	22	0.46	0.00	0.17

P. I. = Aqu =0.50, Eth =0.33, Chl = 0.42 **KEY: Aqu = Aqueous, Eth = Ethanol, Chl = Chloroform, P. I. Proportionate Index**

4.4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Extract

The result of the MIC and MBC of extracts of *P. guajava, M. indica, C. papaya,* and *C. aurantifolia* as well as that of the test antibiotic amoxicillin is presented in Table 4.7, 4.8, 4.9 and 4.10 respectively.

For *P. guajava* the aqueous, ethanolic and the chloroform extracts showed MIC ranges of (6.25µ/ml-50µ/ml), (25µ/ml-50µ/ml) respectively while chloroform maintained MIC of 25µ/ml for all the tested bacteria. Likewise *P. guajava* showed MBC values ranging from (12.5µg/ml - 50µg/ml), (25µg/ml-100 µg/ml and 50µg/ml - 200µg/ml) for aqueous, ethanolic and chloroform extracts respectively.

For *M. indica* both the aqueous, ethanolic and the chloroform extracts showed MIC range of (12.5µg/ml -50µg/ml) and MBC range of (50µg/ml - 100µg/ml, 25µg/ml - 400µg/ml and 25µg/ml - 100µg/ml) for aqueous, ethanolic and chloroform extracts respectively.

C. papaya extracts showed MIC ranges of (6.25µg/ml – 50µg/ml) for aqueous extract while ethanolic and chloroform extract showed the same rage of (25µg/ml - 50µg/ml) each. And lastly, *C. aurantifolia* showed MIC (12.5µg/ml - 25µg/ml, 12.5µg/ml - 50µg/ml and 25µg/ml - 100µg/ml) for aqueous, ethanolic and chloroform extracts respectively. The *C. papaya* extracts also showed MBC range of (25µg/ml-100µg/ml and 50µg/ml - 100µg/ml) for ethanolic and chloroform extracts respectively while aqueous extract had MBC of 50µg/ml against all the test bacteria

4.5 Thin layer chromatography Result

The result of the thin layer chromatography of the aqueous, ethanolic and chloroform extract of all the plants is presented in (Table 4.11 to 4.21). The TLC chromatogram revealed 3 bands for aqueous extract of *P. guajava*, 4 bands for ethanolic and 6 bands for chloroform extract. Aqueous extract of *M. indica* revealed 2 bands, ethanolic 6 and chloroform 4. Aqueous extract of *C. papaya* revealed 5 bands and chloroform 6 bands. Ethanolic extract was excluded because it lacks antibacterial activity. While aqueous extract of *C. aurantifolia* revealed 3 bands, ethanol 3 and chloroform 5 bands both with different Rf values. The result of the TLC was performed on 20 x 20 Silica Gel Plate. Other features considered in each TLC fraction include appearance of each band at 254nm and 365nm wave length as well as the weight of the scrapped band prior to been eluted and the weight of the extract (mg) recovered from the fractions.

Table 4.7 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract of *P. guajava* Against the Test Bacteria

S/N	Test Organisms	Aqueous Extract		Ethanolic Extract		Chloroform Extract		Amoxicillin	
		MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
1	*S. typhi*	50	100	25	100	25	50	12.5	25
2	*S. paratyphi A*	50	100	50	100	25	50	6.25	25
3	*S. paratyphi B*	6.25	50	25	50	25	50	6.25	12.5

Table 4.8 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract of *M. indica* Against the Test Bacteria

S/N	Test Organisms	Aqueous Extract		Ethanolic Extract		Chloroform Extract		Amoxicillin	
		MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
1	*S. typhi*	50	100	50	100	50	100	12.5	25
2	*S. paratyphi A*	12.5	50	12.5	400	12.5	50	6.25	25
3	*S. paratyphi B*	50	100	12.5	25	12.5	25	6.25	12.5

Table 4.9 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Extract of *C. papaya* against the Test Bacteria

S/N	Test Organisms	Aqueous Extract		Ethanolic Extract		Chloroform Extract		Amoxicillin	
		MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
1	*S. typhi*	50	100	50	100	50	100	12.5	25
2	*S. paratyphi A*	6.25	12.5	25	50	25	50	6.25	25
3	*S. paratyphi B*	12.5	25	50	100	25	100	6.25	12.5

Table 4.10 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract of *C. aurantifolia* against the Bacteria

S/N	Test Organisms	Aqueous Extract		Ethanolic Extract		Chloroform Extract		Amoxicillin	
		MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
1	*S. typhi*	25	50	50	100	50	100	12.5	25
2	*S. paratyphi A*	12.5	50	12.5	25	50	100	6.25	25
3	*S. paratyphi B*	25	50	12.5	25	25	50	6.25	12.5

`Table 4.11 TLC Separation of Aqueous Extract of *P. guajava* on Silica gel using Chloroform: Methanol (4:6) solvent system

Rf No.	Rf Value	Appearance of the band under UV at		Weight of the scrapped band (mg)	Weight of the extract recovered from the band (mg)
		254nm	365nm		
1	0.1272	Dark Brown	Brown	22.87	10.16
2	0.7572	Purple	Brown	34.01	11.96
3	0.9769	Orange	Yellow	23.56	10.74

Table 4.12 TLC Separation of Ethanolic Extract of *P. guajava* on Silica gel Using Hundred Percent Chloroform Solvent System

Rf No.	Rf Value	Appearance of the band under UV at		Weight of the scrapped band (mg)	Weight of the extract recovered from the band (mg)
		254nm	365nm		
1	0.1316	Brown	Light brown	65.22	26.10
2	0.1404	Green	Green	19.00	8.02
3	0.1520	Purple	Light purple	20.02	7.89
4	0.1637	Pale yellow	Pale yellow	19.87	9.24

Table 4.13 TLC Separation of Chloroform Extract of *P. guajava* on Silica gel using Hundred Percent Chloroform Solvent System

Rf No.	Rf Value	Appearance of the band under UV at		Weight of the scrapped band (mg)	Weight of the extract recovered from the band (mg)
		254nm	365nm		
1	0.1389	Brown	Brown	50.23	20.10
2	0.1667	Light green	Light Green	23.09	10.11
3	0.2778	Green	Green	42.34	17.89
4	0.3611	Dark green	Dark Green	55.01	19.32
5	0.5278	Orange	Pale orange	20.61	8.12
6	0.7222	Yellow	Yellow	19.22	10.99

Table 4.14 TLC Separation of Aqueous Extract of *M. indica* on Silica gel using Chloroform: Methanol (4:6) Solvent System

Rf No.	Rf Value	Appearance of the band under UV at		Weight of the scrapped band (mg)	Weight of the extract recovered from the band (mg)
		254nm	365nm		
1	0.1714	Brown	Deep Brown	43.09	21.00
2	0.9429	Orange	Pale Yellow	26.05	16.10

Table 4.15 TLC of the Ethanol Extract of *M. indica* on Silica gel Using Hundred Percent Chloroform Solvent System

Rf No.	Rf Value	Appearance of the band under UV at		Weight of the scrapped band (mg)	Weight of the extract recovered from the band (mg)
		254nm	365nm		
1	0.1316	Dark brown	Pale Brown	75.09	42.65
2	0.1684	Purple	Light Purple	16.45	6.31
3	0.1842	Dark Green	Dark Green	34.90	13.66
4	0.2105	Yellowish green	Yellowish Green	19.64	11.00
5	0.2895	Yellow	Orange	23.00	8.89

Table 4.16 TLC Separation of Chloroform Extract of *M. indica* on Silica gel Using Hundred Percent Chloroform Solvent System

Rf No.	Rf Value	Appearance of the band under UV at		Weight of the scrapped band (mg)	Weight of the extract recovered from the band (mg)
		254nm	365nm		
1	0.2059	Brown	Light brown	43.11	17.43
2	0.2941	Dark green	Dark Green	55.12	23.57
3	0.4118	Light brown	Brown	20.11	8.05
4	0.9412	Maroon	No absorbance	16.56	9.50

Table 4.17 TLC Separation of Aqueous Extract of *C. papaya* on Silica gel Using Chloroform: Methanol (4:6) Solvent System

Rf No.	Rf Value	Appearance of the band under UV at		Weight of the scrapped band (mg)	Weight of the extract recovered from the band (mg)
		254nm	365nm		
1	0.1875	Brown	Brown	65.90	23.37
2	0.8125	Orange	Light brown	87.67	45.00
3	0.9063	Yellowish green	Light green	21.67	9.76
4	0.9750	Yellow	Yellow	15.34	5.61

Table 4.18 TLC Separation of Chloroform Extract of *C. papaya* on Silica gel Using Hundred Percent Chloroform Solvent System

Rf No.	Rf Value	Appearance of the band under UV at		Weight of the scrapped band (mg)	Weight of the extract recovered from the band (mg)
		254nm	365nm		
1	0.1579	Brown	Brown	54.10	38.00
2	0.2368	Dark Green	Green	71.09	21.01
3	0.2895	Ash	Ash	24.45	9.12
4	0.3947	Greenish Yellow	Yellowish green	36.12	12.12
5	0.7105	Reddish Brown	Reddish brown	19.17	8.71
6	0.9737	Orange	Pale Orange	40.90	16.00

Table 4.19 TLC Separation of Aqueous Extract of *C. aurantifolia* on Silica gel Using Chloroform: Methanol (4:6) Solvent System

Rf No.	Rf Value	Appearance of the band under UV at		Weight of the scrapped band (mg)	Weight of the extract recovered from the band (mg)
		254nm	365nm		
1	0.1667	Brown	Brown	42.00	28.01
2	0.2333	Green	Dark green	20.12	11.09
3	0.9867	Dark brown	Reddish brown	19.90	8.00

Table 4.20 TLC Separation of Ethanolic Extract of *C. aurantifolia* on Silica gel Using Hundred Percent Chloroform Solvent System

Rf No.	Rf Value	Appearance of the band under UV at		Weight of the scrapped band (mg)	Weight of the extract recovered from the band (mg)
		254nm	365nm		
1	0.1100	Yellowish brown	Yellowish brown	20.87	18.16
2	0.1250	Dark green	Green	36.01	11.66
3	0.9250	Reddish yellow	Reddish yellow	20.56	11.74

Table 4.21 TLC Separation of Chloroform extract of *C. aurantifolia* on Silica gel using Hundred Percent Chloroform Solvent System

Rf No.	Rf Value	Appearance of the band under UV at		Weight of the scrapped band (mg)	Weight of the extract recovered from the band (mg)
		254nm	365nm		
1	0.1374	Brown	Light brown	60.10	48.00
2	0.1923	Dark green	Green	70.09	31.01
3	0.2747	Purple	Light purple	25.45	10.12
4	0.4505	Light brown	Brown	30.12	12.12
5	0.7142	Reddish Yellow	No absorbance	19.17	9.11

4.6 Bio-autography Result

The result of the bio-autography carried out on both the aqueous, ethanolic and chloroform extract of *P. guajava, M. indica, C. papaya* and *C. aurantifolia* against the test bacteria revealed that, out of the forty five (45) fractions tested only seven (7) showed activity on the test bacteria as shown in (Table 22). However, the level of their activity varies with the fractions. While some of the fractions appeared active against all the 3 bacteria some appeared to be active against only one or two of the test bacteria. The most bioactive fractions appeared to be Rf3 of aqueous extract of *P. guajava* and Rf2 and Rf23 of Ethanolic extract of *M. indica*. They all appeared to be bioactive against all the tested bacteria. Followed by Rf2 and Rf3 of chloroform extract of *C. papaya* which was active against *S. typhi* and *S. paratyphi* B and lastly the least bioactive among them was chloroform extract of *C. aurantifolia* which appeared to be active against only *S. paratyphi A*. Moreover, even among the most bioactive fractions, Rf3 of ethanolic extract of *M. indica* showed a wider zone of inhibition in comparison to the other bioactive fractions.

Table 4.22 Direct Contact Bioautography Showing the Activity of the Extracts Against the Test Bacteria

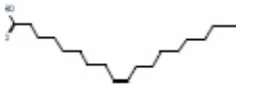
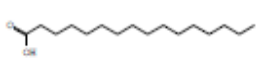
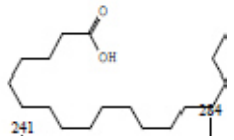
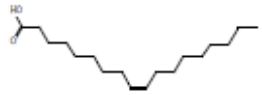
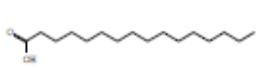
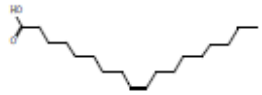
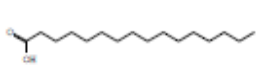
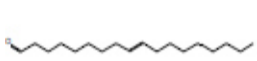
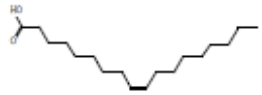
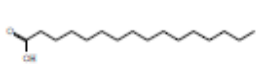
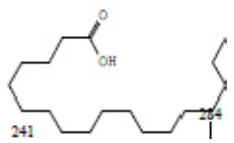
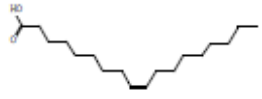
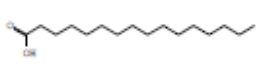
Extract	Bacteria	Rf1	Rf2	Rf3	Rf4	Rf5	Rf6
P. guajava in Aqueous	*S. typhi*	-	-	+	N	N	N
	S. paratyphi A	-	-	+	N	N	N
	S. paratyphi B	-	-	+	N	N	N
P. Guava in Ethanol	*S. typhi*	-	-	-	-	N	N
	S. paratyphi A	-	-	-	-	N	N
	S. paratyphi B	-	-	-	-	N	N
P. guava in Chloroform	*S. typhi*	-	-	-	-	-	-
	S. paratyphi A	-	-	-	-	-	-
	S. paratyphi B	-	-	-	-	-	-
M. Indica in Aqueous	*S. typhi*	-	-	N	N	N	N
	S. paratyphi A	-	-	N	N	N	N
	S. paratyphi B	-	-	N	N	N	N
M. indica in Ethanol	*S. typhi*	-	+	+	-	-	N
	S. paratyphi A	-	+	+	-	-	N
	S. paratyphi B	-	+	+	-	-	N
M. indica in Chloroform	*S. typhi*	-	-	-	-	N	N
	S. paratyphi A	-	-	-	-	N	N
	S. paratyphi B	-	-	-	-	N	N
C. papaya in Aqueous	*S. typhi*	-	-	-	-	-	N
	S. paratyphi A	-	-	-	-	-	N
	S. paratyphi B	-	-	-	-	-	N
C. papaya in Chloroform	*S. typhi*	-	+	+	-	-	-
	S. paratyphi A	-	-	-	-	-	-
	S. paratyphi B	-	+	+	-	-	-
C. aurantifolia in Aqueous	*S. typhi*	-	-	-	N	N	N
	S. paratyphi A	-	-	-	N	N	N
	S. paratyphi B	-	-	-	N	N	N
C. aurantifolia in Ethanol	*S. typhi*	-	-	+	N	N	N
	S. paratyphi A	-	-	-	N	N	N
	S. paratyphi B	-	-	-	N	N	N
C. aurantifolia in chloroform	*S. typhi*	-	-	-	-	-	N
	S. paratyphi A	-	-	-	-	+++	N
	S. paratyphi B	-	-	-	-	-	N

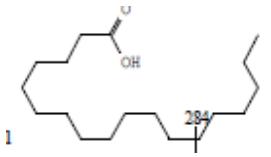
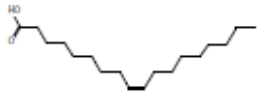
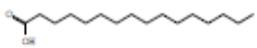
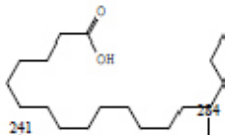
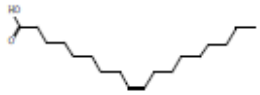
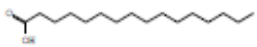
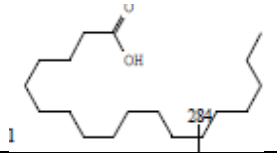
Key: + indicates activity, - indicates no activity, N indicates limit of the retention factor, Rf = Retention Factor

4.7 GC-MS Analysis of the Most Bioactive TLC Fractions

The total ion chromatograms (TIC) revealed the presence of different constituents including fatty acids, heterocyclic compounds and esters among others. The seven different TLC fractions showed wide distinctions in terms of their chemical composition. The major similarities were observed in the case of oleic acid and palmitic acids which were present in all the fractions followed by stearic acid which was present in four out of the seven fractions. Chloroform fraction has the highest number of constituents, followed by ethanolic fractions and eventually the aqueous fraction. The detailed tabulation of the GC-MS analysis of the most bioactive compounds was presented in Table 4.23. All the compounds have been elucidated and effectively matched and identified. For RF3 of *P. guajava* (aqueous) the major constituents were Oleic acid (Peak 6, 35.56% peak area), Palmitic acid (Peak 2, 20.26% peak area) followed by stearic (peak 7, 9.84% peak area). For Rf5 of *C. aurantifolia* (chloroform) the major constituents were Stearolic acid (Peak 13, 20.16 peak area), then Oleic acid (peak 14, 18.23% peak area) and lastly palmitic acid (Peak 10, 14.95% peak area). Rf3 of C. aurantifolia (ethanolic) have oleic acid, palmitic acid and 9-Octadecenal as major constituents with 12, 7 and 16 peaks and 36.57%, 19.67% and 8.08% peak area respectively. For Rf3 of *C. papaya* (chloroform) the major constituents were oleic acid, palmitic acid, stearic acid and 9-octadecenal with peaks of 9, 5, 10 and 15 and 36.65%, 16.68%, 9.78% and 7.44% peak area. For Rf3 of M. indica (ethanol) the major constituents were oleic acid, palmitic acid and stearic acid with peaks of 8, 3 and 9 and peak area of 39.48%, 19.32% and 10.47% respectively. For Rf3 of *C. papaya* (chloroform) the major constituents were oleic acid, palmitic acid, and stearic acid with peaks of 11, 6 and 9 and peak area of 35.98, 16.201 and 9.012 respectively. And lastly Rf3 of M. indica (ethanolic) have oleic acid, palmitic acid and stearic acid as major constituents with peaks of 7, 5, and 9 and peak areas of 40.157, 19.982 and 11.127 respectively.

Table 4.23 GC-MS Analysis of the Most Bioactive TLC Fractions

Leaves Extract	Peak No.	Retention (min)	% composition By area	Compound name	Molecular Formula	Chemical structure
P. guajava (Aqueous) Rf3	6	20.846	35.56	Oleic acid	$C_{18}H_{36}O_2$	
	2	18.014	20.26	Palmitic acid	$C_{16}H_{32}O_2$	
	7	21.079	9.84	Stearic acid	$C_{18}H_{36}0_2$	
C. aurantifolia (Chloroform) Rf5	13	20.396	20.16	Stearolic acid	$C_{18}H_{32}O_2$	
	14	20.825	18.23	Oleic acid	$C_{18}H_{36}O_2$	
	`10	18.012	14.95	Palmitic acid	$C_{16}H_{32}O_2$	
C. aurantifolia (Ethanolic) Rf3	12	20.852	36.57	Oleic acid	$C_{18}H_{36}O_2$	
	7	18.016	19.67	Palmitic acid	$C_{16}H_{32}O_2$	
	16	23.803	8.08	9-Octadecenal	$C_{18}H_{34}O$	
C. papaya (Chloroform) Rf3	9	20.788	36.68	Oleic acid	$C_{18}H_{36}O_2$	
	5	17.935	16.68	Palmitic acid	$C_{16}H_{32}O_2$	
	10	21.030	9.78	Stearic acid		
	15	23.791	7.44	9-Octadecenal	$C_{18}H_{34}O$	
M. indica (Ethanol) Rf3	8	20.854	39.48	Oleic acid	$C_{18}H_{36}O_2$	
	3	18.004	19.32	Palmitic acid	$C_{16}H_{32}O_2$	

	9	21.910	10.47	Stearic acid	$C_{18}H_{36}0_2$	
C. papaya (Chloroform) Rf2	11	20.152	35.98	Oleic acid	$C_{18}H_{36}O_2$	
	6	16.986	16.201	Palmitic acid	$C_{16}H_{32}O_2$	
	9	20.139	9.012	Stearic acid		
M. indica (Ethanolic) Rf2	7	21.895	40.157	Oleic acid	$C_{18}H_{36}O_2$	
	5	18.456	19.982	Palmitic acid	$C_{16}H_{32}O_2$	
	9	20.997	11.127	Stearic acid	$C_{18}H_{36}0_2$	

4.8 Brine Shrimp Lethality Assay of the Extracts

The result of the brine shrimp lethality is presented in (Table 24). From the result chloroform extracts appeared to be the most toxic followed by aqueous extracts of *C. aurantifolia* and chloroform extract of *M. indica* respectively. Chloroform extract of *C. papaya* was not toxic with LC_{50} value of (11,641µg/ml). The less toxic ones include ethanolic extract of *C. papaya* (179.505µg/ml), aqueous extracts of *M. indica* and ethanolic extracts of *C. aurantifolia*. All the rest appeared to be toxic with values lower than 100µg/ml.

Table 4.24 Brine Shrimp Lethality Assay of the Extracts

Plant Extract	Organic solvent	Concentration (ppm or µg/ml)	No. of Shrimps	No. of Survivors	% Mortality	LC_{50} (µg/mL) Brine Shrimp Lethality
P. guajava	Aqueous	1000	10	0	100	49.534
		100	10	3	70	
		10	10	9	10	
	Ethanol	1000	10	0	100	39.903
		100	10	2	80	
		10	10	9	10	
	Chloroform	1000	10	0	100	31.112
		100	10	2	80	
		10	10	8	20	
M. indica	Aqueous	1000	10	1	90	100.00
		100	10	5	50	
		10	10	9	10	
	Ethanol	1000	10	1	90	60.203
		100	10	4	60	
		10	10	8	20	
	Chloroform	1000	10	0	100	41.378
		100	10	3	70	
		10	10	7	30	
C. papaya	Aqueous	1000	10	0	100	57.450
		100	10	2	80	
		10	10	10	0	
	Ethanol	1000	10	5	50	179.505
		100	10	6	40	
		10	10	10	0	
	Chloroform	1000	10	8	20	11,651.01
		100	10	9	10	
		10	10	10	0	
C. aurantifolia	Aqueous	1000	10	0	100	36.044
		100	10	4	60	
		10	10	7	30	
	Ethanol	1000	10	1	90	154.318
		100	10	7	30	
		10	10	9	10	

CHAPTER FIVE

5.0 DISCUSSION

The physical properties and percentage yield of the aqueous, ethanolic and chloroform extract is shown in Table 4.1. The highest percentage yield of the extract was observed in aqueous extract which was 46.24%w/w of the total sample extracted, followed by ethanolic extract with 17.86% w/w and lastly chloroform extract with the least of 13.4%w/w of the total sample. This indicates that the plants components are more soluble in high polar solvents. The yield was calculated on the basis of weight of crude extract taken for extraction and the amount of solid residue obtained after lyophilization. However, according to the above finding, it can be deduced that the amount of extracts recovery is polarity dependant.

Phytochemical screening for the bioactive components present in the aqueous, ethanolic and chloroform extracts of the leaves of *P. guajava, C. papaya, M. indica* and *C. aurantifolia* revealed the presence of numerous secondary metabolites including; alkaloids, carbohydrates, saponins, tannins, flavonoids, steroids, terpenoids, phenols, and xanthoproteins as shown in (Table 4.2). Ethanolic extracts has the highest number of phytochemical components having nine different compounds in all the extract while the aqueous and chloroform extracts were found to contain the same number of components having eight different compounds each. Tannins and flavonoids were present in each of the extracts, while anthraquinones and glycosides were absent in all the extracts. Xanthoproteins were detected in the ethanolic extract of *M. indica* only. Alkaloids has addictive or pain killing or poisonous effect and sometimes help in important cure. Saponins may help to prevent colon cancer. Flavonoids possess antiallergic, anti-inflammatory, antiviral and antioxidant activities. Steroids are used to suppress various allergic, inflammatory and autoimmune disorders (Pritesh and Zara 2015)

This finding agrees with similar finding of Pintu and Arna (2014) in which they reported that water extract of *Mangifera indica* young leaves contain tannins, alkaloids, steroid, carbohydrate, glycoside and flavonoid that may be responsible for the anti-diarrheal properties of the crude extract. Similarly Harborne and Williams (2000) revealed that, Terpenoids are attributed for analgesic and anti-inflammatory activities and flavonoids have been reported to possess many useful properties, including anti-inflammatory, estrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic anti tumour activity. Preliminary qualitative test is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development.

The results of phytochemical screening of ethanol, chloroform and water extracts and fractions of *C. papaya* revealed the presence of alkaloids, flavonoids, saponins, steroids and tannins. These metabolites have been reported to possess antimicrobial activity (Yusha'u *et al.,* 2009). Flavonoids might also play a role in disease resistance. Some flavonoids such as quercetin and rutin, are known to support human health by serving antiinflammatory, antihistaminic and antiviral agents (Okwu, 2004). Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids, such as glycyrrhizin and chrysin (Duraipandiyan *et al.,* 2006) against HIV.

Flavonoids have been referred to as nature's biological response modifiers, because of inherent ability to modify the body's reaction to allergies. It possesses various pharmacological roles including anti-allergic, antiinflammatory, cardio-protective, anti-microbial and anticancer activities (Duraipandiyan *et al.,* 2006).

The result of the antibacterial activity of the extracts is shown in Table 4.3 to 4.6. Form the results*P. guajava* against the test bacteria, showed zones of inhibition of (18mm)against*S.*

paratyphi B, followed by *S. paratyphi A* (16mm) and the least was *S. typhi* (14mm). *S. Paratyphi B* was resistant to both ethanolic and chloroform extracts while *S. typhi* was found resistant to chloroform extract as well. *S. paratyphi A* appeared to be the most sensitive to all the extracts regardless of extraction solvent. Therefore the susceptibility pattern of the test bacteria to the *P. guajava* extract is; *S. paratyphi B >S.paratyphi A >S. typhi*.

For *M. indica* extract highest zone of inhibition was observed in ethanolic extract with 13.0mm for *S. paratyphi A*, 17mm for *S. paratyphi B*, and 15.75mm for *S. typhi*. This is followed by aqueous extract with 16.5mm for *S.paratyphi B*and 16mm for *S. typhi*. *S. paratyphi A*, was resistant to both aqueous and chloroform extract of *M. indica*. *S. paratyphi B* was also resistant to chloroform extract. It can therefore, be deduced that, chloroform extract of *M. indica* was the least bioactive. It is pertinent however, to state that, lowest concentration of 50 and 100µ/ml showed little or no activity on the test bacteria. The hierarchy of the susceptibility pattern of the tested bacteria to the extract is: *S. paratyphi A >S. paratyphi B >S. paratyphi C*.

For *C. papaya* extract, highest bioactivity was observed in chloroform extract with zones of inhibition of 17mm for *S. paratyphi B*, and 16mm for *S. typhi.* This is followed by aqueous extract with zones of inhibition of 16mm for *S. typhi* and 15mm for *S. paratyphi B*. *S. paratyphi A* was resistant to all the three extract. *S. paratyphi B* was also resistant to ethanolic extract. It is important to note that, all the tested bacteria showed resistance to ethanolic extract. Therefore, the bioactivity pattern of the extract to the tested bacteria followed the sequence: Chloroform Extract >Aqueous Extract >Ethanolic Extract. And the hierarchy of the susceptibility of the bacteria to the extract followed the order: *S. paratyphi B >S. typhi >S. paratyphi A*.

In similar research work Odunbakuand Ashidi(2012)reported that the two trees extracts *(Mangifera indica* and *Citrus aurantifolia)* have considerable inhibitory effects on *Staphylococcus albus, Pseudomonas aeruginosa, Aspergillus terreus, Aspergillus niger*and *Penicillium oxalicum.* For *C. aurantifolia,* aqueous extract produced highest zone of inhibition of 18.5mm against *S. paratyphi A*, followed by chloroform extract with zone of inhibition of 13mm against the same bacteria. *S. typhi* was resistant to both aqueous and chloroform extract of the *C. aurantifolia* while being sensitive to ethanolic extract of the *C. aurantifolia* even at lowest concentration. Both *S. paratyphi A* and *S. paratyphi B* showed resistance to the ethanolic extract of *C. aurantifolia*. The sequence of bioactivity of the extract against the bacteria therefore is: aqueous extract > chloroform extract > ethanolic extract. While the hierarchy of the susceptibility of the bacteria to this extract is: *S. paratyphi A >S. paratyphi B >S. typhi*.

Shanjida*et al.,* (2015) reported that, different concentrations of the methanolic extracts of the leaves and barks of *Psidium guajava*, leaves of *Mangifera indica* and fruits and seeds of *Carica papaya* exhibited antimicrobial activities against all the isolates of bacteria (*Bacillus cereus, Bacillus subtilis, Escherichia coli* and *Salmonella typhi*). And the findings (Abegaz *et al.,* 1996; Jairaj, 1999; Esimone *et al.,* 2003) relates that extracts from the leaves of P. guajava, showed significant antimicrobial activity against *P. aeruginosa*, *S. aureus*, *Proteus mirabilis*, *C. albicans*, *A. niger and A. flavus*.

The result of the MIC and MBC of extracts of *P. guajava, M. indica, C. papaya,* and *C. aurantifolia* as well as that of the test antibiotic amoxicillin is presented in (Table 4.7 to 4.10) respectively.

For *P. guajava* the aqueous, ethanolic and the chloroform extracts have MIC range of (6.25µ/ml-50µ/ml), (25µ/ml-50µ/ml) respectively while chloroform maintained MIC of 25µ/ml for all the

tested bacteria. Likewise *P. guajava* have MBC values ranging from (12.5µg/ml -50µg/ml), (25µg/ml-100 µg/ml and 50µg/ml - 200µg/ml) for aqueous, ethanolic and chloroform extracts respectively.

For *M. indica* both the aqueous, ethanolic and the chloroform extracts have MIC range of (12.5µg/ml -50µg/ml) and MBC range of (50µg/ml - 100µg/ml, 25µg/ml - 400µg/ml and 25µg/ml - 100µg/ml) for aqueous, ethanolic and chloroform extracts respectively.

C. papaya extracts had MIC ranges of (6.25µg/ml – 50µg/ml) for aqueous extract while ethanolic and chloroform extract had the same rage of (25µg/ml - 50µg/ml) each. And lastly, C. aurantifolia had an MIC (12.5µg/ml - 25µg/ml, 12.5µg/ml - 50µg/ml and 25µg/ml - 100µg/ml) for aqueous, ethanolic and chloroform extracts respectively. The *C. papaya* extracts also had MBC range of (25µg/ml-100µg/ml and 50µg/ml - 100µg/ml) for ethanolic and chloroform extracts respectively while aqueous extract had MBC of 50µg/ml against all the test bacteria

The result of the thin layer chromatography of the aqueous, ethanolic and chloroform extract of all the plants is presented in (Table 4.11 to 4.21). The TLC chromatogram revealed 3 bands for aqueous extract of *P. guajava*, 4 bands for ethanolic and 6 bands for chloroform extract. Aqueous extract of *M. indica* revealed 2 bands, ethanolic 6 and chloroform 4. Aqueous extract of *C. papaya* revealed 5 bands and chloroform 6 bands. Ethanolic extract was excluded because it lacks antibacterial activity. While aqueous extract of *C. aurantifolia* revealed 3 bands, ethanol 3 and chloroform 5 bands both with different Rf values.

The result of the direct contact bioautography carried out on both the aqueous, ethanolic and chloroform extract of *P. guajava, M. indica, C. papaya* and *C. aurantifolia* against the test bacteria revealed that, out of the forty five (45) fractions tested only seven (7) showed activity on

the test bacteria as shown in (Table 4.22). However, the level of their activity varies with the fractions. While some of the fractions appeared active against all the 3 bacteria some appeared to be active against only one or two of the test bacteria. The most bioactive fractions appeared to be Rf3 of aqueous extract of *P. guajava* and Rf2 and Rf3 of Ethanolic extract of *M. indica*. They all appeared to be bioactive against all the tested bacteria. Followed by Rf2 and Rf3 of chloroform extract of *C. papaya* which was active against *S. typhi* and *S. paratyphi* B and lastly the least bioactive among them was chloroform extract of *C. aurantifolia* which appeared to be active against only *S. paratyphi A*. Moreover, even among the most bioactive fractions, Rf3 of ethanolic extract of *M. indica* showed a wider zone of inhibition in comparison to the other bioactive fractions.

For GC-MS analysis of the most bioactive compounds the total ion chromatograms (TIC) revealed the presence of different constituents including fatty acids, heterocyclic compounds and esters among others. The seven different TLC fractions showed wide distinctions in terms of their chemical composition. The major similarities were observed in the case of oleic acid and palmitic acids which were present in all the fractions followed by stearic acid which was present in four out of the seven fractions. Chloroform fraction has the highest number of constituents, followed by ethanolic fractions and eventually the aqueous fraction. The detailed tabulation of the GC-MS analysis of the most bioactive compounds was presented in Table 4.23. All the compounds have been elucidated and effectively matched and identified. For RF3 of *P. guajava* (aqueous) the major constituents were Oleic acid (Peak 6, 35.56% peak area), Palmitic acid (Peak 2, 20.26% peak area) followed by stearic acid (peak 7, 9.84% peak area). For Rf5 of *C. aurantifolia* (chloroform) the major constituents were Stearolic acid (Peak 13, 20.16 peak area), then Oleic acid (peak 14, 18.23% peak area) and lastly palmitic acid (Peak 10, 14.95% peak

area). Rf3 of *C. aurantifolia* (ethanolic) have oleic acid, palmitic acid and 9-Octadecenal as major constituents with 12, 7 and 16 peaks and 36.57%, 19.67% and 8.08% peak area respectively. For Rf3 of *C. papaya* (chloroform) the major constituents were oleic acid, palmitic acid, stearic acid and 9-octadecenal with peaks of 9, 5, 10 and 15 and 36.65%, 16.68%, 9.78% and 7.44% peak area. For Rf3 of *M. indica* (ethanol) the major constituents were oleic acid, palmitic acid and stearic acid with peaks of 8, 3 and 9 and peak area of 39.48%, 19.32% and 10.47% respectively. For Rf3 of *C. papaya* (chloroform) the major constituents were oleic acid, palmitic acid, and stearic acid with peaks of 11, 6 and 9 and peak area of 35.98, 16.201 and 9.012 respectively. And lastly Rf3 of M. indica (ethanolic) have oleic acid, palmitic acid and stearic acid as major constituents with peaks of 7, 5, and 9 and peak areas of 40.157, 19.982 and 11.127 respectively. Kabara (1972) reported that fatty acids such as oleic, palmitic, stearic, myristic, linoleic and linolenic acids were active against *Clostridium perfringens* and *Staphylococcus pyogens*. Some *in vitro* studies have indicated that the fatty acid composition could either directly or indirectly affect the aflatoxin contamination (Passi *et al.,* 1984; Doehlert *et al.,* 1993; Burrow *et al.,* 1997). Reports by Dilika *et al.,* (2000); and Sun *et al.,* (2003) that long-chain unsaturated fatty acids, including linoleic acid, are well known to inhibit bacteria like *E. coli* is also a barking to this study. Galbraith and Miller (1973) reported that long-chain fatty acids have higher antimicrobial activity against Gram-positive bacteria than Gram-negative bacteria. The difference in the fatty acid sensitivity between Gram-positive and Gram-negative bacteria may result from the impermeability of the outer membrane of Gram negative bacteria since it is an effective barrier against hydrophobic substances (Sheu and Freese, 1973; Sheu *et al.,* 1975). Linolenic, linoleic and palmitic acids isolated from *Schotia brachypetala,*

Pelargonium sp. and *Pentanisia prunelloides*, respectively, were found to have antibacterial activity (McGraw *et al.,* 2002; Seidal and Taylor, 2004; Yff *et al.,* 2002).

The result of the brine shrimp lethality is presented in (Table 4.24). From result chloroform extracts other than that of *C. papaya* appeared to be the most toxic followed by aqueous extract of *C. aurantifolia* and chloroform extract of *M. indica* respectively. Chloroform extract of *C. papaya* was not toxic with LC_{50} value of (11,641µg/ml). The less toxic ones include ethanolic extract of *C. papaya* (179.505µg/ml), aqueous extract of *M. indica* and ethanolic extract of *C. aurantifolia*. All the rest appeared to be toxic with values lower than 100µg/ml. From the phytochemical screening, the presence of alkaloids and steroids was observed. So the observed cytotoxic action may be due to the presence of such compounds. Again, reports exist on the role of alkaloids and steroids in cytotoxic activity of plant extracts (Dhar *et al.,* 1973; Vijayan *et al.,* 2004; and Badami *et al.,* 2003). However, phenolics and flavonoids are also known to show cytotoxicity in Hoechst 33258 fluorescence assay by inhibiting cellular DNA in a concentration-dependent manner (Chang *et al.,* 2000).

5.1 Conclusion

It can be concluded from the above finding that ethanol was found to be the best solvent of extraction of bioactive phytochemicals from the leaves of *P. guajava, M. indica C. papaya*, and *C. aurantifolia.*The study indicated that the leaves extracts of *P. guajava, M. indica, C. papaya*, and *C. aurantifolia* had antibacterial activities against some of the test bacteria and as such provided scientific support to the traditional use of these plants in treating typhoid fever.The plants extract have oleic and palmitic acids as their common and major constituents even though some had stearic acid and 9-octedecenal in addition to the two major constituents.The most bioactive compounds responsible for the antibacterial activity of leaves of *P. guajava, M. indica, C. papaya*, and *C. aurantifolia* can be further purified to provide chemical analog that can be

synthesized commercially for the treatment of bacterial infections especially those caused by *Salmonella typhi* and *Salmonella paratyphi*.The study also revealed that, all the extracts are toxic with exception of chloroform extract of *C. papaya*.

5.2 Recommendation

Based on the observation from this study, it is recommended that:

- The bioactive compounds identified by GC-MS analysis can be purified and used as precursor for new antibiotics for the treatment of typhoid and paratyphoid fevers.

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