USMANU DANFODIYO UNIVERSITY, SOKOTO STATE

(POST GRADUATE SCHOOL)

EVALUATION OF ANTIBACTERIAL ACTIVITY OF *GARCINIA KOLA* (BITTER KOLA), GINGER AND HONEY ON SOME HOSPITAL ISOLATES FROM SOKOTO

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DEDICATION

This dissertation is dedicated to my beloved parents, brothers, sisters, and my lovely wife and daughter who prayed for me tirelessly for the success of this work. May the almighty God bless them.

CERTIFICATION

This dissertation by okeh George rapuruchukwu (14211228014) has met the requirements for the award of the Degree of master of Medical Laboratory Sciences (medical microbiology) of the Usmanu Danfodiyo University, Sokoto State and is approved for its contribution to knowledge.

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LIST OF ABBREVIATIONS

D/W	Distilled water
EA	Ethyl acetate
EABK	Ethyl acetate bitter kola extract
EAG	Ethyl acetate ginger extract
ERY	Erythromycin
H	Honey
MBC	Minimum bactericidal concentration
MBK	Methanol bitter kola extract
Met	Methanol
MG	Methanol ginger extract
MIC	Minimum inhibitory concentration
NEG. CON.	Negative control
NH	N-hexane
NHBK	N-hexane bitter kola extract
WHO	Word health organization
SEM	Standard error mean
STD DRUGS	Standard drugs
TET	Tetracycline
W/V	Weight per volume
NHG	N-hexane ginger extract
G	Growth
T/S	Turbidity seen
N/T	No turbidity
N/G	No growth

ABSTRACT

The combination of honey, bitter kola and ginger in our society have become routine act without enough research work backing the combination as regard to whether they are synergistic, additive or antagonistic. The present study aimed to determine the in vitro antibacterial potential of combining honey, bitter kola, and ginger against isolated pathogens, which include *S. aureus, E. coli and P. aeruginosa* using well diffusion method. Honey, ginger and seeds of bitter kola were purchased from kara kasua (kara market) in Sokoto State, Nigeria. The methanol, ethyl acetate and n-hexane extract of bitter kola and ginger were obtained using maceration method at 1:5 g/v ratio of plant and solvent. Zone of inhibition of various extracts and honey was tested on *S. aureus, E. coli and P. aeruginosa* individually and in combination forms. Undiluted honey indicated highest zone of inhibition on *S. aureus* (14.0±0.3mm) and lowest on *P. aeruginosa* (7.5±0.3mm). Bitter kola extract indicated highest inhibition on *S. aureus* (17.0±0.6 mm) and lowest on *E. coli* (11±0.0mm) and *P. aeruginosa* (11±0.0mm)from 166.7mg/ml ethyl acetate concentration. The combination of honey and bitter kola extracts unveiled a synergistic effect on *S. aureus* . The combined methanol bitter kola extracts was observed to show a synergistic effect among *S. aureus*when compared with individual action of honey and methanol bitter kola. A negligible zone of inhibition was observed from ginger combination with honey. Finally, the study suggests the antimicrobial potential of bitter kola, honey or its combination as an antibiotic.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of study

There has been a steady rise in antibiotic resistance of bacteria that urgently calls for the discovery of alternative therapeutic agents. Plants are traditionally used for treatment of bacterial infections though they are not clinically regulated due to lack of awareness and enough data to support the reported therapeutic claims. Honey possesses therapeutic potentials which include antimicrobial activity. The antimicrobial activity of honey has been effectively established against an extensive spectrum of microorganisms.Microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* frequently are isolated from skin wounds (Tan *et al.,* 2009). There are many reports of honey being very effective as an adjunct in the treatment of wounds, burns, skin ulcers and as an anti-inflammatory agent (Lusby, 2002). Honey can inhibit the growth of a wide range of bacteria, fungi, protozoa and viruses (Molan, 1992). Honey also contains various constituents such as water, carbohydrates, proteins, vitamins, amino acid and minerals. Honey in general has high sugar content but a low water content and acidity, which prevent microbial growth (Tan *et al.,* 2009). Most types of honey generate hydrogen peroxide when diluted due to the activation of the enzyme glucose oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide (Bang *et al.,* 2003). The bactericidal action could also be ascribed to the normal acidity of honey, its high sugar content, nitrogenous or other compounds (Basualdo *et al.,* 2007).

Garcinia kola (Bitter kola), "*Namijin Goro"* in Hausa *"Orogbo*" in Yoruba; *"Akili"* in Ibo, is a member of the family Gattiferae . It is also commonly called false or male kola. It is a

tree plant that is commonly found in tropical West African countries such as Nigeria and Sierra Leone, They have, in chewing a bitter astringent and resinous taste, somewhat resembling that of the raw coffee bean, with a residual slight sweetness. The seeds are chewed as stimulants among the native. Medicinally, the seeds are said to prevent or relieve colic and particularly they are curative for colds in the head or chest, relieving cough and hoarseness and improving the singing voice. Other medicinal uses of the plant include, its use as an anti-hypertensive, treatment of urinary tract infections,treatment of liver disorders, aphrodisiac and as a chewing stick.

Ginger (*Zingiber officinale*) is a native plant of Southeast Asia. It is reported to have number of pharmacological activities. Its efficacy is due to its aromatic, Carminative and absorbent properties (Ali *et al.,*2008).Ginger (*Zingiber officinale*) is a flowering plant, in the familyZingiberaceae whose rhizome,ginger root or simplyginger, is widely used as a spice or a folk medicine.Ginger originated in the lush tropical jungles in Southern Asia. Although ginger no longer grows wild, it is thought to have originated on the Indian subcontinent. The ginger plants grown in India show the largest amount of genetic variation. The larger the number of genetic variations, the longer the plant has grown in that region. (*“Zingiber officinale information from NPGS/GRIN”. Ars-grin.gov. Retrieved 3 March 2008;“10 Health Benefits of Ginger Root”. Retrieved 2 Feb 2016.)*.In line with the generally accepted methods of searching for biological active substances from plants (Fatope *et al.,* 1993) the research is aimed at screening for in-vitro antibacterial activity of the methanol, ethyl acetate and N-hexane soluble extracts of seeds of *Garcinia kola*, Ginger and honey against selected pathogen. The objective of this study is to evaluate

theindividual and combinedantibiotic activity of ginger, bitter kola and honey against selected pathogens using a broth dilution method and agar well diffusion method.

1.2Statement of the problem

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is diminished, leading to very serious threat to public health, and for all kinds of antibiotics, including the major last-resort drugs, the frequencies of resistance are increasing worldwide(Levy and Marshall, 2004; Mandal *et al.,* 2009). Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products, including honey (Mandal *et al.,*2010; Basualdo *et al.,* 2007). Over the past two decades, there have been increase developments of drug resistance in pathogenic microorganisms as well as the occurrence of undesirable side effects of certain antibiotics and the emergence of previously uncommon infections. This has led to an increase search for drugs replacement with plants possessing antimicrobial properties and a continuous search for medicinal plants to provide a new source of possible effective and faster antimicrobial drugs to combatresistance bacterial.Traditional medical practice has assumed exalted status in various communities around the world. According to theBalick *et al.,* (1996) an estimated 80% of people living in developing countries rely on harvested wild plants for their primary health care. Several reports on the antibacterial activities of medicinal plants against pathogenic organisms abound in literatures.

1.3Justification

The continuous spread of multidrug-resistant pathogens has become a threat to public health and a major concern for infection control practitioners worldwide. In addition to increasing the cost of drug regimens, this scenario has paved way for re-emergence of previously controlled diseases and has contributed substantially to high frequency of opportunistic and chronic infection cases in developing countries Fauci, (2001). For long people has been using this herb individually or combination as antimicrobial agent in our society, without adequate knowledge of it pharmacokinetics activity. And this include the use of honey in combination with bitter kola, Ginger etc. This calls for alternative means of treatment, continuous search for new drugs, improvement, maintenance of plant and other source for treatment. This will lead to improvement in safety use of herbsand it may serve to get remedy for long resistance antibacterial organism.

1.4.0 Aims and objectives of the study.

1.4.1Aims

To determine the in vitro evaluationof antibacterial potential of bitter kola, ginger and honey against isolated pathogen.

1.4.2 Objective of the study

1. To determine theindividual antibacterial potential of honey, ginger and bitter kola extracts at different concentration on isolated pathogens.

2. To determine the combined antibacterial potential of honey and bitter kola extracts, honey and ginger extract at different concentration on isolated pathogens.

3. To determine thephytochemical analysis of bitter kola and ginger extracts.

CHAPTER TWO

2.0 LITERATURE REVIEW

The use of “natural” or alternative medicines has increased markedly over the last few years. More and more older adults are using complementary and alternative medicine dietary supplements and herbal remedies without advice from a physician on the assumption that these substances will have a beneficial effect (Cohen *et al.,* 2002). However, this might not be a safe or advisable practice. For example, at least one recent survey revealed a significant problem with herb-chemotherapeutic drug interactions in cancer patients and, notably, at least half of the herbal remedies taken by these patients lacked research data documenting their potential interactions (Engdal*et al.,* 2009). Regrettably, a great deal of the information regarding the effectiveness and safety of these remedies has been gathered from short stories or historical accounts, and much of the information offered is generally misleading and might even be detrimental (Ernst and Schmidt, 2002).

2.1 Taxonomy of honeybee

Honeybees have long been known by the people of Indonesia, this state can be known by the various names of bees in different local languages, for example nyiruan (Sundanese), tawon (Javanese), labah (Minang) and loba (Batak) (Apiari Scout Beekeeping Center, 2003).

In general, the classification of honeybees can be explained as follows:

Kingdom : Animalia

Phylum : Arthropods

Class : Insecta

Order : Hymenoptera

Family : Apidae

Genus : Apis

Species : *Apis andreniformis*, *Apis cerana*, *Apis dorsata*, *Apis florae, Apis laboriosa*, *Apis mellifera*

According toHailm and Suharno, (2001)honeybees consist of five types, namely *Apis florae*, *Apis trigona*, *Apis cerana*, *Apis mellifera* and *Apis dorsata*. However, not all honeybees can be cultivated (Apiari Scout Beekeeping Center, 2003).

2.2 Antibacterial bioactive compounds in honey

Honey bioactive compounds that possesantibacterial activitywere classified into inhibine and non-inhibine compounds. Inhibine was declared as forming enzyme and the accumulation of hydrogen peroxide (H_2O_2) in dilute honey and nectar (White *et al.,* 1963). The hydrogen peroxide has long been known as an effective antibacterial and is the main component of some penicillin especially notatin (glucose oxidase). Various bacteria are very sensitive to inhibine and Gram-negative bacteria are more sensitive than Gram-positive bacteria (Willix *et al.,* 1992). However, inhibine is very sensitive to heat and its presence in honey is determined by the type, age, and condition and processing of honey. Non-inhibine compounds are bioactive compounds that have the potential of honey as an antibacterial. The types of antibacterial compounds belonging to the compound group include alkaloids, flavonoids, glycosides and other compounds. The content of bioactive compounds in each type of honey is different between one with another, this difference is influenced by the source of nectar for honeybees (Bogdanov, 1997). The honeybees feed on difference nectarand thismay cause differences in the characteristics of honey such as color, flavor and aroma as well as the antibacterial activity of honey. Differences in inhibition of honey to some tested pathogenic bacteria was thought to be influenced by differences in the levels and types of group of bioactive compounds used, so testing on different species of bacteria can produce different inhibition that characterized by differences in the diameter of inhibition zone.Previous comparative study about antibacterial activity assays from three types of forest honey and other types of cultivated honey against *S. typi* and *E. coli* indicates that all three types of wild honey has better antibacterial activity rather than

cultivated honey. The commonly used honey is obtained from the cultivation of honeybee species,*A. mellifera* that feed with one type of nectar (monoflora) and several types of nectar (multiflora). Previous research that forest honey from Sumbawa, West Nusa Tenggara (SB1) have the highest antibacterial activity against*E. coli* and *S. thypi* compared with other types of honey, with the largest diameter of inhibition zone are 32.5 and 27.5 mm, respectively. While forest honey obtained from Kalimantan Barat (KB1) have the highest antibacterial activity against *S. thypi* compared with other types of cultivated honey with diameter of zone of inhibition which is 12.8 mm. Results from zones of inhibition of forest honey from Sumatra (SM1) are known to have the highest antibacterial activity against *E. coli* and equivalent tothe honey from rubber tree which has the highest antibacterial activity against *E. coli* among the types of other cultivated honey and were classified as very strong with the diameter of the inhibition zone to be 25 mm(Bogdanov, 1997).

2.3 Medicinal property of honey

Honey is an ancient remedy for the treatment of infected wounds, which has recently been ‘rediscovered’ by the medical profession, particularly where conventional modern therapeutic agents fail. The first written reference to honey, a Sumerian tablet writing, dating back to 2100-2000 BC, mentions honey's use as a drug and an ointment. Aristotle (384-322 BC), when discussing different honeys, referred to pale honey as being “good as a salve for sore eyes and wounds”. Manuka honey has been reported to exhibit antimicrobial activity against pathogenic bacteria such as *Staphylococcus aureus* and *Helicobacter pylori* making this honey a promising functional food for the treatment of wounds or stomach

ulcers (French *et al.,* 2005).The honey has been used from ancient times as a method of accelerating wound healing (Vandenberg *et al.,* 2008), and the potential of honey to assist with wound healing has been demonstrated repeatedly, (Simon *et al.*, 2008). Honey is gaining acceptance as an agent for the treatment of ulcers, bed sores and other skin infections resulting from burns and wounds (Cooper *et al.*, 2002). The healing properties of honey can be ascribed to the fact that it offers antibacterial activity, maintains a moist wound environment that promotes healing, and has a high viscosity which helps to provide a protective barrier to prevent infection (Lusby *et al.*, 2005). There are many reports of honey being very effective as dressing of wounds, burns, skin ulcers and inflammations; the antibacterial properties of honey speed up the growth of new tissue to heal the wound (Lusby *et al.,* 2002). The medihoney and manuka honey have been demonstrated to have *in vivo* activity and are suitable for the treatment of ulcers, infected wounds and burns(Lusby *et al*., 2005; Al-Waili *et al*.,2005). The honey when applied topically, rapidly clears wound infection to facilitate healing of deep surgical wounds with infection (Ahmed *et al .,*2003). The application of honey can promote the healing in infected wounds that do not respond to the conventional therapy, *i.e.*, antibiotics and antiseptics(Ahmed *et al.,* 2003), including wounds infected with methicillin-resistant *S. aureus*(Natarajan*et al.,* 2001; Dunford *et al.,* 1999). Moreover, it can be used on skin grafts and infected skin graft donor sites successfully (Misirlioglu *et al*., 2003).The manuka, jelly bush and pasture honeys are capable of stimulating the monocytes, the precursors of macrophages, to secrete TNF-α (Tonks *et al.,* 2003; Tonks *et al.,* 2001). On the other hand, glycosylated proteins can induce TNF-α secretion by macrophages, and this cytokine is known to induce the mechanism of wound repairing.Furthermore, the ability of honey to reduce 'reactive

intermediates release' (Tonks *et al.,*2001) may well limit tissue damage by activated macrophages during wound healing. Thus, the immunomodulatory property of honey is relevant to wound repair.The support for using honey as a treatment regimen for peptic ulcers and gastritis comes from traditional folklore as well as from reports in modern times. Honey may promote the repair of damaged intestinal mucosa, stimulate the growth of new tissues and work as an anti-inflammatory agent. Raw honey contains copious amounts of compounds such as flavonoids and other polyphenols which may function as antioxidants(Blassa *et al.,* 2006). Clinical observations have been reported of reduced symptoms of inflammation when honey is applied to wounds. The removal of exudate in wounds dressed with honey is of help in managing inflamed wounds (Ahmed*et al.,* 2003).

2.4Antibacterial activity of honey

The use of honey as a traditional remedy for microbial infections dates back to ancient times. Research has been conducted on manuka (*L. scoparium*) honey, which has been demonstrated to be effective against several human pathogens, including *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium*, *S. aureus*(Lusby *et al.,* 2005). Laboratory studies have shown that the honey is effective against methicillin-resistant *S. aureus* (MRSA), β-*haemolytic streptococci* and vancomycin-resistant *Enterococci* (VRE) (Allen*et al.,* 2000). However, the newly identified honeys may have advantages over or similarities with manuka honey due to enhanced antimicrobial activity, local production (thus availability), and greater selectivity against medically important organisms (Lusby*et al.*, 2005). The coagulase-negative *staphylococci* are very similar to *S. aureus*(Cooper *et*

al., 2002) in their susceptibility to honey of similar antibacterial potency and more susceptible than *Pseudomonas aeruginosa* and *Enterococcus* species (Cooper*et al.,* 2002).

2.5Zone diameter of inhibition of different honey

The zone diameter of inhibition (ZDI) of different honey samples (5%–20%) has been determined against *E. coli* O157: H7 (12 mm – 24 mm) and *S. typhimurium* (0 mm – 20 mm). The ZDIs of Nilgiris honeys were found to be (20–21) mm, (15-16) mm and (13–14) mm for *S. aureus*, *P. aeruginosa* and *E. coli*, respectively (Rajeswari*et al.,*2010). According to Agbagwa and Frank-Peterside(2010) investigation of different honey samples: Western Nigerian honey, Southern Nigerian honey, Eastern Nigerian honey and Northern Nigerian honey, and compared their abilities to inhibit the growth of *S. aureus*, *P. aeruginosa*, *E. coli* and *Proteus mirabilis* with an average of ZDIs (5.3–11.6) mm, (1.4–15.4) mm, (4.4–13.5) mm and (9.1–17) mm, respectively, and with honey concentrations of 80%–100%. The extracts of raw and processed honey showed ZDI (6.94–37.94) mm, against gram-positive bacteria *viz.*, *S. aureus*, *Bacillus subtilis*, *Bacillus cereus*, as well as gram-negative bacteria like *E. coli*, *P. aeruginosa* and *S. enterica* serovar Typhi.

2.6.0 Antibacterial activity of ulmo and manuka honeys based on the ZDI produced for clinical (C) MRSA and standard (S) MRSA, *E. coli* and *P. aeruginosa* isolates.

2.6.1 Minimum inhibitory concentration

The MIC assay showed that a lower MIC was observed with ulmo (*Eucryphia cordifolia*) honey (3.1% – 6.3% v/v) than with manuka honey (12.5% v/v) for MRSA isolates; for the

E. coli and *Pseudomonas* strains equivalent MICs were observed (12.5% v/v)(Sherlock *et al.,*2010). The MICs for Tualang honey ranged 8.75% - 25%, while those for manuka honey ranged 8.75% – 20% against many pathogenic gram-positive and gram-negative bacteria(Tan *et al.,* 2009). The MICs of manuka, heather, khadikraft and local honeys against clinical and environmental isolates of *P. aeruginosa* were recorded as 10% – 20%, 10% – 20%, 11% and 10% – 20%, respectively(Mullai*et al.,* 2007). The MICs of *A. mellifera* honey ranged (126.23 – 185.70) mg/mL and of *Tetragonisca angustula* honey (142.87 - 214.33) mg/mL against *S. aureus*. The *Egyptian clover* honey MIC was 100 mg/mL for *S. typhimurium* and *E. coli* O157:H7. The Nilgiri honey MICs were 25%, 35% and 40% for *S. aureus*, *P. aeruginosa* and *E. coli*, respectively. MIC values of honey extracts were found in the range of (0.625–5.000) mg/mL, for *S. aureus*, *B. subtilis*, *B. cereus*, and gram-negative bacteria (*E. coli*, *P. aeruginosa* and *S. typhi*(Chauhan *et al.,* 2010).

By visual inspection, the MICs of Tualang honey ranged 8.75% – 25% compared with those of manuka honey (8.75% – 20%) against wound and enteric microorganisms: *Streptococcus pyogenes*, coagulase-negative *Staphylococci*, MRSA, *Streptococcus agalactiae*, *S. aureus*, *Stenotrophomonas maltophilia* (*S. maltophilia*), *Acinetobacter baumannii* (*A. baumannii*), *S. Typhi*, *P. aeruginosa*, *Proteus mirabilis*, *Shigella flexneri, E. coli*, *Enterobacter cloacae* (*E. cloacae*) (Tan *et al.,* 2009). Six bacterial strains from burn-wound patients, namely, *Aeromonas schubertii* (*A. schubertii*), *Haemophilius paraphrohaemlyticus* (*H. paraphrohaemlyticus*), *Micrococcus luteus* (*M. luteus*), *Cellulosimicrobium cellulans* (*C. cellulans*), *Listonella anguillarum* (*L. anguillarum*) and*A.*

baumannii had MICs of Cirtrus, Clover, Nigella and Eljabaly honeys 35%–40%, 35%–40%, 35%–40%, 25%–30%, respectively, as has been reported by Hassanein *et al.,2011,* The honeys were inhibitory at dilutions down to 3.6% – 0.7 % (v/v), for the pasture honey, 3.4% – 0.5% (v/v), and for the manuka honey, against coagulase-negative *Staphylococci*

(French *et al.,* 2005).

2.7.0 Mechanism and factors affecting antibacterial activityof honey

2.7.1 Mechanism of antibacterial activity of honey

The beneficial role of honey is attributed to its antibacterial property with regards to its high osmolarity, acidity (low pH) and content of hydrogen peroxide (H_2O_2) and non-peroxide components, *i.e.*, the presence of phytochemical components like methylglyoxal (MGO)(Weston, 2000). The antimicrobial agents in honey are predominantly hydrogen peroxide, of which the concentration is determined by relative levels of glucose oxidase, synthesized by the bee and catalase originating from flower pollen(Weston, 2000). Most types of honey generate H_2O_2 when diluted, because of the activation of the enzyme glucose oxidase that oxidizes glucose to gluconic acid and H_2O_2, which thus attributes the antimicrobial activity(Bang *et al*., 2003). But, in some cases, the peroxide activity in honey can be destroyed easily by heat or the presence of catalase.

Besides H_2O_2, which is produced in most conventional honeys by the endogenous enzyme glucose oxidase, several other non-peroxide factors have been found to be responsible for the unique antibacterial activity of honey(Simon *et al.,* 2008). Honey may retain its

antimicrobial activity even in the presence of catalase (absence of glucose oxidase), and thus this type of honey is regarded as "non-peroxide honey". Several components are known to contribute the non-peroxide activity, such as the presence of methyl syringate and methylglyoxal, which have been extensively studied in manuka honey that is derived from the manuka tree (*L. scoparium*) (Mavric *et al.,* 2008; Adams *et al.,* 2008). Unlike manuka honey, the activity of ulmo honey is largely due to H_2O_2 production: 25 % (v/v) solution of ulmo honey had no detectable antibacterial activity when tested in presence of catalase, while, at the same concentration the manuka honey retained its antibacterial activity in the presence of catalase (absence of H_2O_2)(Sherlock *et al.,* 2010). Neither type of activity is influenced by the sterilizing procedure of gamma-irradiation.

Honey is characteristically acidic with pH between 3.2 and 4.5, which is low enough to be inhibitory to several bacterial pathogens. The minimum pH values for growth of some common pathogenic bacteria are:*E. coli* (4.3), *Salmonella* spp. (4.0), *P. aeruginosa* (4.4), *S. pyogenes* (4.5), and thus in undiluted honey the acidity is a significant antibacterial factor. The antibacterial property of honey is also derived from the osmotic effect of its high sugar content and low moisture content, along with its acidic properties of gluconic acid and the antiseptic properties of its H_2O_2(Khan *et al.,* 2007). A recent study examining the antimicrobial properties of honey *in vitro* found that H_2O_2, MGO and an antimicrobial peptide, bee defensin-1, are distinct mechanisms involved in the bactericidal activity of honey(Kwakman *et al.,* 2010).

2.7.2 Factors affecting antibacterial nature of honey

Asadi-Pooya *et al., (*2003) reported that the difference in antimicrobial potency among the different honeys can be more than 100-fold, depending on its geographical, seasonal and botanical source as well as harvesting, processing and storage conditions. The antibacterial nature of honey is dependent on various factors working either singularly or synergistically, the most salient of which are H_2O_2, phenolic compounds, wound pH, pH of honey and osmotic pressure exerted by the honey. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects(Molan,1992). It has further been reported that physical property along with geographical distribution and different floral sources may play important role in the antimicrobial activity of honey (Taormina *et al.,* 2001). Several authors reported that different honeys vary substantially in the potency of their antibacterial activity, which varies with the plant source (Lusby*et al.,* 2005; Wilkinson *et al.,* 2005). Thus, it has been shown that the antimicrobial activity of honey may range from concentrations < 3 % to 50 % and higher (Lusby *et al.,* 2005; French *et al.,* 2005; Wilkinson *et al.,* 2005). The bactericidal effect of honey is reported to be dependent on concentration of honey used and the nature of the bacteria,(Adeleke *et al.,* 2006). The concentration of honey has an impact on antibacterial activity; the higher the concentration of honey the greater its usefulness as an antibacterial agent(Badawy *et al.,* 2004). Taormina *et al.,* (2001) reported that the concentration of honey needed for complete inhibition of *S. typhimurium* growth is <25%.It is important to know thatmicrobial resistance to honey has never been reported (Dixon, 2003), which makes it a very

promising topical antimicrobial agent against the infection of antibiotic-resistant bacteria (*e.g.*, MDR *S. maltophilia*) and in the treatment of chronic wound infections that do not respond to antibiotic therapy. Hence honey has been used as a last-resort medication. Manuka honey has been widely researched and its antibacterial potential is renowned worldwide. The potency of honeys, such as Tualang honey, against microorganisms suggests its potential to be used as an alternative therapeutic agent in certain medical conditions, particularly wound infection.Lusby *et al.,*(2005) reported that honeys other than the commercially available antibacterial honeys (*e.g.*, manuka honey) can have equivalent antibacterial activity against bacterial pathogens. The growth of bacterial species that cause gastric infections, such as *S. typhi*, *S. flexneri* and *E. coli*, are inhibited by Tualang honey at the low concentrations. The Tualang honey has been reported to be effective against *E. coli*, *S. typhi* and *S. pyogenes*(Tumin *et al.,* 2005), and thus, when taken orally in its pure undiluted form, this honey may help speed up recovery from such infections. Honey is effective when used as a substitute for glucose in oral rehydration and its antibacterial activity shortened the duration of bacterial diarrhoea.Currently, the emerging antimicrobial resistance trends in burn wound bacterial pathogens are a serious challenge(Church *et al.,* 2006). Thus, honey with effective antimicrobial properties against antibiotic-resistant organisms such as MRSA and MDR *P. aeruginosa, Acinetobacter* spp. and members of the family *Enterobacteriaceae*, which have been associated with infections of burn wounds and in nosocomial infections, is much anticipated (Church et *et al.,* 2006).Overall, the unpredictable antibacterial activity of non-standardized honey may hamper its introduction as an antimicrobial agent due to variation in the *in vitro* antibacterial activity of various honeys. At present a number of honeys are sold with

standardized levels of antibacterial activity, of which the best known is manuka (*Leptospermum*) honey as well as Tualang (*Koompassia excelsa*) honey. The medical-grade honey (Revamil, medihoney), which has the potential to be a topical antibacterial prophylaxis because of its broad-spectrum bactericidal activity, or to be a treatment for topical infections caused by antibiotic-resistant as well as antibiotic-sensitive bacteria, should be considered for therapeutic use. Moreover, mountain, manuka, capillano and eco-honeys have exhibited inhibitory activity against *H. pylori* isolates at concentration 10% (v/v), demonstrating that locally produced honeys possess excellent antibacterial activity comparable to the commercial honeys. Therefore it is necessary to study other locally produced but yet untested honeys for their antimicrobial activities.

2.8 Origin and distribution of *Garcinia kola*

Bitter kola has also been recognized as an indigenous medicinal plant found in rain forest ofcentral and western Africa, especially Benin, Cameroon, Democratic Republic of Congo, *Cote d'Ivoire*, Gabon, Ghana, Liberia, Nigeria, Senegal and Sierra Leone. Its natural habitat is thus the subtropical or tropical moist lowland forests. It is usually found in the coastal areas and low land plains up to 300 m above sea level with an average of 2500 mm of rainfall per annum. The major places where the plant would be found growing in the wild are forest reserves and free areas of the rain-forest or it is planted or conserved in on-farm oil-palm, cocoyam plantations. These growing regions have low altitude with annual temperature ranging from 32.15^{0}C to 21.4^{0}C anda relative humidity of 76.34% (Ntamag, 1997). In the coastal rainforests of south-western and south-eastern parts of Nigeria the nut is chewed and readily served to visitors as a sign of goodwill. Bitter kola is enjoyed by the

three major ethnic groups in Nigeria (i.e. the Yoruba's, the Igbo's and the Hausa's), from whom it derived the local names *akilu/agbilu*in Igbo`s and *Orogbo* in Yoruba languages.The local market for bitter kola extends beyond the southern production areas to the northern parts of the country. In Nigeria, its trade is as important as that of kola nut (*Cola nitida and C. acuminata*) in major towns and cities in southern parts of the country where the tree is common. *Garcinia kola* or bitter kola is a tree that grows in the rain forests of west Africa. The fruit, seeds, nuts and bark of the plant have been used for centuries in folk medicine to treat ailments from coughs to fever. According to a report from the Center For International Forestry Research, *Garcinia kola* trade is still important to the tribes and villages in Nigeria. As with any herb, never consume *Garcinia kola* without first discussing its use and benefits with your physician, especially if you are currently being treated for other medical conditions or are on any medications(Ntamag, 1997).

Figure 2.1 *Garcinia kola* - MHNT

2.9 Botanical and Agronomic Characteristics of BitterKola

According to an earlier description by Heckel and Schlagdenhauffen, (1884), bitter kola is a tree of variable aspect, well branched, ever-green and grows to a height of about 12m. Towards the base of the branches are large opposite leaves (12" long by 7" broad), with short petioles, while at the extremity of the branches, the leaves are much smaller (5" by 2"). The leaves are oval, slightly dilated at the base, full green on the upper surface and greenish underneath. Ladipo, (1995) reported that the tree produces reddish yellowish or orange colored fruit, with each fruit containing two to four yellow seeds and a sour tasting pulp. The seeds when consumed have bitter astringent taste. The fruit is classified a berry, the size of an apple with a rugos epiderm covered entirely with rough hairs. As a tropical fruit tree species, it is characterized by slow rate of growth (Ladipo, 1995). Cultivation of the plant is limited because of poor germination and the length of time it takes (about 10 -

15 years) to reach reproductive phase. In Nigeria, the demand for bitter kola is high but the production is limited due to problem of seed dormancy; untreated seeds are difficult to germinate. Farmers believe that germination of bitter kola takes about six to twelve months and that only a few seeds germinate. There is also the problem of setting up nurseries. However, Anegheh *et al.,* (2006) developed a pre-nursery treatment to breakdormancy and enhance germination. This work revealed that seed cutting (nicking) was very effective in enhancing germination of *Garcinia kola*. The seed is first raised in the nursery and then transplanted to the field. Fruiting commences in July and ends in October, while harvest continues as ripe fruits fall and are collected for the extraction of seeds. Ladipo, (1995) reported that a mature fruit tree of 10 to 15 years produces 85 to 1717 fruits, with 208 to 6,112 seeds. Taking the mean of these values at 834 fruits and 2,627 nuts per tree he projected a fruit production of 26 tonnes per ha per annum with 278 trees per hectare at 6 x 6m spacing. When ripe, the green pericarp of the fruit turns reddish yellow color and the fruit falls. The fruits are gathered, broken and stored in an open cool place to allow for fermentation of the pericarp and pulpy mesocarp. Thereafter, they are threshed to release the seeds which are washed to remove the sticky mucilaginous material that sheaths them. The seeds or nuts that are not sold fresh are air dried and stored in baskets lined with jute bags. According to Ofor *et al.,* (2004) storage of bitter kola in polyethene bags is favored in terms of shelf life and palatability.

Scientific	Classification
Kingdom	Plantae
(unranked):	Angiosperms

(unranked):	Eudicots
(unranked):	Rosids
Order:	Malpighiales
Family:	Clusiaceae
Genus:	*Garcinia*
Species:	*G. kola*
(unranked):	Angiosperms
Bonomial name	*Garcinia kola*

Heckel and Schlagdenhauffen (1884)

2.10Nutrient composition of *Garcinia kola* nuts

*Garcinia kola*contains nutrients such as proteins, carbohydrates, fiber, minerals, fat and oils. Ibekwe *et al.,* (2007) reported that *Garcinia kola* seed has poor nutrient composition but highly valued in trado-medicine due to its useful active phytochemical composition. Contrary to the nutrient compositions of *Garcinia kola* reported by Ibekwe *et al.,* (2007)in Table2.1, Esiegwu and Udedibie, (2009) reported nutrient compositions of *Garcinia kola*as shown inTable 2.2. Odebunmi *et al.,* (2009) reported the moisture content of the seeds to be 60.48 + 0.06%, dry matter of 39.52 + 0.06%, crude fat of 4.51 + 0.56%, crude protein of 2.48 + 0.10%, ash content of 0.79 + 0.005%, crude fiber of 5.23 + 0.16% and total carbohydrates (+ fiber) of 35.64%. These values are different from what had previously been reported for bitter kola. Eleyinmi *et al.,* (2006) reported a protein content of 3.95%, lipid of 4.33%, ash of 1.14% and a crude fiber content of 11.4% in the seed.

Table 2.1Nutrient composition of Garcinia kola (% of dry matter)

Components	Amount%
Moisture	14.60
Crude protein	0.58
Crude fiber	0.10
Ether extracts	3.00
Ash	5.00
Nitrogen free-extract	91.32

Adapted from Ibekwe *et al.,*(2007).

Table 2.2 Nutrient composition of Garcinia kola(% of dry matter)

Components	Amount%
Dry matter	7.30
Crude protein	2.64
Crude fiber	20.51
Ether extracts	9.47
Ash	1.07
Nitrogen free-extract	57.54

Adepted from Esiegwu and Udedibie, (2009)

2.11Chemical composition of *Garcinia kola*:

Chemical analysis of *Garcinia kola* seed in Nigeria as reported by Okwu, (2005) showed that itcontains a wide range of vitamins and minerals as shown in tables 2.3 and 2.4. According to Odebunmi *et al.,* (2009), *Garcinia kola* has 722.10 mg/100g of potassium (K), 67.07 ± 0.12 mg/kgDM of calcium (Ca), 114.83 ± 3.47 mg/kg DM of magnesium (Mg), 6.10 ± 0.43 mg/KgDM of iron (Fe), 2.30 ± 0.08 mg/kgDM of zinc (Zn), and 188.57 ± 0.37 mg/kg DM of phosphorus (P). Asaolu, (2003) also reported that the fresh seeds of bitter kola (wet weight) contains high moisture content of 75.50% and dry weight of 24.50, while the ash content was found to be 5.90%, crude fat was 14.50%, carbohydrate was 10.85%, crude fat was 14.50% and crude protein was found to be very low (4.25%). Dosunmi and Johnson, (1995) in comparing the nutritive value of the fresh fruit from Nigeria showed that crude protein was higher in the mesocarp (7.8%) than in the pericarp (3.9%), while the pericap was richer in crude fiber (13.9% - 16.5%). The mesocarp was also richer in crude lipid (6.9% - 8.7%). Unsaturated fatty acids (linoleic acid, 40.5%, oleic acid, 30.8%) are the main components of the lipids (4.5%) found in the seeds of this species (Essien *et al.,* 1995; Omode *et al.,* 1995).

Table 2.3 Vitamin composition of Garcinia kola seeds(dry weight basis)

Vitamins	Amount(mg/100g)
Thiamin(vit. B1)	0.5 ± 40.30
Riboflavin(vit. B2)	0.22 ± 0.01
Niacin(nicotinic acid)	1.60 ± 0.01
Ascorbic acids(Vit.C)	9.47±0.7

Adepted from Okwu(2005).

Table 2.4 Mineral composition of Garsinia Kola seeds (dry weight basis)

Vitamins	Amounts(mg/100g)
Macro elements	
Calcium	0.80 ± 0.40
Magnesium	0.42 ± 0.30
Potassium	2.50 ± 0.10
Phosphorus	0.33 ± 0.10
Sodium	0.72 ± 0.10
Iron	17.75 ± 0.30
Zinc	17.75 ± 0.30
Copper	0.78 ± 0.20
Manganese	2.01 ± 0.50
Chromium	0.00±0.50
Cobalt	0.55 ± 0.20
Cadmium	0.29 ± 0.10

Adapted from Okwu (2005)

2.12Phytochemical Constituents of *Garcinia kola*:

The role of phytochemicals in enhancing body cell immunity against diseases in the body cannot be overemphasized. The active constituents contributing to the protective effect of *Garcinia kola* on animals is attributed to the presence of phytochemicals, vitamins and minerals (Okwu and Ekeke, 2003). Phytochemicals exhibit a wide range of biological activities as a result of the anti-oxidant properties of some of these chemicals. Several types of polyphenols (phenolic acid, hydrolysable tannins and flavonoids) show anti-carcinogenic and mutagenic effects (Uruquiaga and Leighton, 2000). Okwu, (2005) and Esiegwu and Udedibie, (2009) reported the phytochemical values as shown in table 2.5 and 2.6.

Table 2.5 Phytochemical constituents of Garcinia kola seeds (Dry weight basis)

Constituents	Amount(mg/100g)
phenols	0.11 ± 0.20
Alkaloids	0.36 ± 0.10
Tannins	0.26 ± 0.20
Flavonoids	1.98± 0.20

Adapted from Okwu(2005).

Table 2.6 Phytochemical constituents of Garcinia kola seeds (Dry weight basis)

Constituents	Amount(mg/100g)
Cyanogenic glycosides	0.54
Tannins	0.34
Saponins	10.06
Alkaloids	4.93

Adapted from Esiegwu and Udedibie (2009)

Garcinia kola stem has been shown to contain a complex mixture of phenolic compounds such as biflavonoids, xanthones and benzophenone (Iwu and Igboko, 1982) which have anti-microbial activity as kolanone (Hussain *et al.,* 1982), kola flavonone and garcinia flavonone. Phytochemical studies have shown that the seeds constituents include biflavonoids, xanthones and benzophenones. Thus, the seeds of *Garcinia kola* are known to have a general antidote effect in traditional medicine in Africa. These possibly explain its reported aphrodisiac properties and in the treatment of catarrh and abdominal colicky pain. In addition, their use is believed to improve singing voice and relieve cough (Irvin, 1961). Investigation by Amalu *et al.,(2014)* showed that the seed of *Garcinia kola*had some degree of inhibitory effects on *Staphylococcus aureus* (Gram +ve) and *E. coli*(Gram – ve), with no inhibitory effect on *Candida albicans.*According to Madubunyi, (2008) investigation with petroleum ether, 70% ethanol and water as extraction solvents. The ethyl acetate fraction of the ethanol extract, which showed the maximum antimicrobial activity, was recovered in a 2.4% w/w yield. Antimicrobial evaluation of the differential solvent extracts of*G. kola*seeds revealed that the petroleum ether, ethanol, the milky layer and ethyl acetate fractions possessed antimicrobial properties. The observed activity was due to the presence of a polyisoprenyl benzophenone (Kolanone) in the petroleum ether extract as well as the hydroxybiflavanonols present in the ethyl acetate fraction. GB1 (a hydroxybiflavanonol) was found to be the main component exhibiting significant ($p<0.005$) antimicrobial activity against Gram-positive and Gram-negative bacteria in a bacteriostatic manner and against Candida albicans and Aspergillus flavus in a fungistatic manner. The MIC of GB1 against*S. aureus*was 3.1×10^{-7} µg/ml and 3.0×10^{-3} µg/ml against*E. coli*. Arekemase *et al.,* (2012) reported that small bitter kola seed ethanol and aqueous (hot water) extracts exhibited more

antimicrobial activity at a concentration of 30 mg/ml, with zones of inhibition ranging from 17 to 23mm for ethanol. The aqueous (hot water) extracts showed zones of inhibition ranging from 20 to 27mm. The extracts also showed antifungal activity against Aspergillus niger. The minimum inhibitory concentration (MIC) showed antimicrobial activity at lowest concentration of 0.008mg/ml and maximum concentration of 5.0mg/ml against *Staphylococcus aureus*. The results imply that the ethanol and aqueous extracts of *Garcinia kola* seed posses strong antibacterial and antifungal properties when compared with standard antibiotics amoxicillin; ciproxin; tetracycline and streptomycin used during the investigation, and hence its potential as a useful chemotherapeutic agent in the treatment of bacterial and fungal infections in humans.

2.13 Traditional uses of *Garcinia kola*

*Garcinia kola*is cultivated throughout West Africa for its edible fruit and seeds which are used as rejuvenating agent for masticatory purposes and as a general antidote (Ibiblio, 1983). Among the Igbos of Nigeria it i*s* presented to visitors as a sign of peace and welcome. It is also used to entertain guests during ceremonies and festivities. Again, it is popularly used among other Nigerian groups for nervous alertness and induction of insomnia when chewed. The kernels of the nuts are widely traded and eaten as a stimulant (Omode *et al.,*1995; Atawodi*et al.,* 1995). It is believed to clean the digestive system, without side effects such as abdominal problems, even when a lot of it is eaten (Onochie and Stanfield, 1960). In traditional medicine, the dried nut is ground and mixed with honey to make a traditional cough mixture. The ground nut may also be mixed with water and given to new born babies to relieve stomach cramps.

Experimentations using *Garcinia kola* kernels as hop substitutes in several indigenous alcoholic drinks as well as flavour enhancer in the beverage industry also exist (US Food and Drug Administration, 2014). Ofor *et al.,* (2004) identified several ethno-botanical uses to which the indigenes of Imo state in South–eastern Nigeria put the *Garcinia kola* seeds. These include as an antidote to snake bites, poison and overdose, for cough, vomiting and as a snake repellent. The seeds which serve as a bitter stimulant also serve as a snake repellent when they are placed round the compound. The seed is used in the treatment of diarrhoea (Braid 1991), bronchitis and throat infections (Orie and Ekon, 1993; Adesina *et al.,* 1995), liver disorders and enjoys a folk reputation in Africa as a poison antidote (Kabangu *et al.,*1987). According to Farombi *et al.,* (2000), the seeds of *Garcinia kola* have pharmacological uses in treating coughs, throat infections, bronchitis, hepatitis and liver disorders.

2.14 Ginger (***zingiber officinale***) is a flowering plant, in the familyZingiberaceae whose rhizome, ginger root or simplyginger, is widely used as a spice or a folk medicine.It is a herbaceousperennial which grows annual stems about a meter tall bearing narrow green leaves and yellow flowers. Ginger is in the family Zingiberaceae, to which also belong turmeric (*Curcuma longa*), cardamom (*Elettaria cardamomum*), and galangal. Ginger originated in the lush tropical jungles in Southern Asia. Although ginger no longer grows wild, it is thought to have originated on the Indian subcontinent. The ginger plants grown in India show the largest amount of genetic variation. The larger the number of genetic variations, the longer the plant has grown in that region.(*"10 Health Benefits of Ginger Root". Retrieved 2 Feb 2016*) Ginger was exported to Europe via India in the first century

AD as a result of the lucrative spice trade and was used extensively by the Romans.(*"What are the benefits of ginger?". Medical News Today 29 August 2014. Retrieved 11 November 2014 accessed 7th may 2016*)India is now the largest producer of ginger.

Table 2.7 Scientific classification of ginger.

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Monocots
Clade:	Commelinids
Order:	Zingiberales
Family:	Zingiberaceae
Genus:	*Zingiber*
Species:	***Z. officinale***

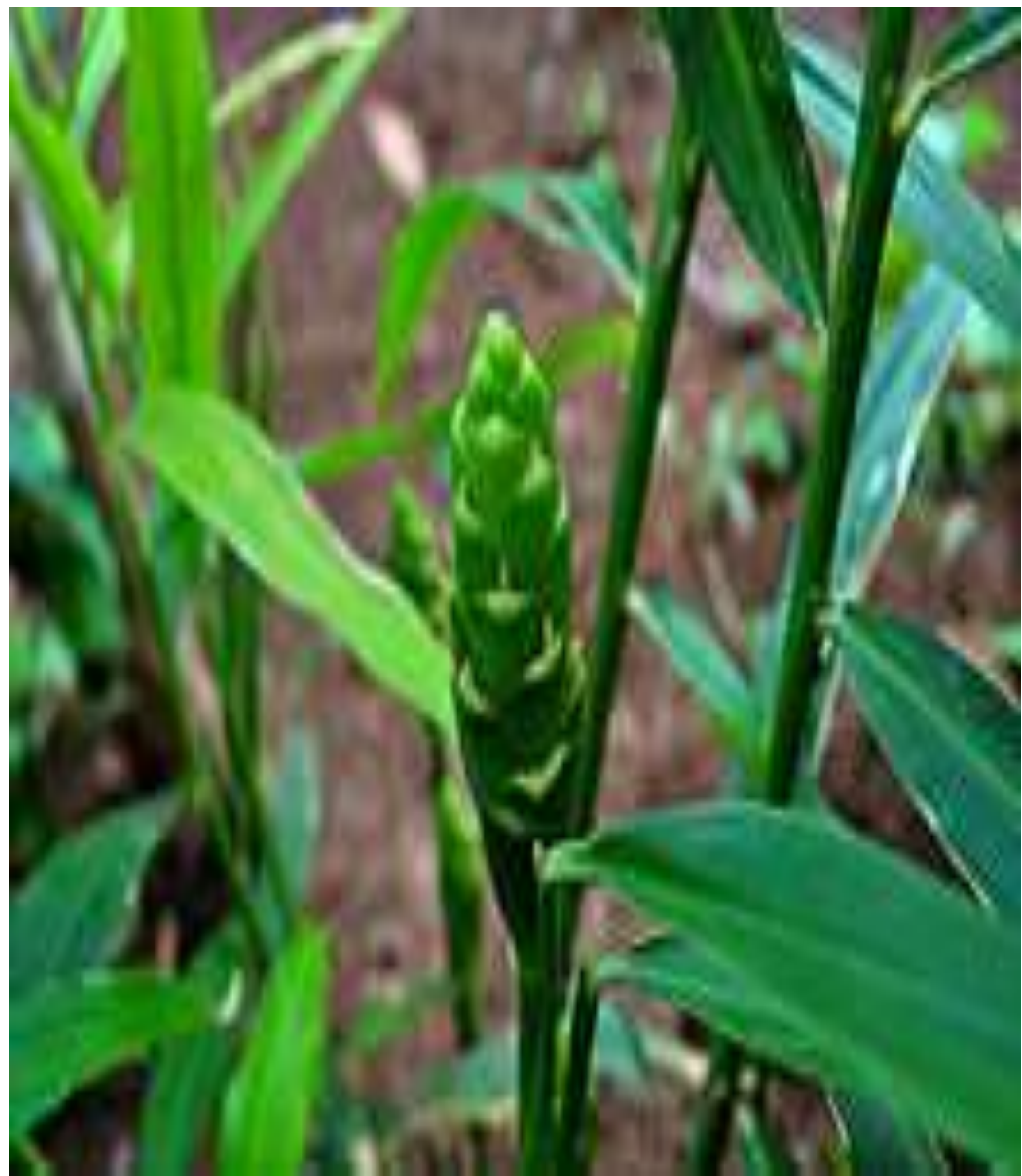
"Zingiber officinale information from NPGS/GRIN". ars-grin.gov. Retrieved 3 March 2008, accessed 7th may 2016.

2.15 History and origin of ginger

Ginger is a member of a plant family that includes cardamom and turmeric. Its spicy aroma is mainly due to presence of ketones, especially the gingerols, which appear to be the primary component of ginger studied in much of the health-related scientific research. The rhizome, which is the horizontal stem from which the roots grow, is the main portion of ginger that is consumed. Ginger's current name comes from the Middle English *gingivere*, but this spice dates back over 3000 years to the Sanskrit word *srngaveram*, meaning "horn root," based on its appearance. In Greek, it was called *ziggiberis*, and in Latin, *zinziberi*. Interestingly, ginger does not grow in the wild and its actual origins are uncertain.

Indians and Chinese are believed to have produced ginger as a tonic root for over 5000 years to treat many ailments, and this plant is now cultivated throughout the humid tropics, with India being the largest producer. Ginger was used as a flavoring agent long before history was formally recorded. It was an exceedingly important article of trade and was exported from India to the Roman Empire over 2000 years ago, where it was especially valued for its medicinal properties. Ginger continued to be a highly sought after commodity in Europe even after the fall of the Roman Empire, with Arab merchants controlling the trade in ginger and other spices for centuries. In the thirteenth and fourteenth centuries, the value of a pound of ginger was equivalent to the cost of a sheep. By medieval times, it was being imported in preserved form to be used in sweets. Queen Elizabeth I of England is credited with the invention of the gingerbread man, which became a popular Christmas treat.

Fig 2.1 Horticulture of ginger



Ginger plant with flower, south India

Ginger produces clusters of white and pink flower buds that bloom into yellow flowers. Because of its aesthetic appeal and the adaptation of the plant to warm climates, ginger is often used as landscaping around subtropical homes. It is a perennialreed-like plant with annual leafy stems, about a meter (3 to 4 feet) tall. Traditionally, the rhizome is gathered when the stalk withers; it is immediately scalded, or washed and scraped, to kill it and prevent sprouting. The fragrant perisperm of Zingiberaceae is used as sweetmeats by Bantu, and also as a condiment and sialagogue.

Table 2.7 Ginger production, 2013

Country	Production(millions of tones)
India	0.683
China	0.390
Nepal	0.235
Indonesia	0.233
Nigeria	0.160

Source: food and agricultural organization of United Nation, FAOSTAT.

In 2013 with a global production of 2.1 million tonnes of ginger, India accounted for 33%, followed by China (19%), Nepal, Indonesia and Nigeria (table 7)(*"Production/Crops for Ginger, World". Food and Agriculture Organization of the United Nations, Statistics Division. 2013. Retrieved 24 December 2015 accesssed 1st june 2016)*

2.16 Bioactive components of ginger

At least 115 constituents in fresh and dried ginger varieties have been identified by a variety of analytical processes. Gingerols are the major constituents of fresh ginger and are found slightly reduced in dry ginger, whereas the concentrations of shogaols, which are the major gingerol dehydration products, are more abundant (Jolad*et al.,*2005) in dry ginger than in fresh ginger. At least 31 gingerol-related compounds have been identified from the methanolic crude extracts of fresh ginger rhizome. Ginger has been fractionated into at least 14 bioactive compounds, including [4]-gingerol, [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-paradol, [14]-shogaol, [6]-shogaol,1-dehydro-[10]-gingerdione, [10]-gingerdione, hexahydrocurcumin, tetrahydrocurcumin, gingerenone A, 1,7-bis-(4′ hydroxyl-3′ methoxyphenyl)-5-methoxyhepthan-3-one, and methoxy-[10]-gingerol (Koh*et al.,* 2009). The proportion of each individual component in a sample of ginger depends on country of origin, commercial processor, and whether the ginger is fresh, dried, or processed (Schwertner *et a.,l* 2006). Of the bioactive pungent components of Jamaican ginger, including [6]-, [8]-, and [10]-gingerols and [6]-shogaol, [6]-gingerol appears to be the most abundant pungent bioactive compound in most of the oleoresin samples studied (Bailey*et al.,* 2008). Although phylogenetic analysis has showed that all ginger samples from widely different geographical origins are genetically indistinguishable, metabolic profiling showed some quantitative differences in the contents of [6]-, [8]-, and [10]-gingerols (Jiang*et al.,*2006). An examination of the concentrations of [6]-, [8]-, and [10]-gingerols and [6]-shogaol in 10 different ginger-root dietary supplements purchased randomly from a variety of pharmacies and health food stores yielded some disconcerting results (Schwertner*et*

*al.,*2006). Perhaps not surprisingly, the content of these active components was found to vary extensively from none or very minute amounts to several milligrams per gram. In addition, the suggested serving size ranged from about 250 mg to 4.8 g/day (Schwertner*et al.,* 2006). The basis for the wide range of dosing is not clear. These studies suggest that ginger contains a variety of bioactive compounds and standardization of contents is critically lacking. The characteristic odor and flavor of ginger is caused by a mixture of zingerone, shogaols, and gingerols, volatile oils that compose one to three percent of the weight of fresh ginger.The pungent taste of ginger is due to nonvolatilephenylpropanoid-derived compounds, particularly gingerols and shogaols, which form from gingerols when ginger is dried or cooked. Zingerone is also produced from gingerols during this process; this compound is less pungent and has a spicy-sweet aroma.

2.17Usage, preparation and processing of ginger

Ginger is used in numerous forms, including fresh, dried, pickled, preserved, crystallized, candied, and powdered or ground. The flavor is somewhat peppery and slightly sweet, with a strong and spicy aroma. The concentration of essential oils increases as ginger ages and, therefore, the intended use of the rhizome determines the time when it is harvested. If extracting the oil is the main purpose, then ginger can be harvested at 9 months or longer. Ginger is commonly pickled in sweet vinegar, which turns it a pink color; this form is popular with sushi. Ginger harvested at 8-9 months has a tough skin that must be removed before eating, and the root is more pungent and is used dried or pulverized into ground ginger. This is the form most commonly found in our spice racks and used in cookies,

cakes, and curry mixes. Candied or crystallized ginger is cooked in sugar syrup and coated with granulated sugar. Ginger harvested at 5 months is not yet mature and has a very thin skin, and the rhizomes are tender with a mild flavor and are best used in fresh or preserved forms(Iris and Sissi, 2011).

2.18Health benefits of Ginger.

Ginger root has been in use since ancient times for its anti-inflammatory, carminative, anti-flatulent, and anti-microbial properties. Total antioxidant strength measured in terms of *oxygen radical absorbance capacity* (ORAC) of ginger root is 14840 µmol TE/100 g.It contains health benefiting essential oils such as *gingerol, zingerone, shogaol, farnesene*, and small amounts of *ß-phelladrene, cineol, and citral*. Gingerols help improve the intestinal motility and have been anti-inflammatory, painkiller (analgesic), nerve soothing, anti-pyretic as well as anti-bacterial properties. Studies have shown that it may decrease nausea induced by motion sickness or pregnancy and may help relieve migraine headache.Studies suggest that zingerone**,** a chemical compound which gives pungent character to the ginger root, is effective against *E. coli* induced diarrhea, especially in children.This herb root only has 80 calories per 100 g and contains no cholesterol. Nonetheless, it composes many essential nutrients and vitamins such as pyridoxine (vitamin B-6), pantothenic acid (vitamin B-5) that are required for optimum health.Furthermore, it also holds good amount of minerals like potassium, manganese,copper, and magnesium. Potassium is an important component of cell and body fluids that helps controlling heart rate and blood pressure(Iris and Sissi, 2011).

2.19 Medicinal uses of Ginger.

Ginger root slices, boiled in water with lemon or orange juice, and honey, is a popular herbal drink in ayurvedic medicine to relieve common cold, cough, and sore throat.Its extraction is used as a vehicle to mask bitterness and after-taste in traditional ayurvedic preparations.Gingerols increase the motility of the gastrointestinal tract and have analgesic, sedative, anti-inflammatory, and antibacterial properties. Studies have shown that it may help reduce nausea caused by motion sickness or pregnancy and may help relieve migraine.(www.nutrition-and-you.com, accessed on 1st May, 2016).

2.20 Health effects of Ginger

Because ginger and its metabolites appear to accumulate in the gastrointestinal tract, the consistent observations of ginger exerting many of its effects in this area are not surprising. Ginger has been purported to exert a variety of powerful therapeutic and preventive effects and has been used for thousands of years for the treatment of hundreds of ailments from colds to cancer. Like many medicinal herbs, much of the information has been handed down by word of mouth with little controlled scientific evidence to support the numerous claims. However, in the last few years, more organized scientific investigations have focused on the mechanisms and targets of ginger and its various components(Kim *et al.,* 2002).

2.21General Antioxidant Properties of Ginger

The presence of oxidative stress is associated with numerous diseases and a common mechanism often put forth to explain the actions and health benefits of ginger is associated with its antioxidant properties (Aeschbach*et al.,*1994). Ginger was reported to decrease age-related oxidative stress markersand was suggested to guard against ethanol-induced hepatotoxicity by suppressing oxidative consequences in rats treated with ethanol. Ginger root contains a very high level (3.85 mmol/100 g) of total antioxidants, surpassed only by pomegranate and some types of berries. The phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), promotes oxidative stress by activating the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system or the xanthine oxidase system or both. Ginger was reported to suppress TPA-induced oxidative stress in human promyelocytic leukemia (HL)-60 cells and Chinese hamster ovary AS52 cells (Kim *et al.,* 2002). Others have shown that ginger compounds effectively inhibit superoxide production. Several reports indicate that ginger suppresses lipid peroxidation and protects the levels of reduced glutathione (Shobana and Naidu, 2000; El-Sharaky *et al.,*2009).Reactive nitrogen species, such as nitric oxide (NO), influence signal transduction and cause DNA damage, which contributes to disease processes. Nitric oxide is produced by inducible nitric oxide synthase (iNOS), which is stimulated in response to various stresses. [6]-gingerol was reported to dose-dependently inhibit NO production and reduce iNOS in lipopolysaccharide (LPS)-stimulated mouse macrophages (Ippoushi *et al.,*2003). [6]-gingerol also effectively suppressed peroxynitritemediated oxidative damage (Ippoushi*et al.,* 2003). Ippoushi *et al.,* (2003) later proposed that [6]-gingerol and peroxynitrite form a symmetric dimer with [6]-

gingerol covalently linked at the aromatic ring of peroxynitrite, attenuating peroxynitrite-induced oxidation and nitration reactions. [6]-shogaol, 1-dehydro-[10]-gingerdione, and [10]-gingerdione also decreased LPS-induced NO production, and [6]-shogaol and 1-dehydro-[10]-gingerdione were reported to effectively reduce iNOS expression. In the bromobenzene (BB)-induced hepatotoxicity model, orally given ginger extract (100 mg/kg body weight [BW]) normalized NO levels and total and reduced glutathione levels, and also decreased the level of lipid peroxidation (El-Sharaky *et al.,* 2009). Ginger consumption has also been reported to decrease lipid peroxidation and normalize the activities of superoxide dismutase and catalase, as well as GSH and glutathione peroxidase, glutathione reductase, and glutathione-S-transferase, in rats. Ginger supplementation before ischemia/reperfusion resulted in a higher total antioxidant capacity (i.e., normalized glutathione peroxidase and superoxide dismutase activities) and lower total oxidant (lower tissue malondialdehyde, NO, and protein carbonyl contents) status levels compared to an untreated group of Wistar albino rats. Overall, the rats fed ginger (5%) experienced less kidney damage due to oxidative stress induced by ischemia/reperfusion.Ginger extract has been reported to exert radioprotective effects in mice exposed to gamma radiation (Jagetia *et al.,* 2004), and the effect was associated with decreased lipid peroxidation and protection of GSH levels. [6]-gingerol pretreatment also decreased oxidative stress induced by ultraviolet B (UVB) and activated caspase-3, -8, -9, and Fas expression (Kim*et al.,* 2007). Evidence does seem to suggest that ginger and some of its components are effective antioxidants in vitro. However, whether the physiological activity occurs in humans in vivo is not clear, and the specific mechanism and cellular targets are still to be determined.

2.22Anti-Inflammatory effects of ginger

One of the many health claims attributed to ginger is its purported ability to decrease inflammation, swelling, and pain. [6]-gingerol (Young*et al.,* 2005), a dried ginger extract, and a dried gingerol-enriched extract (Minghetti*et al.,* 2007) were each reported to exhibit analgesic and potent anti-inflammatory effects. Earlier animal studies suggest that rat hind limbs perfused with [6]-gingerol showed increased heat production that was associated with increased oxygen consumption and lactate efflux (Eldershaw*et al.,* 1992). The thermogenesis was at least partly associated with vasoconstriction independent of adrenergic receptors or secondary catecholamine release. In contrast, larger doses of ginger components inhibited oxygen consumption, which was attributed to disruption of mitochondrial function (Eldershaw*et al.,* 1992). These results were supported in a later study in which rats that were given a single intraperitoneal injection of [6]-gingerol (2.5 or 25 mg/kg) exhibited a rapid, marked drop in body temperature and a significant decrease in metabolic rate.Data suggest that ginger may exhibit anti-inflammatory effects through the modulation of calcium levels mediated through transient receptor potential vanilloid subtype 1 (TRPV1), which is a heat-and pain-sensitive receptor that can interact with [6]-gingerol (Dedov*et al.,* 2002). [6]-gingerol has been reported to induce a substantial rise in intracellular calcium levels in Madin-Darby canine kidney renal tubular cells by stimulating both extracellular calcium influx and thapsigargin (an endoplasmic reticulum Ca^{2+} pump inhibitor)-sensitive intracellular calcium release (Chen*et al.,* 2008). The gingerols are known to be TRPV1 agonists (Dedov *et al.,* 2002), and the [6,8,10]-gingerols and [6,8,10]-shogaols can increase the intracellular calcium concentration in TRPV1-expressing

HEK293 cells through TRPV1 (Iwasaki*et al.,* 2006). Shogaols appear to be more potent than the gingerols, and most of the compounds cause aversive or nociceptive responses mediated by TRPV1 when applied to the eye or following subcutaneous injection to the hind paw, respectively (Iwasaki*et al.,* 2006). In this case, most of the ginger compounds also promoted adrenal catecholamine secretion, which influences energy consumption (Iwasaki *et al.,* 2006).Ginger has been suggested to be effective against inflammation, osteoarthritis, and rheumatism. However, inconsistencies in clinical studies have led to debate regarding the effectiveness and safety of ginger for treatment of arthritis (Marcus and Suarez-Almazor, 2001). An earlier study showed that ginger oil (33 mg/kg), administered orally to rats for 26 days, caused a significant repression of paw and joint swelling associated with severe chronic adjuvant arthritis. More recently, the effectiveness of a crude ginger extract was compared with a fraction containing only gingerols and derivatives to inhibit joint swelling in the streptococcal cell wall-induced arthritis animal model of rheumatoid arthritis (Funk *et al.,* 2009). Results indicated that although both extracts could prevent joint inflammation, the crude dichloromethane extract, which also contained essential oils and more polar compounds, was more effective (when normalized to gingerol content) in preventing both joint inflammation and destruction (Funk*et al.,* 2009). In humans, one study showed no difference between placebo and ginger in patients with osteoarthritis of the hip or knee (Bliddal*et al.,* 2000). In contrast, patients suffering from osteoarthritis of the knee showed a consistently greater response to treatment with ginger extract compared with the control group. In addition, relief from pain and swelling was reported in patients suffering from rheumatoid arthritis, osteoarthritis, or general muscular discomfort when using powdered ginger as a dietary supplement for 3 months to

2 years (Srivastava and Mustafa, 1992). Besides pain relief from arthritis, results of a double-blind comparative clinical trial indicated that ginger (250-mg capsules) was as effective as the nonsteroidal anti-inflammatory drugs mefenamic acid (250 mg) and ibuprofen (400 mg) in relieving pain in women with primary dysmenorrhea (Ozgoli *et al.,* 2009). In contrast, consumption of 2 g of ginger before 30 minutes of cycling exercise (60% VO_2) had no effect on quadriceps muscle pain, rating of perceived exertion, work rate, heart rate, or oxygen uptake.Researchers have hypothesized that the anti-inflammatory effects of ginger might be related to its ability to inhibit prostaglandin and leukotriene biosynthesis (Srivastava and Mustafa, 1992). Some others have showed that gingerols actively inhibit arachidonate 5-lipoxygenase, an enzyme of leukotriene biosynthesis (Kiuchi *et al.,* 1992). [8]-gingerol, but not [6]-gingerol, was shown to inhibit cyclooxygenase-2 (COX-2) expression, which is induced during inflammation to increase formation of prostaglandins (Tjendraputra*et al.,*2001). Others have also reported that ginger extract suppresses the activation of tumor necrosis factor α (TNF-α) and expression of COX-2 in human synoviocytes. Proinflammatory cytokines such as TNF-α, interleukin (IL)-1β, and IL-12, which are produced primarily by macrophages, play an important role in sepsis, ischemia/reperfusion injury, and transplant rejection. [6]-gingerol was reported to inhibit the production of proinflammatory cytokines from LPS-stimulated peritoneal macrophages, but to have no effect on the function of antigen presenting cells (APC) or the LPS-induced expression of proinflammatory chemokines (Tripathi*et al.,* 2007). However, this same group later reported that a ginger extract attenuated the production of IL-12, TNF-α, and IL-1β proinflammatory cytokines and RANTES (regulated upon activation, normal T cell expressed and secreted) and monocyte chemoattractant protein 1 (MCP-1)

proinflammatory chemokines in LPS-stimulated murine peritoneal macrophages (Tripathi *et al.,*2008). In general, ginger extract inhibited macrophage activation and APC function, and indirectly suppressed T-cell activation (Tripathi *et al.,* 2008). Other stable [6]-gingerol metabolites or analogs were reported to suppress LPS-induced NO production in murine macrophages mainly by reducing *inos* gene and iNOS protein production (Aktan *et al.,* 2006). Some of ginger's anti-inflammatory effects appear to be associated with decreased IκBα degradation and impaired nuclear factor κB (NF-κB) nuclear translocation of p65 (Aktan *et al.,* 2006). The majority of scientific evidence does seem to suggest that ginger and its various components have anti-inflammatory effects both in vitro and in vivo. However, the data supporting ginger as an effective anti-inflammatory agent in humans in vivo are still contradictory and incomplete.

2.23 Ginger as an Antinausea Agent

The most common and well-established use of ginger throughout history is probably its utilization in alleviating symptoms of nausea and vomiting. The effectiveness of ginger as an antiemetic has been attributed to its carminative effect, which helps to break up and expel intestinal gas. This idea was supported by the results of a randomized, double-blind trial in which healthy volunteers reported that ginger effectively accelerated gastric emptying and stimulated antral contractions (Wu *et al.,* 2008). Previously, [6]-gingesulfonic acid, isolated from ginger root, was showed to be effective against HCl/ethanol-induced gastric lesions in rats. This compound showed weaker pungency but more potent antiulcer activity than [6]-gingerol or [6]-shogaol.Ginger root is commonly recommended for preventing seasickness (Schmid *et al.,* 1994) and is found to be superior

to dimenhydrinate (Dramamine) or placebo against symptoms of motion sickness (Mowrey and Clayson, 1982). A follow-up study also indicated that 1 g of ginger might be effective in reducing the subjective severity of seasickness in naval cadets on the high seas (Grontved *et al.,* 1988). On the other hand, additional research studies showed no benefits of using ginger for treating motion sickness (Stewart *et al.,* 1991), and at least one group reported that patients receiving ginger extract for treating osteoarthritis experienced more, although mild, gastrointestinal adverse events compared to a placebo-treated group (Altman and Marcussen, 2001). The exact antiemetic mechanism of ginger is not clear although some evidence suggests that it inhibits serotonin receptors and exerts its antiemetic effects directly on the gastrointestinal system and in the central nervous system. Although the antiemetic effects of ginger are the most well-studied effects of this condiment and have been reviewed extensively, the effectiveness and safety of ginger for treating nausea and vomiting have been questioned in the past because the findings reported were often contradictory (Wilkinson, 2000b). At the same time, ginger continues to be recommended for alleviating nausea and vomiting associated with pregnancy, chemotherapy, and certain surgical procedures.Nausea and vomiting during pregnancy affects most pregnant women, and over the years ginger has been used to try to alleviate the condition. At least one survey indicated that the overall use of dietary supplements in pregnant women appears to be low, but ginger is commonly recommended and used to prevent nausea (Tsui *et al.,* 2001). Several double-blind, randomized, placebo-controlled clinical trials have indicated that ginger consumption is effective and safe in helping to prevent nausea and vomiting during pregnancy (Portnoi *et al.,* 2003; Willetts *et al.,* 2003). Randomized trials suggest that although ginger might not be as potent as some treatments, its consumption for treating

nausea or vomiting or both in early pregnancy has very few or no adverse side effects and seems to be effective (Vutyavanich *et al.,* 2001). In fact, ginger has been reported to be as effective as dimenhydrinate (i.e., Dramamine) in treating nausea and vomiting in pregnancy with fewer side effects (Pongrojpaw and Somprasit,2007). Women who received ginger (250-mg capsules) appeared to experience less vomiting and nausea compared to those receiving placebo (Ozgoli *et al.,*2009) and ginger also relieved pain from primary dysmenorrhea (Ozgoli *et al.,*2009). The effectiveness of ginger has been compared with that of vitamin B6 (another recommended therapy) in randomized, double-blind, controlled trials. Results indicated that ginger and vitamin B6 therapy were equally effective in reducing nausea and the number of vomiting episodes during pregnancy (Sripramote and Lekhyananda, 2003; Smith *et al.,* 2004). In a later randomized, double-blind, controlled trial, pregnant women were randomly divided to receive either 650 mg of ginger or 25 mg of vitamin B6 (3xd/4 days). In this case, ginger actually appeared to be more effective than vitamin B6, with only minor side effects. These results were supported in an additional trial in which pregnant women with nausea were randomized into groups to receive either 1 g of ginger/day or 40 mg of vitamin B6/day for 4 days. Results of this trial indicated that compared with a baseline, nausea and vomiting in the ginger group were significantly less than those reported by the vitamin B6 group (Ensiyeh and Sakineh, 2009). A systematic review of the results of other double-blind, randomized, controlled trials, uncontrolled trials, case reports, and observational studies indicated that ginger is superior to placebo and as effective as vitamin B6 in relieving the severity of nausea and vomiting, with no reported side effects or adverse effects on pregnancy. A similar review of the literature regarding the safety and efficacy of ginger in the management of nausea and vomiting

during pregnancy revealed that ginger appears to be a relatively low-risk and effective treatment for these symptoms (Boone and Shields, 2005). Importantly, no differences in birth weight, gestational age, or frequencies of congenital abnormalities have been observed between ginger-treated and untreated mothers (Willetts *et al.,* 2003).

2.24Cardiovascular and Other Disease-Preventive Effects of Ginger

At least one group found that administration or consumption of standardized ginger extract decreased aortic atherosclerotic lesion areas, plasma triglycerides and cholesterol, low-density lipoprotein (LDL)-associated lipid peroxides, and LDL aggregation in mice (Fuhrman *et al.,* 2000). In rabbits that were fed a high-cholesterol diet, administration of ginger extract resulted in a significant antihyperlipidemic effect and a lower degree of atherosclerosis compared to the group that was fed cholesterol alone. Importantly, ginger powder (3 g/day in 1-g capsule 3xd) significantly lowered lipid levels in volunteer patients in a double-blind, controlled clinical trial study (Alizadeh-Navaei *et al.,* 2008). Triglyceride and cholesterol were substantially decreased as was LDL levels compared to placebo group. Notably, the high-density lipoprotein (HDL) level of the ginger group was higher than that of the placebo group, whereas the very-low-density lipoprotein (VLDL) level of the placebo group was higher than that of the ginger group (Alizadeh-Navaei *et al.,* 2008). Dried ginger powder (0.1 g/kg BW, per oral administration for 75 days) significantly lowered (50%) the development of atheroma in the aorta and coronary arteries of rabbits that were fed cholesterol (Verma*et al.,* 2004). This effect was associated with decreased lipid peroxidation and increased fibrinolytic activity with ginger, but blood lipid levels were not different from control animals (Verma *et al.,* 2004). Another compound isolated

from ginger, (E)-8 β,17-epoxylabd-12-ene-15,16-dial, was reported to inhibit cholesterol biosynthesis, and ginger meal (1%) decreased serum cholesterol levels significantly (Dias *et al.,* 2006). Ginger was also reported to slightly reduce retinoid-binding protein mRNA expression levels in liver and visceral fat in male rats that were fed cholesterol to induce hyperlipidemia (Matsuda *et al.,* 2009). These results hint that ginger consumption might improve lipid metabolism (Matsuda *et al.,* 2009).Antiplatelet therapy is an effective approach for preventing coronary heart disease. Ginger components are suggested as a potential new class of platelet-activation inhibitors without the potential side effects of aspirin, which is most commonly used in this approach. In a comparison of gingerols and analogs with aspirin, ginger compounds were found to be less potent compared to aspirin in inhibiting arachidonic acid-induced platelet release and aggregation and COX activity (Koo *et al.,* 2001). However, several analogs had a significant inhibitory effect, suggesting that further development of more potent gingerol analogs might have value as an alternative to aspirin therapy in preventing ischemic heart disease (Koo *et al.,* 2001). Consumption of ginger (5 g) inhibited platelet aggregation induced in men who consumed 100 g of butter daily for 7 days (Verma *et al.,* 1993), and a later study showed that ginger enhanced fibrinolytic activity (Verma and Bordia, 2001). An evaluation of the antiplatelet activity of 20 pungent constituents of ginger revealed that [8]-paradol was the most potent COX-1 inhibitor and antiplatelet aggregation agent (Nurtjahja-Tjendraputra *et al.,* 2003). [8]-gingerol and [8]-shogaol were also found to be effective antiplatelet aggregation agents (Nurtjahja-Tjendraputra *et al.,* 2003). Ginger and nifedipine (a calcium-channel blocker) were reported to have a synergistic effect on antiplatelet aggregation in normal human volunteers and hypertensive patients (Young *et al.,* 2006). Ginger oil (24% citral)

effectively lowered spontaneous or prostoglandin F2-alpha (PGF2-alpha)-2α-induced rat myometrial (uterus) contractility, and increases in external calcium concentration reversed the relaxant effects of ginger oil (Buddhakala *et al.,* 2008). Ginger compounds have been reported to directly stimulate myocardial sarcoplasmic reticulum (SR) calcium uptake (Antipenko *et al.,* 1999; Maier *et al.,*2000), but its therapeutic use in treating heart failure has not been advocated (Maier *et al.,* 2000). Ginger is also used to treat asthma, diabetes, and other conditions.Asthma is a chronic disease characterized by inflammation and hypersensitivity of airway smooth muscle cells to different substances that induce spasms, and ginger has been used for centuries in treating respiratory illnesses. Components of ginger rhizomes are reported to contain potent compounds capable of suppressing allergic reactions and might be useful for the treatment and prevention of allergic diseases (Chen *et al.,*2009). Ghayur *et al.,* (2008) reported that a ginger extract inhibits airway contraction and associated calcium signaling, possibly by blocking plasma membrane calcium channels. In a mouse model of Th2-mediated pulmonary inflammation, an intraperitoneal injection of a ginger extract mainly comprised of gingerols markedly decreased the recruitment of eosinophils to the lungs in ovalbumin-sensitized mice and also suppressed the Th2 cell-driven response to allergen (Ahui *et al.,* 2008).Ginger has been suggested to have antidiabetic effects. In the streptozotocin-induced diabetic rat model, rats that were fed ginger exhibited better glucose tolerance and higher serum insulin levels than untreated rats, suggesting that it can help control blood sugar levels.

2.25 Metabolism of ginger

Although ginger is one of the most widely consumed spices in the world, not a great deal is known regarding its metabolism or metabolites. Evaluating the bioactivity of ginger is necessary for completely understanding its mechanism of action and potential therapeutic effects. Although many food-derived supplements are consumed today with little knowledge of their activity or safety, more attention is beginning to be given to addressing these issues. The most well-studied bioactive component of ginger is probably [6]-gingerol (Surh *et al.,* 1999). The careful isolation of several metabolites of [6]-gingerol following its oral administration (50 mg/kg) to rats was reported (Nakazawa and Ohsawa, 2002). A primary metabolite, (S)-[6]-gingerol-4′-*0*-β-glucuronide, was detected in the bile and several minor metabolites were found in β-glucuronidase-treated urine, suggesting that [6]-gingerol undergoes conjugation and oxidation of its phenolic side chain (Nakazawa and Ohsawa, 2002). Gingerol is rapidly cleared from rat plasma following intravenous administration (3 mg/kg)(Ding *et al.,* 1991), and it was reported to be metabolized enzymatically in a stereospecific reduction to gingerdiol (Surh and Lee, 1994).A method has been developed for the simultaneous quantification of [6]-, [8]-, and [10]-gingerol and [6]-shogaol in rat plasma in pharmacokinetic studies after oral administration of ginger oleoresin (Wang *et al.,* 2009). The investigators were able to identify a glucuronide of [6]-gingerol after hydrolysis of β-glucuronidase, and the intestinal glucuronidation was further confirmed by comparing plasma samples of hepatic portal vein and femoral vein (Wang *et al.,* 2009). This method was also used to obtain pharmacokinetics, tissue distribution, and excretion studies of 6-gingerol after oral or intraperitoneal administration in rats (Wang *et*

*al.,*2009). In a study in which a ginger extract (approximately 53% [6]-gingerol) was administered to rats by oral ingestion, [6]-gingerol was absorbed rapidly into the plasma, with a maximal concentration (4.23 μg/mL) being reached after 10 minutes (Jiang *et al.,* 2008). The [6]-gingerol was distributed to various tissues and the most concentration was found in the gastrointestinal tract. Peak concentrations of [6]-gingerol were reached in most tissues at about 30 minutes, and the concentration in tissues was higher than that in plasma (Jiang *et al.,* 2008).At least one clinical trial focused on the pharmacokinetics of [6]-, [8]-, and [10]-gingerols and [6]-shogaol along with their respective conjugate metabolites (Zick *et al.,*2009). In this case, human volunteers were given ginger at doses ranging from 100 mg to 2 g and blood samples were taken at 15 minutes to 72 hours after a single oral dose. Results indicated that the free forms of [6]-, [8]-, and [10]-gingerols or [6]-shogaol were not detectable, whereas the respective glucuronide of each compound was detected, suggesting that these ginger components are readily absorbed after oral consumption and can be detected as glucuronide conjugates (Zick *et al.,*2009). Although progress in determining the active components and metabolites of ginger and understanding their pharmacokinetics has been made, more work is clearly needed.Sebiomo*et al.,* (2011) reported that ginger root ethanol extract showed the greatest effect on both *S. aureus*and *S. pyogene* compared to the leaf and root water extract and the leaf ethanol extract. This is an indication that ginger is effective against *S. aureus*and *S.pyogene* infections. The phytoconstitutuents of ginger have longed been known as its antibacterial properties have been widely reported. The data from this study showed that ethanol extract were more effective than the water extract. This may be attributed to the fact that the three antibiotics as a conventional antibiotic, is prepared by a reproducible manufacturing processes and

procedures. Extract of herbal medicines are subject to degradation and decomposition on storage (El – Mahmood and Amey,2007). Gamil *et al.,* (2016) study on Antimicrobial Effect of Honey and Some Herbal Plant Extracts Against Multidrug Resistance Bacteria Isolated from Patient in Local Riyadh Hospital indicated that ethanolic extracts had no activity against *S. aureus* and *E. coli*. which on the other hand disagrees with (Hegazi, 2011; Gull *et al.,* 2012) who found that the ginger ethanolic extracts inhibit the growth of *S. aureus* and *E. coli*. The results indicated that the highest bacterial resistance to antibiotics *Escherichia coli* (57.14 %) followed by *Klebsiella pneumonia* (42.28 %) and *Staphylococcus aureus* (28.57 %). The Egyptian and Saudi laguanza honey were showed strong antibacterial effects and inhibitory effect with concentrations of 50, 20% and 10% followed by Clove and Black cumin ethanolic extracts against tested bacteria. Combination honey with antibiotics or plant extracts mixture (v/v) revealed synergistic effects on tested bacteria. Kianoush *et al.,* (2013) investigation on Antifungal and Anti-bacterial Synergistic Effects of Mixture of Honey and Herbal Extracts reported that different honey from different parts of Iran have different effects on growth inhibition of *Staphylococcus aureus, Pseudomonasaeruginosa*, *Eschershia coli* and *candida albicans* due to different sources which lead to having different compounds.Yahaya *et al.,* (2012) investigation on Combined Antimicrobial Effect of Ginger and Honey on Some Human Pathogensshowed that the ginger extract had activity on all the tested organisms at varying degree. The highest ginger activity was recorded on *Candida albican* at concentrations of 50% and above, followedby *E. coli*and *Salmonella typhi* at 60% and above. *S. typhi* was more sensitive to honey at 50% and above**.** While *E. coli*showed least sensitivity. All the test organisms were sensitive to the combine effect of ginger and honey at all concentrations

tested (30% and above). The zones of inhibitions increased tremendously to the synergistic effect. The results obtained in this study agrees with that of Ficker *et al.,* (2003), Grange and Davey, (1990) and Zahra *et al.,* (2009)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area:

Sokoto, usually referred to as Sokoto State to distinguish it from city of Sokoto, is located in the extremes northwest of Nigeria, near to the Sokoto River and the Rima River with coordinate 13°05´N 05°15´E. Sokoto State is mainly populated by Hausa people and very few non Hausa people. Most Sokoto State residents are Sunni Muslims, with a Shia minority and very few others. Sokoto is a dry Sahel, surrounded by sandy savannah and isolated hills with total area of about 25,973km^{2}(10,028sqi) rank 16th of 36 states. Very hot area with annual average temperature of 28.3^{o} c(82.9^{o} F) and recorded over 4,392,391 population during 1999 census, 4,244,399 estimated population in 2005 rank 14th out of 36 States in Nigeria. (http://www.onlinenigeria.com/map.gif accessed 1st july, 2016)**.**

3.2 Study Design

This is an analytical and experimental study aimed to determine combined antibacterial effect of honey, ginger and bitter kola extract against selected pathogens.

3.3 Test organism and their sources

The test organisms were isolated from Specialist Hospital Sokoto, *S. aureus, E. coli and P. aeruginosa* were isolated from wound and urine samples to represent both gram positive

and gram negative bacterial. *P. aeruginosa*was chosen to represent gram negative resistance bacterial.

3.4 Sample collection of plants materials and honey

The seeds of bitter kola, ginger and honey were purchased from Kara Kasua (market) in sokoto state, Nigeria. They were botanically identified and confirmed at the herbarium of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria with I.D UDUH/ANS/0123,UDUH/ANS/0127,and UDUH/ANS/0128 respectively. The bitter kola and ginger were sliced into smaller sizes, air-dried and grounded into powder.

3.5Selection of extraction solvents base on polarity

Methanol, Ethyl acetate and N-hexane are polar protic solvent (dielectric constant:33), polar aprotic solvent (dielectric constant:6.02) and non-polar solvent (dielectric constant:1.88) respectively. They were chosen base on polarity using dielectric constant scale for rough measurement of solvents polarity (Malmberg and Maryott, 1956; Lowery and Richardson, 1987).

3.6Extraction procedure (simple maceration method)of plants material

Methanol, Ethyl acetate and N-hexane were used as extraction solvent.100g of the air-dried, grounded bitter kola and ginger were weighed with weighing balance S.METLER and were extracted using 500ml of ethyl acetate, 500ml of 100% methanol and 500ml of N-hexane in the ratio of 1:5(w/v) at room temperature for 72 hours with occasional shaking. The mixtures were then filtered into clean dry glass containers using Whitman’s No. 1 filter

paper (9 cm) and the filtrates were evaporated (temp. $40^{o}c$-$44^{o}c$) to dryness and stored at room temperature in desiccator with plastic until needed for the test.

3.7 Percentage(%) yield of plant extract

The percentage yield is equal to weight of extract divided by original weight of extract times one hundred. Percentage(%) yield= $E/O \times 100$

Where, E= weight of the extract and

O= weight of the original sample.

3.8.0 Preparation of honey concentrations and plants extracts concentrations

3.8.1 Preparation of honey concentrations

Hundred percent pure honey was obtained by filtration using sterile gauze and a total of five tubes were set with the first tube containing 6ml of pure honey and others 3ml of distilled water.1275mg/ml concentration of honey was prepared by dissolving 3.825g(3ml) of honey from the first tube into 3ml of distilled water in the next tube. 637.5mg/ml concentration of honey was prepared by pipetting 3ml of 1275mg/ml concentration of honey into 3ml of distilled water in the next tube. 318.75mg/ml concentration of honey was prepared by pipetting 3ml of 637.5mg/ml concentration of honey into 3ml of distilled water in next tube and finally, 106.25mg/ml concentration of honey was prepared by pipetting 3ml of 318.75mg/ml concentration of honey into 3ml of distilled water in the last tube. In each step, proper shacking of the mixture was made before transfer of 3ml to the next tube. The concentrations prepared were 1275mg/ml, 637.5mg/ml, 318.75mg/ml, 106.25mg/ml and 53.125mg/ml respectively(Ochei and Kolhtkar, 2008).

3.8.2Preparation of ginger extracts concentration

A total of five tubes were set with each containing 3ml of distilled water. 0.5g of ginger extracts was measured and dissolved in 3ml of sterile distilled water in the first tube to make up the highest working concentration (166.67mg/ml). To prepare, 83.33mg/ml concentration of ginger, 3ml of 166.67mg/ml concentration of ginger from the first tube was pipetted into 3ml of distilled water in the next tube. 41.67mg/ml concentration of ginger was prepared by pipetting 3ml of 83.33mg/ml concentration of ginger into 3ml of distilled water in the next tube. 20.82mg/ml concentration of ginger was prepared by pipetting 3ml of 41.67mg/ml concentration of ginger into 3ml of distilled water in the next tube and finally, 10.42mg/ml concentration of ginger was prepared by pipetting 3ml of 20.82mg/ml concentration of ginger into 3ml of distilled water in the last tube. In each step, proper shacking of the mixture was made before transfer of 3ml to the next tube. Thus, concentrations prepared were; 166.67mg/ml, 83.33mg/ml, 41.67mg/ml, 20.82mg/ml, and 10.42mg/ml respectively(Ochei and Kolhtkar, 2008).

3.8.3Preparation of bitter kola extracts concentration

A total of five tubes were set with each containing 3ml of distilled water. 0.5g of bitter kola extracts was measured and dissolved in 3ml of sterile distilled water in the first tube to make up the highest working concentration(166.67mg/ml). To prepare, 83.33mg/ml concentration of bitter kola, 3ml of 166.67mg/ml concentration of bitter kola from the first tube was pipetted into 3ml of distilled water in the next tube. 41.67mg/ml concentration of bitter kola was prepared by pipetting 3ml of 83.33mg/ml concentration of bitter kola into

3ml of distilled water in the next tube. 20.82mg/ml concentration of bitter kola was prepared by pipetting 3ml of 41.67mg/ml concentration of bitter kola into 3ml of distilled water in the next tube and finally, 10.42mg/ml concentration of bitter kola was prepared by pipetting 3ml of 20.82mg/ml concentration of bitter kola into 3ml of distilled water in the last tube. In each step, proper shacking of the mixture was made before transfer of 3ml to the next tube. The concentrations prepared were the same as in ginger above (Ochei and Kolhtkar, 2008).

3.9 Media preparation

Mueller-Hinton agar (MHA) and Nutrient broth (oxoid) were prepared according to manufacturers instruction.

3.10 Sterility test of seed extract and honey

The extract and honey were tested for growth of contaminants. One milliliter (1ml) of standard seed extract and honey were inoculated aseptically unto Nutrient Agar and incubated at 35° C for 24hrs. The plates were observed for any sign of visible growth. The absence of growth on the

Plates signified that the extracts weresterile. The extracts were then assessed for antimicrobial activity (Ochei and Kolhtkar, 2008).

3.11.0Preparation of pure culture and identification of isolated pathogens

The clinical isolates selected for this research include *Staphylococcus aureus,P. aeruginosa and Escherichia coli* from wound and urine, details on how it were selected were given in

section 3.3. The isolates were obtained from Medical Microbiology Laboratory, Specialist Hospital Sokoto, Nigeria as pure clinical isolates and were maintained on fresh Nutrient agar slants. The colonies were picked with a sterile wire loop from the cultured plate and were used in making streaks on the surface of a newly prepared sterile Mueller-Hinton agar plates, the plates were incubated at 37^0c for 24hrs. Discrete colonies of a particular species of bacteria were then obtained at the end of incubation. It was then picked with a sterile wire loop and streaked into the prepared agar slants after which it was incubated for growth and latertransferred into refrigerator for further use. Biochemical test was conducted for proper identification of pathogen before use (Ochei and Kolhtkar, 2008)

3.11.1Gram's stain for smears

A smear was made and was allowed to air dried before head fixing. The smear was taken to staining rack and was stained with 1% aqueous solution of crystal violet for 1minute. It was washed with water and mordant with logul's iodine for 1minute. It was washed with water again and decolorized with acetone for 1second. Then washed well with water and counterstained with 0.5% aqueous solution of neutral red for 1minute. Finally, washed with water, blot dried and examined with oilimmersion (Ochei and Kolhtkar, 2008).

3.11.2 Catalase test

Few colonies of the gram positive cocci were emulsified in distilled water on a cleaned slide and was placed in a petri dish. Then, 2 drops of H_20_2 was added, the petri dish was covered. Gas bubbles indicate a positive reaction. *Staphylococcus* species were positive control organism while *Streptococcus* species were negative control organism(Ochei and Kolhtkar, 2008)

3.11.3 Coagulase test

Slide test to detect bound coagulase was done; two separate drops of saline were placed on a slide. Two colonies of the organism were emulsified on each of the drops to make thick suspensions. Dipped the tip of a straight wire into the undiluted plasma and mixed the adhering traces of plasma into one of the bacterial suspensions. Look for immediate coarse clumping of the mixture (5-10 seconds). The clumping indicates positive coagulase test. No plasma was added to the other suspension, it serves as negative control to the test and to differentiate non-specific granular appearance from true coagulase clumping (Ochei and Kolhtkar, 2008)

3.11.4 Citrate utilization test

Made light suspension of the organism in saline. Stab inoculated simmom's citrate agar. A growth indicated by a growth of blue colour in simmom's agar, indicates positive result. This means citrate has been utilized. *Klebsiella pneumonia* was used aspositive control organism (Ochei and Kolhtkar, 2008)

3.11.5 Urease test

The entire surface of a urea slope agar was inoculated and incubated at 37^0C in an incubator. It was examined after overnight of incubation. Then observe the red-pink colour of urease positive test (Ochei and Kolhtkar, 2008).

3.11.6 Hanging drop preparation

A ring was made with plasticine of about 2cm in diameter on a clean slide. Place a loopful of culture in the centre of a clean 22mm square cover-slip. Carefully pressed the ring of

plasticine on the cover-slip with the drop of culture in the centre of the ring and not touching the slide. With a quick movement the slide was inverted so that the cover-slip gets to the uppermost. It was examined under x10 and x40 microscopy. At the end of examination, the whole preparation was discarded into a jar of disinfectant and was allowed to soak to kill the micro-organism.

3.11.7 Kligler iorn agar (KIA)

KIA was incubated with a well isolated colony, picked with a long straight wire loop, stabbing into the butt of the tube. The wire extended to 3-5mm of the bottom of the tube. Then, the slant surface was streaked with the same wire. It was incubated at 37^0C for 24hrs and changes were recorded for identification of organism(Ochei and Kolhtkar, 2008).

.3.11.8 Oxidase test

Whatman's no 1 filter paper was cut into strips and soaked in freshly prepared 1% solution of tetramethyl-paraphynylene diamine dihydrochlo-ride. Drainedstrips for about 30 seconds and freezed dried. The papers have a tint of purple. Stored in a dark container with tight fitting cover. They can be kept for several months. For use, a strip was removed and moistens with a drop of distilled water. Picked the suspected colony with a plastic or platinum wire loop and smear over the moist area of the paper. A deep purple colour appearing within 5-10 seconds indicates positive oxidase test. *P. aeruginosa* is a positive control organism (Ochei and Kolhtkar, 2008).

3.12.Preparation of McFarland standard

About 0.5 McFarland equivalent turbidity standard was prepared by adding 0.5 ml of 1% barium chloride solution ($B_aCl_z2H_2O$) to 9.5ml of 1% sulphuric acid solution (H_2SO_4). A small volume of the turbid solution was transferred to cap tube of the same type that was used to prepare the test and of control inocula. This was stored in the dark at room temperature(Azu and Onyeagba, 2007; Garc and Bruc, 1993).

3.12.1 Standardization of Inoculium

The inoculum size of all bacterial isolates to be tested were standardized by the use of overnight broth cultures prepared by inoculating 3 loopfuls of well- isolated colonies of test bacteria in 10ml of Nutrient broth which were incubated at 35°C for 24 hours. A loopful of the overnight broth culture was diluted in 4ml of sterile physiological saline (0.8% W/V), such that its turbidity matched with that of 0.5 Mac Farland standard considered to have a bacterial density of 1.5 x 10^6 CFU /ml. This was gauged by comparing the turbidity of the test suspension with the turbidity standard against the background of a printed black paper (Cheesbrough, 2002)

3.12.2 Determination of antibacterial activity of crude extracts usingwell diffusion assay

Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of anti-bacterialactivities of the plant extract and honey (Perez *et al.,* 1990). A sterile swab was used to evenly distribute bacterial isolate on the Mueller-Hinton agar plate. The Mueller-Hinton agar plates were allowed to dry for 15 minutesbefore use in the test. Wells of 6mm were then created. A molten Mueller Hinton agar was used to seal the bottom of wells created and allowed to set before applying the test substance, syringe were

used to fill the individual extract and honeyinto each wells in accordance with the labeling. A total of six wells were made for each concentration with a negative control of distilled water. Positive control disc of 30ul/disc of erythromycin and tetracycline were used. The plates were incubated at 35°C for 24 hours after which they were examined forinhibition zones. Each experiment was repeated three times to ensure reliability.

3.12.3 Determination of minimum inhibitory concentration (MIC)

This was done using the broth doubling dilution method, a total of 13 tubes were set up in a rack and 1ml of nutrient broth were pipetted into tubes 2-10, and 11. 2ml of broth was added in tube 12, as broth control. 1ml of working inoculums was added into tube 1 and 11, using tube 11 as inoculums control. 1ml of extracts or honey was added into tubes 1 and 2 using tube 1 as positive control for extracts or honey.Doubling dilution was prepared from tube 2 up to 10 and the last 1ml from tube 10 was discarded. then 1ml of 10mg/ml of standard drugs was added to tube 13 as positive control. Finally, 1ml of the working inoculums was added into tubes 2-10 and 13. All the tube were then incubated at 35°c for 24 hrs. Minimum inhibitory concentration was checked by first identifying the controls to ensure credibility and reliability of the process. Turbidity or cloudness in the growth medium indicates growth. The lowest concentration showing no growth was read as minimum inhibitory concentration of the antibacterial agent for the test organism (Cheesbrough, 2002).

3.12.4 Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentrations (MBCs) were determined by sub culturing the contents of nutrient broth used for MIC tests on MHA media using sterile wire loop and making a strike on the media to see bacteria growth after incubating at 37°C for 24 hours. Absence of growth indicated the minimum bactericidal concentrations (MBCs) of extracts or honey.Combined antimicrobial effects were tested by combining different concentration of honey, ginger and bitter kola in an equal volume (Cheesbrough, 2002).

3.13.0 Combination preparation of honey with ginger extract

This was done by combining the individual concentrations of honey and ginger as prepared in 3.9.1 and 3.9.2 respectively in the following form. A total of fifteen tubes were set in a rack with each row containing five tubes. The first two rows were used in preparation of individual concentractions of honey and ginger as described in 3.9.1 and 3.9.2. The following combined concentration of honey and ginger was made by mixing 0.4ml of honey and 0.4ml ginger using 2ml syringe as follows, 106.25mg/ml of honey with 166.67mg/ml of ginger, 318.75mg/ml of honey with 83.33mg/ml of ginger, 637.5mg/ml of honey with 41.67mg/ml of ginger, 1275mg/ml of honey with 20.82mg/ml of ginger, and finally, undiluted honey with 10.42mg/ml of ginger. Proper shacking was made before use(Ochei and Kolhtkar, 2008).

3.13.1 Combination preparation of honey with bitter kola extract

This was done by combining the individual concentrations of honey and bitter kola as prepared in 3.9.1 and 3.9.3 respectively in the following form. A total of fifteen tubes were set in a rack with each row containing five tubes. The first two rows were used in preparation of individual concentractions of honey and bitter kola as described in section 3.9.1 and section 3.9.3. The following combined concentration of honey and bitter kola was

made by mixing 0.4ml of honey and 0.4ml bitter kola using 2ml syringe as follows, 106.25mg/ml of honey with 166.67mg/ml of bitter kola, 318.75mg/ml of honey with 83.33mg/ml of bitter kola, 637.5mg/ml of honey with 41.67mg/ml of bitter kola, 1275mg/ml of honey with 20.82mg/ml of bitter kola, and finally, undiluted honey with 10.42mg/ml of bitter kola. Proper shacking was made before use(Ochei and Kolhtkar, 2008).

3.14.0 Phytochemical analysisof extracts

This was done to identify the compounds which were secondary metabolites that qualitatively indicated by the intensity of the color produced by phytochemical reagents (Kukate., 2005). They include anthraquinones, cardiac glycoside, saponins, alkaloids, tannins, carbohydrates, flavoniods and steroid/triterpenes.

3.14.1 Screening for alkaloids

This was done by stirring 3grams of bitter kola or ginger extract with ethanol containing 3% tartaric acids. The filtrate was shared into 3 beakers and tested for alkaloids as follows- In the first beaker, haggar's reagent was added and in the second beaker, Mayer's reagent was added and in the last beaker, Marguins reagent was added. Precipitations in any of 3 tests indicate the presence of alkaloid (Odebiyi and Sofowora, 1978; Banso and Ngbede, 2006).

3.14.2 Screening of saponins

About 0.5g of the bitter kola or ginger extract was shaked with water in a test tube, frothing which persists on warming considered as preliminary evidence for the presence of saponins. Few drops of olive oil were added to 0.5g of extract and vigorously shaked, formation of soluble emulsion in the extract indicatesthe presence of saponins (Odebiyi and sofowora, 1978).

3.14.3 Screening for tannins

A 10ml freshly prepared 10% potassium hydroxide (KOH) in a beaker was added with 0.5g of bitter kola or ginger extract, it was shaked to dissolve. A dirty precipitate was observed which indicates the presence of tannins (Odebiyi and Sofowora, 1978; Willimson *et al*., 1996).

3.14.4 Screening for steroids

100mg of bitter kola or ginger extract was dissolved in 2ml of chloroform. sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface was indicative of the presence of steroidal ring (Sofowora, 1982).

3.14.5 Screening for flavonoids

About 2g of the powdered bitter kola or ginger, completely detanned with acetone. The residue extracted in warm water after evaporating the acetone in water bath. The mixture was filtered while still hot, allowed to cool before used (filtrate). Sodium hydroxide test- 5ml of 20% sodium hydroxide was added to equal volume of the detanned water extract. A yellow solution indicates the presence of flavonoids.

3.14.6 Screening for anthraquinones

About 0.5g of bitter kola or ginger extract was added to dry test tube, then add 5ml chloroform and shake for 5min. filter the bitter kola or ginger extract and then shake filtrate with an equal volume of 100% ammonia solution. A pink violet or red colour in the ammoniacal layer(lower layer) indicates the presence of free anthraquinones.(Bontrager's test)

3.14.7 Screening for cardiac glycoside

100mg of bitter kola or ginger extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution, then underlayered with 1ml of concentrated sulphuric acid. A browm ring obtained at the interface indicates the presence of de-oxysugar characteristics of cardenolides(Keller killiani test).

3.15. Preparation of control solution

Positive control; 10mg/ml Erythromycin and 10mg/ml tetracycline were used for broth dilution. Negative control: distilled water was used.

3.16Drug susceptibility test

Drug susceptibility of *Staphylococcus aureus* , *Pseudomonas auroginosa and Escherichia coli* cultures were determined using Tetracycline and Erythromycin using agar disc diffusion method (Ochei and Kolhtkar, 2008).

3.17Statistical analysis

Data werepresented as mean± Standard error mean. The one way analysis of variants (1ANOVA) was done and F ratio were compared to detect the significant changes between the groups. The tukey’s multiple comparison method was used to compare the means of different groups and the significant denoted by P-values. All the analysis was carried out in personnel computer using statistical package for social science version 21.

CHAPTER FOUR

4.0 RESULTS

4.1.0 Antibacterial activity of various concentrations of individual solvent extract and honey

4.1.1 Antibacterial activity of various concentrations of methanol extractsof bitter kola against the test bacteria.

The highest antibiotic activity of methanolextract of bitter kola was observed on *S. aureus* (14.5±0.0mm) and *P. aeruginosa* (10.5±0.3mm) at 166.67mg/ml concentration of methanol extract of bitter kola. Antibiotic activity was also detected on *S. aereus* (14.0±0.0mm), *E. coli* (8.0±0.0mm)and *P. aeruginosa* (7.0±0.0mm) at 83.33mg/ml concentration of methanol extract of bitter kola. Interestingly, antibiotic activity was expressed till the last concentration (10.42mg/ml) of methanol extractof bitter kola on *S. aureus* (9.8±0.2mm) unlike *E. coli and P. aeruginosa* that resist antibiotic activity from 41.67mg/ml concentration of methanol extractof bitter kola. The tetracycline and erythromycin were used as positive control(Table 4.1.1).

4.1.2 Antibacterial activity of various concentrations of ethyl acetate extracts of bitter kola against the test bacteria.

The ethyl acetate bitter kola (EABK) extract indicated highest zone of inhibition on *S. aureus*(17.0±0.6mm), lowest on *E. coli*(11.0±0.0mm) and *P. aeruginosa* (11.0±0.0mm). Antibiotic activity was also expressed on*S. aureus, E. coli and P. aeruginosa* in a decreasing order in almost all the concentration. The last antibiotic activity recorded on *P. aeruginosa* was at 83.33mg/ml concentration of ethyl acetate bitter kola (EABK) extract(Table 4.1.2).

Table 4.1.1 Antibacterial activity of various concentrations of methanol extracts of bitter kola against the test bacteria.

Test Bacteria	Concentrations of plants extracts in solvents in mg/ml/zone of inhibition in mm							
	Concentrations of methanol bitter kola extracts in mg/ml					Standard Drugs		N/cont.
	166.67	83.33	41.67	20.82	10.42	Eryth	Tetra	D/W
S. aureus	14.5±0.0	14.0±0.0	13.0±0.3	12.0±0.3	9.8±0.2	15.4±1.8	11.0±0.6	0.0±0.0
E. coli	8.5±0.3	8.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	6.0±3.1	0.0±0.0
P.aero	10.5±0.3	7.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	7.0±3.6	0.0±0.0

Data are presented as mean±SEM. Values $\geq$ 6±SEM indicates antibiotic activity.

Key: *S. aureus=Staphylococcus aureus, E. coli= Escherichia coli, P.aero= Pseudomonas auroginosa,* D/W= distilled water, Eryth=erythromycin, Tetra=tetracycline, N/cont.=negative control.

Table 4.1.2 Antibacterial activity of various concentrations of ethyl acetate extracts of bitter kola against the test bacteria.

Test Bacteria	Concentrations of plants extracts in solvents in mg/ml/zone of inhibition in mm							
	Concentration of Ethyl acetate bitter kola extracts in mg/ml					Standard Drugs		N/cont
	166.67	83.33	41.67	20.82	10.42	Eryth	Tetra	D/W
S. aureus	17.0±0.6	15.0±0.6	15.0±0.0	12.0±0.6	12.0±0.6	13.3±1.3	15.0±2.5	0.0±0.0
E. coli	11.0±0.0	9.0±0.0	8.0±0.0	8.0±0.0	0.0±0.0	0.0±0.0	7.3±3.7	0.0±0.0
P.aero	11.0±0.0	8.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	8.7±0.9	0.0±0.0

Data are presented as mean±SEM. Values $\geq$ 6±SEM indicates antibiotic activity.

KEY:*S. aureus=Staphylococcus aureus, E. coli= Escherichia coli, P.aero= Pseudomonas auroginosa,* D/W= distilled water, Eryth=erythromycin, Tetra=tetracycline, N/cont.=negative control.

4.1.3 Antibacterial activity of various concentrations of n-hexane extracts of bitter kola against the test bacteria.

A slight antibiotic activity was noticed from n-hexane bitter kola extract on *S. aureus* (8.0±0.0mm) and there was no antibiotic activity recorded on *E. coli*or*P. aeruginosa*(Table 4.1.3).

4.1.4 Antibacterial activity of various concentrations of methanolextracts of ginger against the test bacteria.

The methanol ginger extract presented no antibiotic activity on all the test bacteria except a slight antibiotic activity on*S. aureus*(7.0±0.0mm)(Table 4.1.4).

4.1.5 Antibacterial activity of various concentrations of n-hexane extracts of ginger against the test bacteria.

There was no antibacterial activity shown by various concentrations of N-hexane ginger extracts on the test bacteria at all(Table 4.1.5).

4.1.6 Antibacterial activity of various concentrations of ethyl acetateextracts of ginger against the test bacteria.

There was no antibacterial activity shown by various concentrations ofethyl acetate ginger extracts on the test bacteria at all(Table 4.1.6).

Table 4.1.3 Antibacterial activity of various concentrations of N-hexaneextracts of bitter kola against the test bacteria.

Test Bacteria	Concentrations of plants extracts in solvents in mg/ml/zone of inhibition in mm							
	Concentration of n-hexane bitter kola extracts in mg/ml					Standard Drugs		N/con
	166.67	83.33	41.67	20.82	10.42	Eryth	Tetra	D/W
S. aureus	8.0±0.0	7.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	13.0±2.4	15.0±2.2	0.0±0.0
E. coli	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
P.aero	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	5.3±2.73	0.0±0.0	0.0±0.0

Data are presented as mean±SEM. Values $\geq$ 6±SEM indicates antibiotic activity.

KEY:*S. aureus=Staphylococcus aureus, E. coli= Escherichia coli, P.aero= Pseudomonas auroginosa,* D/W= distilled water, Eryth=erythromycin, Tetra=tetracycline, N/cont.=negative control.

Table 4.1.4 Antibacterial activity of various concentrations of methanol extracts of ginger against the test bacteria.

Test Bacteria	Concentrations of plants extracts in solvents in mg/ml/zone of inhibition in mm							
	Concentration of methanolginger extracts in mg/ml					Standard Drugs		N/cont
	166.67	83.33	41.67	20.82	10.42	Eryth	Tetra	D/W
S. aureus	7.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	11.0±0.0	12.3±1.2	0.0±0.0
E. coli	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	7.0±3.5	0.0±0.0
P.aero	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	9.7±0.0	0.0±0.0

Data are presented as mean±SEM. Values $\geq$ 6±SEM indicates antibiotic activity.

KEY:*S. aureus=Staphylococcus aureus, E. coli= Escherichia coli, P.aero= Pseudomonas auroginosa,* D/W= distilled water, Eryth=erythromycin, Tetra=tetracycline, N/cont.=negative control.

Table 4.1.5 Antibacterial activity of various concentrations of N-hexaneextracts of ginger against the test bacteria.

Test Bacteria	Concentrations of plants extracts in solvents in mg/ml/zone of inhibition in mm							
	Concentration of N-hexane ginger extracts in mg/ml					Standard Drugs		N/cont
	166.67	83.33	41.67	20.82	10.42	Eryth	Tetra	D/W
S. aureus	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	11.0±0.0	12.3±1.2	0.0±0.0
E. coli	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	7.0±3.5	0.0±0.0
P.aero	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	9.8±1.2	0.0±0.0

Data are presented as mean±SEM. Values $\geq$ 6±SEM indicates antibiotic activity.

KEY: *S. aureus=Staphylococcus aureus, E. coli= Escherichia coli, P.aero= Pseudomonas auroginosa,* D/W= distilled water, Eryth=erythromycin, Tetra=tetracycline, N/cont.=negative control.

Table 4.1.6 Antibacterial activity of various concentrations of ethyl acetateextracts of ginger against the test bacteria.

Test Bacteria	Concentrations of plants extracts in solvents in mg/ml/zone of inhibition in mm							
	Concentration of Ethyl acetate ginger extracts in mg/ml					Standard Drugs		N/cont
	166.67	83.33	41.67	20.82	10.42	Eryth	Tetra	D/W
S. aureus	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	11.0±0.0	12.3±1.2	0.0±0.0
E. coli	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	7.0±3.5	0.0±0.0
P.aero	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	9.8±1.2	0.0±0.0

Data are presented as mean±SEM. Values $\geq$ 6±SEM indicates antibiotic activity.

KEY: *S. aureus=Staphylococcus aureus, E. coli= Escherichia coli, P.aero= Pseudomonas auroginosa,* D/W= distilled water, Eryth=erythromycin, Tetra=tetracycline, N/cont.=negative control.

4.1.7 Antibacterial activity of various honey concentrations against the test bacteria.

The undiluted honey was observed to show antibiotic activity on *Staphylococcus aureus*(14.0±0.3mm), *Escherichia coli*(11.5±0.3mm) and *Pseudomonas auroginosa*(7.5±0.3mm) with the highest zone of inhibition expressed on *S. aureus* and the lowest activity on *P. aeruginosa*. At 1275mg/ml concentration, honey was still able to show antibiotic activity on *S. aureus*(9.5±1.2mm) andE. coli(8.0±0.0mm).

4.1.8 Minimum inhibitory concentration and minimum bactericidal concentration of bitter kola and honey.

In this study, the MIC and MBCfor *S. aureus* was observed to be 318.75mg/ml & 1275mg/ml, 20.82mg/ml & 83.33mg/ml, 10.42mg/ml & 83.33mg/ml for honey, methanol bitter kola extract and ethyl acetate bitter kola extract respectively. And the MIC and MBC for *E. coli* was observedto be 637.5mg/ml, 83.33mg/ml for honey and ethyl acetate bitter kola extract respectively.

4.2.0 Antibacterial activity of solvent extracts in combination with honey.

4.2.1 Antibacterial activity of methanol extractsof bitter kola (MBK) in various concentration in combination with honey against the test bacteria.

The combined antibacterial potential of 166.67mg/ml conc. of MBK with (H_1), reveals antibiotic activity on *S. aureus*(18.0±1.5mm), *E. coli* (7.0±0.0mm) and *P. aeruginosa*(2.16±1.8mm) with the highest activity displayed on *S. aureus* without any activity on *P.aeruginosa*. At 83.33mg/ml concentration of methanol bitter kola extract with honey (H_2) down to 10.42mg/ml concentration of methanol bitter kola extract with undiluted honey; antibiotic activity was only detected on*S. aureus* throughout the combination without any activity on *E. coli* or*P. aeruginosa*.

Table 4.1.7 Antibacterial activity of various honey concentrations against the test bacteria.

Test Bacteria	Concentrations of honey in solvent in mg/ml/zone of inhibition in mm							
	Various honey concentrations express in mg/ml					Standard Drugs		N/cont
	undiluted	1275	637.5	318.75	106.25	Eryth	Tetra	D/W
S. aureus	14.0±0.3	9.5±1.2	0.0±0.0	0.0±0.0	0.0±0.0	11.0±0.6	12.3±1.2	0.0±0.0
E. coli	11.5±0.3	8.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	6.1±3.5	0.0±0.0
P.aero	7.5±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	9.7±1.2	0.0±0.0

Data are presented as mean±SEM. Values $\geq$ 6±SEM indicates antibiotic activity.

KEY: *S. aureus=Staphylococcus aureus, E. coli= Escherichia coli, P.aero= Pseudomonas auroginosa,* D/W= distilled water, Eryth=erythromycin, Tetra=tetracycline, N/cont.=negative control.

Table 4.1.8 Minimum inhibitory concentration and minimum bactericidal concentration of bitter kola and honey.

Minimum inhibitory concentration and minimum bactericidal concentration of bitter kola and honey in mg/ml							
Test bacteria	MIC & MBC	honey	Plant extracts		Positive control		Neg. con
		H	MBK	EABK	ERY	TET	broth
S. aureus	MIC	318.75	20.82	10.42	n/t	n/t	t/s
	MBC	1275	83.33	83.33	n/g	n/g	g
E. coli	MIC	637.5	t/s	83.33	n/t	n/t	t/s
	MBC	g	g	g	n/g	n/g	g
P.aero	MIC	t/s	t/s	t/s	t/s	t/s	t/s
	MBC	g	g	g	g	g	g

KEY: MBC=methanol bitter kola, EABK=ethyl acetate bitter kola, ERY= erythromycin, TET=tetracycline, g=growth, t/s=turbidity seen, n/t=no turbidity, n/g=no growth, *P.aero=P.aeruginosa, S. aereus= Staphylococcus aereus, E. coli= Escherichia coli,*

Table 4.2.1 Antibacterial activity of methanol extracts of bitter kola in various concentration in combination with honey against the test bacteria

Test Bacteria	Combinations of different concentration of methanol extracts of bitter kola with honey in mg/ml/zone of inhibition in mm							
	Combinations of Honey and Methanol bitter kola extract in mg/ml					Standard Drugs		N/cont
	166.67 +H_1	83.33 +H_2	41.67 +H_3	20.82 +H_4	10.42 +H_5	Eryth	Tetra	D/W
S. aureus	18.0±1.5	15.5±0.0	13.0±1.5	13.0±1.5	11.0±0.6	11.0±0.0	11.0±0.6	0.0±0.0
E. coli	7.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	6.0±3.5	0.0±0.0
P.aero	2.16±1.8	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	8.3±0.6	0.0±0.0

Data are presented as mean±SEM. Values $\geq$ 6±SEM indicates antibiotic activity.

KEY: *S. aureus=Staphylococcus aureus, E. coli= Escherichia coli, P.aero= Pseudomonas auroginosa,* D/W= distilled water, Eryth=erythromycin, Tetra=tetracycline, N/cont.=negative control H_1= 106.25mg/ml concentration of honey,H_2=318.75mg/ml concentration of honey, H_3= 637.5mg/ml concentration of honey, H_4=1275 mg/ml concentration of honey, H_5= undiluted honey.

4.2.2 Antibacterial activity of ethyl acetate extracts of bitter kola in various concentration in combination with honey against the test bacteria.

The combined antibacterial potential of honey and ethyl acetate bitter kola extract gave the following outcome; at 166.67mg/ml concentration of ethyl acetate extract of bitter kola with 102.25mg/ml concentration of honey, zone of inhibition was observed on *S. aureus*(9.0±1.2mm), *E coli*(8.67±0.7mm) and*P. aeruginosa* (7.0±0.6mm). Antibiotic activity was observed on *S. aureus*though out the concentrations of the mixture(Table 4.2.2).

4.2.3 Antibacterial activity of n-hexane extracts of bitter kola in combination with honey against the test bacteria.

The n-hexane bitter kola was observed to show no antibiotic activity apart from combination with undiluted honey which shows activity on*S. aureus*(8.3±0.0mm) and *E. coli*(9.3±0.0mm)(Table 4.2.3).

4.2.4 Antibacterial activity of ginger extracts in various solvents in combination with honey against the test bacteria

The combined antibiotic activity of honey and ginger in all the extract (methanol, n-hexane and ethyl acetate) at different concentration was observed to show no antibiotic activity(Table 4.2.4).

Table 4.2.2 Antibacterial activity of ethyl acetate extracts of bitter kola in various concentration in combination with honey against the test bacteria

Test Bacteria	Combinations of different concentration of ethyl acetate extracts of bitter kola with honey in mg/ml/zone of inhibition in mm							
	Combinations of Honey and ethyl E. bitter kola extract in mg/ml					Standard Drugs		N/cont
	166.67 +H_1	83.33 +H_2	41.67 +H_3	20.82 +H_4	10.42 +H_5	Eryth	Tet	D/W
S. aureus	19.0±1.2	16.0±0.0	12.0±0.0	17.0±0.6	17.0±0.6	13.3±2.5	15.0±2.4	0.0±0.0
E. coli	8.67±0.7	8.0±0.0	2.8±2.7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
P.aeroginosa	7.0±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	5.3±2.7	0.0±0.0	0.0±0.0

Data are presented as mean±SEM. Values $\geq$ 6±SEM indicates antibiotic activity.

KEY: *S. aureus=Staphylococcus aureus, E. coli= Escherichia coli, P.aero= Pseudomonas auroginosa,* D/W= distilled water, Eryth=erythromycin, Tetra=tetracycline, N/cont.=negative control H_1= 106.25mg/ml concentration of honey,H_2=318.75mg/ml concentration of honey, H_3= 637.5mg/ml concentration of honey, H_4=1275 mg/ml concentration of honey, H_5= undiluted honey.

Table 4.2.3 Antibacterial activity of N-hexaneextracts of bitter kola in combination with honey against the test bacteria

Test Bacteria	Concentrations of ethyl acetate bitter kola extracts in mg/ml/zone of inhibition in mm							
	Concentration of N-hexane bitter kola extracts in mg/ml					Standard Drugs		N/cont
	166.67 +H_1	83.33 +H_2	41.67 +H_3	20.82 +H_4	10.42 +H_5	Ery	Tet	D/W
S. aureus	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	8.3±0.0	11.0±0.6	12.3±1.3	0.0±0.0
E. coli	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	9.3±0.0	0.0±0.0	6.0±0.0	0.0±0.0
P.aeroginosa	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

KEY:*S. aureus=Staphylococcus aureus, E. coli= Escherichia coli,P.aeroginosa= Pseudomonas auroginosa,* D/W= distilled water,concs= concentrations,H_1= 106.25mg/ml concentration of honey,H_2=318.75mg/ml concentration of honey, H_3=637.5mg/ml concentration of honey, H_4=1275mg/ml concentration of honey, H_5= undiluted honey, N/cont=negative control.

Table 4.2.4 Antibacterial activity of ginger extracts in various solvents in combination with honey against the test bacteria

Test Bacteria	Concentrations of ginger plants extracts in solvents in mg/ml/zone of inhibition in mg/ml											
	Met. Ext & honey		S / Drugs		N-hex & honey		S / Drugs		Eth acet & honey		S / Drugs	
	166.67 $+H_1$	83.33 $+H_2$	Ery	Tet	166.67 $+H_1$	83.33 $+H_2$	Ery	Tet	166.67 $+H_1$	83.33 $+H_2$	Ery	Tet
S. aureus	0.0±0.0	0.0±0.0	7.0±0.0	12.3±1.2	0.0±0.0	0.0±0.0	7.0±0.0	12.3±1.2	0.0±0.0	0.0±0.0	7.0±0.0	12.3±1.
E. coli	0.0±0.0	0.0±0.0	0.0±0.0	7.0±3.5	0.0±0.0	0.0±0.0	0.0±0.0	7.0±3.5	0.0±0.0	0.0±0.0	0.0±0.0	7.0±3.5
P.aero	0.0±0.0	0.0±0.0	0.0±0.0	9.7±1.2	0.0±0.0	0.0±0.0	0.0±0.0	9.7±1.2	0.0±0.0	0.0±0.0	0.0±0.0	9.7±1.2

KEY:*S. aureus=Staphylococcus aureus, E. coli= Escherichia coli, P.aero= Pseudomonas auroginosa,* Met. Ext=methanol extracts, N-hex=N-hexane extract, Eth acet=ethyl acetate extract,S/Drugs=standard drugs,H_1= 106.25mg/ml concentration of honey,H_2=318.75mg/ml concentration of honey.

4.3.0 Percentage yield and phytochemical screening of bitter kola and ginger extracts.

4.3.1 Percentage yield of 100gginger and 100g bitter kola in 500 volume of solvent.

The methanol bitter kola extractions and N-hexane bitter kola extractions yielded a general highest and lowest values of 15.12% and1.16% respectively. N-hexane ginger extracts and methanol ginger extracts yielded 3.06% and 7.29% respectively. Ethyl acetate bitter kola extract and Ethyl acetate ginger extract gave a percentage yield of 7.90% and 4.49% per each extraction respectively(Table 4.3.1).

4.3.2Results obtained from the preliminary phytochemical screening of the crude bitter kola and ginger.

The results of the preliminary phytochemical screening of the extracts are shown in Table 4.3.2. the methanol extracts of bitter kola and ginger reveal the presence of carbohydrate, cardiac glycoside, saponins, alkaloids, flavonoids and steroids/triterpenes whereas n-hexane bitter kola and ginger reveals the presence of cardiac glycoside and steroids/triterpenes. in other hand, ethyl acetate extracts of bitter kola and ginger revealed the presence of carbohydrate, cardiac glycoside, saponins, alkaloids, tannins, flavonoids and steroids/triterpenes(Table 4.3.2).

4.4 The identified clinical bacterial isolates and their source.

This table indicates different method employed to identify *Staphylococcus aureus,Escherichia coli and Pseudomonas auroginosa*(Table 4.4).

Table 4.3.1 Percentage yield of 100g Ginger and 100g Bitter kola in 500 volume of solvent

Plant extract	Method of extraction	Percentage Yield(%)
Methanol bitter kola	maceration	15.12
Methanol ginger	maceration	7.29
N-hexane bitter kola	maceration	1.16
N-hexane ginger	maceration	3.06
Ethyl acetate bitter kola	maceration	7.90
Ethyl acetate ginger	maceration	4.49

Table 4.3.2 Phytochemical Screening of Bitter kola and Ginger extracts.

Bitter kola extract	Ginger extract						
constituents	**Test**	**NH**	**EA**	**met**	**NH**	**EA**	**Met**
Carbohydrates	Mollisch's test	-	+	+	-	+	+
	Fehling´s test	-	-	+	-	+	+
Anthraquinones	Bontrager´s test	-	-	-	-	-	-
Cardiac glycoside	Salkwoski test	+	+	+	+	+	+
	Kella killiani´s test	+	+	+	+	+	+
Saponins	Froathing test	-	+	+	_	_	+
Alkaliods	Wagner´s test	-	+	±	-	-	+
	Meyer´s test	-	-	±	-	-	+
Tannins	$Fecl_3$ test	-	-	-	-	-	-
	Strong lead subovetete test	-	+	+	-	-	+
Flavonoids	Na0H test	-	+	+	-	-	+
	Shinodas´s test	-	+	+	-	+	+
Steroid/triterpenes	inliberman-burchad	++	++	++	+/s	+/s	+/S

Key: += Present, - = Absent, +/s= steroid present only, ±=patialy positive, NH= n-hexane, EA= ethyl acetate, met= methanol.

Table 4.4 The identified clinical bacterial isolates and their source

Bacteria isolates	N	Source	Gram	Cat	Coag	urea	cit	Oxi	KIA			
									Slope	Butt	Gas	HS
Staphylococcus aureus	3	Wound	GPC	+	+							
Pseudomonas auroginosa	3	urine	GNB	+				+	+	+	+	-
Escherichia coli	3	urine	GNB			–	–	–	Y[6]	Y	+[2]	–

Key: GPC=gram positive cocci, GNB= gram negative bacilli, + = positive, -= negative, N=number of isolate, Cat= catalase, Coag= coagulase, Oxi= oxidase, Cit= citrate, Y=yellow.

CHAPTER FIVE

5.0 DISCUSSIONS

The bitter kola extractions from different solvent was observed to show the following result; The methanol bitter kola (MBK) extract in this study was observed to show its highest antibiotic activity on *S. aureus*(14.5±0.0mm) and*P. aeruginosa* (10.5±0.3mm) at 166.67mg/ml concentration of methanol bitter kola extract and antibiotic activity was also detected on *S. aereus*(14.0±0.0mm)*, E. coli* (8.00±0.0mm)and*P. aeruginosa* (7.0±0.0mm) at 83.33mg/ml concentration of methanol bitter kola (MBK) extract. interestingly, antibiotic activity was expressed till the last concentration (10.42mg/ml) of methanol bitter kola (MBK) extract on *S. aureus*(9.8±0.2mm) unlike *E. coli and P. aeruginosa*that resist antibiotic activity from 41.67mg/ml concentration of MBK extract, indicating more activity on gram positive organism than on gram negative organism and this could be due to the nature of phytochemical compound extracted by methanol. This is suggestive that methanol bitter kola extract can be used in treatment of bacterial infection and preferably in *S. aureus*wound infection. These findings have come to terms with the work done by Arekemase *et al.,* (2012).;Egwuatu*et al.,*(2014).; Ukaoma *et al*.,(2013).; Christinah and Roland, (2012).;Amalu*et al.,*(2014) who described antibacterial effect of bitter kola methanol extract to be in line with this finding in terms of broad spectrum antibacterial activity except the work of Ukaoma *et al*.,(2013) who stated that the extract were more active on *P. aeruginosa* than *S. aereus*(Table 4.1.1)

The ethyl acetate bitter kola (EABK) extract of this study indicated highest zone of inhibition on *S. aureus*(17.0±0.6mm), lowest on *E. coli*(11.0±0.0mm) and *P. aeruginosa*

(11.0±0.0mm). Antibiotic activity was also expressed on*S. aureus, E. coli and P. aeruginosa* in a decreasing order in almost all the concentration demonstrating a greater antibiotic activity of ethyl acetate bitter kola extract than methanol bitter kola extract. This implies that ethyl acetate bitter kola extract contains more phytochemical compound than methanol bitter kola extract and will be better use in treatment of bacterial infection. This could be due to differences in their dielectric constant power of extraction of the different solvent.This report is buttressed by similar work and report of Madubunyi, (2008).; Durand *et al.,*(2015).;Christinah and Roland , (2012)(Table 4.1.2).

A slight antibiotic activity was noticed from n-hexane bitter kola extract on *S. aureus* (8.0±0.0mm) which could be due to non-polar nature of n-hexane, despite high antibiotic activity observed in other solvent extract. The above statement implies that the nature, quality and type of solvent used in extraction of plant materials can increase or decrease the antibacterial contents of the plant. This is in line with report of Uzondu*et al.,*(2010).; Uzondu*et al.,*(2014) who reported no antibacterial activity on *E. coli*and more antibacterial activity on *S. aureus* apart from increased antibacterial activity reported(Table 4.1.3)

The ginger extracts (methanol, ethyl acetate and n-hexane solvent) of this study presented no antibiotic activity apart from methanol extract that show antibiotic activity on*S. aureus*(7.0±0.0mm) and these could be due to the extraction solvent, method of extraction, nature of extract, species of ginger and method of testing the extract. This implies that ginger species might have a very small antibacterial effect. These finding contradicted report of*Hiba et al.,* (2015); Yahaya *et al.,*(2012) who reportedclear antibacterial activity of ginger extract against pathogenic organism and agreed with Gamil *et al.,* (2016). The

soxhlet extraction method was used in most of the work in ginger extraction and this might be the reason for the variations in the report(Table 4.1.4, 4.1.5 and 4.1.6).

According to this study, undiluted honey was observed to show antibiotic activity on *Staphylococcus aureus*(14.0±0.3mm), *Escherichia coli*(11.5±0.3mm) and *Pseudomonas auroginosa*(7.5±0.3mm) with the highest zone of inhibition expressed on *S. aureus* and the lowest activity on *P. aeruginosa*. At 1275mg/ml concentration, honey was still able to show antibiotic activity on *S. aureus*(9.5±1.2mm) and*E. coli*(8.0±0.0mm). Resistance of *P. aeruginosa* to the extract was observed starting from 1275mg/ml concentration of honey while 637.50mg/ml, 318.75mg/ml and 106.25mg/ml concentration of honey did not show any activity against all the bacteria. This antibiotic activity could be due to phytochemical compound contained in honey.The above indicated that honey has a broad spectrum antibacterial activity and can be used in treatment of bacterial infection. These outcomes coincide with the work of Ewnetu *et al.,* (2013).; Swapna *and* Warangal, (2013).; Julie*et al.,* (2011) who reported broad spectrum antibacterial activity of honey with higher activity on gram positive than gram negative organism and the work of Saad*et al.,*(2016).; Rohand *et al.,*(2007) who reported that honey exhibit antibacterial activity(Table 4.1.7).

In this study, the MIC and MBC for *S. aureus* was observed to be 318.75mg/ml & 1275mg/ml, 20.82mg/ml & 83.33mg/ml, 10.42mg/ml & 83.33mg/ml for honey, methanol bitter kola extract and ethyl acetate bitter kola extract respectively. This is slightly in line with the work of Sherlock*et al.,* (2010).; Tan *et al.,* (2009).; Mallai*et al.,* (2007).; Chauhan*et al.,* (2010) apart from high value of MIC and MIC for *P. aeruginosa* and *E. coli* reported for honey. And the MIC and MBC for *E. coli* was observedto be 637.5mg/ml,

83.33mg/ml for honey and ethyl acetate bitter kola extract respectively. The MIC for ethyl acetate bitter kola of this work disagreed with the work Arekemase *et al.*, (2012).; Madubunyi *et al.,* (2008). For *P. aeruginosa,* turbidity and growth was observed for MIC and MBC (Table 4.1.8).

The combined antibacterial potential of 166.67mg/ml concentration of methanol bitter kola extract with 106.25mg/ml concentration of honey, reveals antibiotic activity on *S. aureus*(18.0±1.5mm), *E. coli* (7.0±0.0mm) and *P. aeruginosa*(2.2±1.8mm) with the highest activity displayed on *S. aureus* followed by *E. coli* without any activity on *P.aeruginosa*. At 83.33mg/ml concentration of methanol bitter kola extract with 318.75mg/ml concentration of honey down to 10.42mg/ml concentration of methanol bitter kola extract with undiluted honey; antibiotic activity was only detected on*S. aureus* throughout the combination without any activity on *E. coli* or*P. aeruginosa* indicating a serious antibiotic activity against gram positive organism than gram negative organism(Table 4.2.1).

The zone of inhibition of methanol bitter kola extract and ethyl acetate bitter kola extract conveys similar result indicating additive or synergistic effect of the combination while that of n-hexane bitter kola was observed to show no antibiotic activity apart from combination with highest undiluted honey which shows activity on*S. aureus*(8.3±0.0mm) and *E. coli*(9.3±0.0mm)(Table 4.2.1, 4.2.2 and 4.2.3). This zone of inhibition from the combination of n-hexane bitter kola extract is of no doubt to have emanated from the presences of honey in the combination. Akinnibosun and Itedjere, (2013) concurs with this work in terms of synergistic effect of the combination and disagreed on the order of gram negative and gram positive action.

The combined antibacterial potential of honey and ethyl acetate bitter kola extract gave the following outcome; at 166.67mg/ml concentration of ethyl acetate bitter kola extract with 102.25mg/ml concentration of honey have produced zone of inhibition on *S. aureus*(19.0±1.2mm), *E coli*(8.7±0.7mm) and*P. aeruginosa* (7.0±0.6mm). The antibiotic activity was also observed in various combinations in a similar pattern as above with more activity on*S. aureus* and less activity on*E. coli and P. aeruginosa*. From the above, the mixture can be preferably use in wound infection treatment. These zone of inhibition above when compared with the individual antibiotic activity unveiled that the combination effect to be synergistic and in some cases additive. These report agrees with the work of Akinnibosun and Itedjere, (2013) who reported that there is a synergistic effect from combination of bitter kola and honey when compared with their individual effect and disagreed wth Adeleye *et al*., (2003) who reported that no antibacterial activity of the combination(Table 4.2.1, 4.1.2 and 4.1.7).

The combined antibiotic activity of honey and ginger in all the extract (methanol, n-hexane and ethyl acetate) at different concentration was observed to show no antibiotic activity. These could be due to the oily nature of the extract and method of ginger extraction. This report disagreed with Manasa *et al.,*(2013).; Roopal and Patel, (2011).; Yahaya *et al.,*(2012).; Omoya and Akharaiyi, (2011).; Yalemwork *et al.,* (2014) report which observed varying degree of activity in almost all the tested bacteria and concurred with Gamil *et al.,* (2016) who reported no antibacterial activity of the extracts (Table 4.2.3).

All the ginger extract and n-hexane bitter kola extract in combination with honey shows an negligible zone of inhibition which contradicted report of Malu *et al.,*(2009) who reported

broad antibacterial activity of the extracts and agreed with Gamil *et al.,* (2016) who reported no antibacterial activity of the extracts(Table 4.2.3).

The percentage yield of 100g ginger and 100g of bitter kola in 500m volume of solvents is discussed based on the dielectric constant power of the solvents and the polarity nature of the plants substances. Methanol (polar protic solvent with dielectric constant:33) bitter kola extractions and N-hexane (non-polar solvent with dielectric constant: 1.88) bitter kola extractions yielded a general highest and lowest values of 15.12% and1.16% respectively. This indicates that bitter kola is 92.87% soluble in polar protic solvents and 7.12% soluble in non-polar solvents. N-hexane ginger extracts and methanol ginger extracts yielded 3.06% and 7.29% respectively. This indicates that ginger is 70.43% soluble in polar protic solvents and 29.57% in non-polar solvents. Ethyl acetate (polar aprotic solvent with dielectric constant: 6.02) bitter kola extract and Ethyl acetate ginger extract gave a percentage yield of 7.90% and 4.49% per each extraction respectively. The value of ethyl acetate bitter kola extract (7.90%) indicate dielectric power of polar aprotic in dissolving both polar protic soluble substances and non-polar soluble substances in equal strength. This is further indicated by 4.49% value of ethyl acetate ginger extraction yield, this statement is justified when we compare the sum individual half yield of the polar protic solvent extract and non-polar solvent extract of ginger and bitter kola.This work is in line with dielectric constant scale for rough measurement of solvents polarity reported by Malmberg and Maryott, (1956); Lowery and Richardson,(1987)(Table 4.3.1).

The phytochemical analysis of methanol extracts of bitter kola and ginger revealed the presence of carbohydrate, cardiac glycoside, saponins, alkaloids, flavonoids and

steroids/triterpenes whereas n-hexane bitter kola and ginger reveals the presence of cardiac glycoside and steroids/triterpenes. In the other hand, ethyl acetate extracts of bitter kola and ginger revealed the presence of carbohydrate, cardiac glycoside, saponins, alkaloids, tannins, flavonoids and steroids/triterpenes. The presence of tannins and alkaloids in bitter kola as detected in this study is in line with the work of Okwu and Ekeke , (2003)(Table 4.3.2).

CHAPTER SIX

6.1 CONCLUSION

The outcome of this study revealed honey and bitter kola extracts to have broad spectrum antibacterial activity in their individual and combined forms. And more significant effect has being noticed in *S. aureus*than *E. coli*and*P. aeruginosa.* The leading antibacterial inhibition were produced by bitter kola extracts. Ginger antibiotic activity in this study did not show any reasonable effect both on its own and in combination with honey.

6.2 RECOMMENDATION

Further study should be conducted using advanced technique in the method of testing plant antibiotic activity that will separate and purify chemical compound that are responsible for antibiotic activity especially in ginger study. There will be need for separation and purification of bitter kola chemical compound for its use in modern medicine.

That for honey to be used in treatment of infection, there will be need for the testing of efficacy of such honey before use.

Combination of honey and bitter kola in treatment of infection is also recommended.

6.3 LIMITATION OF THE STUDY

Quality control stains were intended to be used to monitor the performance of the test but could not be used due to time constraint.

REFERENCE

Adams, C. J., Boult, C. H., Deadman, B. J., Farr, J. M., Grainger, M. N., Manley-harris, M. & Snow, M. J. 2008. Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (Leptospermum scoparium) honey. *Carbohydrate research,* 343**,** 651-659.

Adeleke OE, Olaitan JO, Okepekpe EI2006. Comparative antibacterial activity of honey and gentamicin against *Escherichia coli* and *Pseudomonas aeruginosa*. Annals Burn Fire Disasters.;19:n4. [PMC free article]

Adeleye,I.A. & Opiah,L. 2003. Antimicrobial activity of extracts of local cough mixture on upper respiratory tract bacterial pathogen. *West indian Med. journal,*52,3, 188-90.

Adesina, S., Gbile, Z., Odukoya, O., Akinwusi, D., Illoh, H. & Jayeola, A. 1995. Survey of indigenous useful plants of West Africa with special emphasis on medicinal plants and issues associated with management. *The United Nations Programme on Natural Resources in Africa,* 2**,** 84-85.

Aeschbach, R., Löliger, J., Scott, B., Murcia, A., Butler, J., Halliwell, B. & Aruoma, O. 1994. Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food and Chemical Toxicology,* 32**,** 31-36.

Agbagwa, O. & Frank-peterside, N. 2010. Effect of raw commercial honeys from Nigeria on selected pathogenic bacteria. *African Journal of Microbiology Research,* 4**,** 1801-1803.

Ahmed, A. K. J., Hoekstra, M. J., Hage, J. J. & Karim, R. B. 2003. Honey-medicated dressing: transformation of an ancient remedy into modern therapy. *Annals of plastic surgery,* 50**,** 143-148.

Ahui, M. L. B., Champy, P., Ramadan, A., Van, L. P., Araujo, L., André, K. B., Diem, S., Damotte, D., Kati-coulibaly, S. & Offoumou, M. A. 2008. Ginger prevents Th2-mediated immune responses in a mouse model of airway inflammation. *International immunopharmacology,* 8**,** 1626-1632.

Akinnibosun, I. & Itedjere, E. 2013. Evaluation of the antibacterial properties and synergistic effect of Garcinia kola heckel (Family: Guttiferae) seed extract and honey on some bacteria. *Afr J Microbiol Res,* 7**,** 174-180.

Aktan, F., Henness, S., Tran, V. H., Duke, C. C., Roufogalis, B. D. & Ammit, A. J. 2006. Gingerol metabolite and a synthetic analogue Capsarol™ inhibit macrophage NF-κB-mediated iNOS gene expression and enzyme activity. *Planta medica,* 72**,** 727-734.

Al-waili, N., Akmal, M., Al-waili, F., Saloom, K. & Ali, A. 2005. The antimicrobial potential of honey from United Arab Emirates on some microbial isolates. *Medical Science Monitor,* 11**,** BR433-BR438.

Ali, B. H., Blunden, G., Tanira, M. O. & Nemmar, A. 2008. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. *Food and chemical Toxicology,* 46**,** 409-420.

Alizadeh-navaei, R., Roozbeh, F., Saravi, M., Pouramir, M., Jalali, F. & Moghadamnia, A. A. 2008. Investigation of the effect of ginger on the lipid levels. A double blind controlled clinical trial. *Saudi medical journal,* 29**,** 1280-1284.

Allen, K., Hutchinson, G. & Molan, P. The potential for using honey to treat wounds infected with MRSA and VRE. FirstWorld Wound Healing Congress, 2000. 10-13.

Altman, R. D. & Marcussen, K. 2001. Effects of a ginger extract on knee pain in patients with osteoarthritis. *Arthritis & Rheumatism,* 44**,** 2531-2538.

Amalu, P.C., Chukwuezi, F. O & Ugwu, O. P.C.2014. Antimicrobial effects of bitter kola (Garcinia kola) nut on *Staphylococcus aureus, Escherichia coli and Candida albican.Journal of Dental and Medical Sciences*, 13,29-32.

Anegbeh, P., Iruka, C. & Nkirika, C. 2006. Enhancing germination of Bitter Cola (Garcinia kola) Heckel: Prospects for agroforestry farmers in the Niger delta. *Sci. Afr,* 5**,** 1-7.

Antipenko A. Y, Spielman A. I, Kirchberger M. A.1999. Interactions of [6]-gingerol and ellagic acid with the cardiac sarcoplasmic reticulum $Ca2^{+}$-AT Pase. J Pharmacol Exp Ther.;290(1):227–34.

Apiari Scout Beekeeping Center, 2003. Pusat Perlebahan Apiari Pramuka Lebah Madu: Cara Beternak dan Pemanfaatan. Penebar Swadaya, Depok.

Arekemase, M., Babandoko, A. M., Ojo, K. R. M., Elizabeth, A. A. & Kamoldeen, A. A. 2012. Antimicrobial effects of Garcinia kola (bitter kola) on some selected

pathogens from University of Ilorin teaching hospital Ilorin, Nigeria. *Journal of Asian Scientific Research,* 2**,** 159.

Asadi-pooya, A.A., Pnjehshahin, M.R., Beheshti, S. 2003. The antimyco-bacterial effect of honey. Rivista di biologia, 96,491-495.

Asaolu, M. 2003. Chemical compositions and phytochemical screening of the seeds of Garcinia kola (Bitter kola). *Pakistan Journal of Scientific and Industrial Research,* 46**,** 145-147.

Atawodi, S., Mende, P., Pfundstein, B., Preussmann, R. & Spiegelhalder, B. 1995. Nitrosatable amines and nitrosamide formation in natural stimulants: Cola acuminata, C. nitida and Garcinia cola. *Food and chemical toxicology,* 33**,** 625-630.

Azu, N.C & Onyeagba, R.A. 2007. Antimicrobial of extract of allium copa(onions) and zingiber bacillum subtitis. International Journal Trop Med, 3, 1-4.

Badawy, O., Shafii, S., Tharwat, E. & Kamal, A. 2004. Antibacterial activity of bee honey and its therapeutic usefulness against Escherichia coli O157: H7 and Salmonella typhimurium infection. *Revue scientifique et technique (International Office of Epizootics),* 23**,** 1011-1022.

Bailey-shaw, Y. A., Williams, L. A., Junor, G.-A. O., Green, C. E., Hibbert, S. L., Salmon, C. N. & Smith, A. M. 2008. Changes in the contents of oleoresin and pungent bioactive principles of Jamaican ginger (Zingiber officinale Roscoe.) during maturation. *J. Agric. Food Chem,* 56**,** 5564-5571.

Balick, M. J., Elisabetsky, E. & Laird, S. A. 1996. *Medicinal resources of the tropical forest: biodiversity and its importance to human health*, Columbia University Press.

Bang, L. M., Buntting, C. & Molan, P. 2003. The effect of dilution on the rate of hydrogen peroxide production in honey and its implications for wound healing. *The Journal of Alternative & Complementary Medicine,* 9**,** 267-273.

Banso A & Ngbde, J.E.2006. phytochemical screening and invitro antifungal properties of fagara zannthoxyloides. JFA & E (3,4,) 8-9.

Basualdo, C., Sgroy, V., Finola, M. S. & Marioli, J. M. 2007. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Veterinary microbiology,* 124**,** 375-381.

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M. P., Albertini, M. C. & Piatti, E. 2006. Raw Millefiori honey is packed full of antioxidants. *Food Chemistry,* 97**,** 217-222.

Bliddal, H., Rosetzsky, A., Schlichting, P., Weidner, M., Andersen, L., Ibfelt, H.-H., Christensen, K., Jensen, O. & Barslev, J. 2000. A randomized, placebo-controlled, cross-over study of ginger extracts and ibuprofen in osteoarthritis. *Osteoarthritis and Cartilage,* 8**,** 9-12.

Bogdanov, S. 1997. Nature and origin of the antibacterial substances in honey. *LWT-Food Science and Technology,* 30**,** 748-753.

Boone, S. A. & Shields, K. M. 2005. Treating pregnancy-related nausea and vomiting with ginger. *Annals of Pharmacotherapy,* 39**,** 1710-1713.

Buddhakala, N., Talubmook, C., Sriyotha, P., Wray, S. & Kupittayanant, S. 2008. Inhibitory effects of ginger oil on spontaneous and PGF2α-induced contraction of rat myometrium. *Planta medica,* 74**,** 385-391.

Chauhan, A., Pandey, V., Chacko, K. & Khandal, R. 2010. Antibacterial activity of raw and processed honey. *Electron J Biol,* 5**,** 58-66.

Cheesbrough, M. (2002). District Laboratory practices in Tropical Countries (part 2).Cambridge Univ. Press. Pp. 137-139.

Chen C. Y, Chen C. H, Kung C. H, Kuo S. H, Kuo S. Y.2008. [6]-gingerol induces Ca2+ mobilization in Madin-Darby canine kidney cells. J Nat Prod.;71(1):137–40.

Chen, B.-H., Wu, P.-Y., Chen, K.-M., Fu, T.-F., Wang, H.-M. & Chen, C.-Y. 2009. Antiallergic potential on RBL-2H3 cells of some phenolic constituents of Zingiber officinale (Ginger). *Journal of natural products,* 72**,** 950-953.

Christinah, T.S & Roland, N.N. 2012. Identification and antibacterial evolution of bioactive compounds from Garcinia kola(Heckel) seeds. *Journal molecules*, 17,6.

Church, D., Elsayed, S., Reid, O., Winston, B. & Lindsay, R. 2006. Burn wound infections. *Clinical microbiology reviews,* 19**,** 403-434.

Cochran, W.G. 1977. Sampling techniques . John wiley and sons, 3rd ed.

Cohen, R. J., Ek, K. & Pan, C. X. 2002. Complementary and Alternative Medicine (CAM) Use by Older Adults A Comparison of Self-Report and Physician Chart

Documentation. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,* 57**,** M223-M227.

Cooper, R., Halas, E. & Molan, P. 2002. The efficacy of honey in inhibiting strains of Pseudomonas aeruginosa from infected burns. *Journal of Burn Care & Research,* 23**,** 366-370.

Dedov, V. N., Tran, V. H., Duke, C. C., Connor, M., Christie, M. J., Mandadi, S. & Roufogalis, B. D. 2002. Gingerols: a novel class of vanilloid receptor (VR1) agonists. *British journal of pharmacology,* 137**,** 793-798.

Dias, M., Spinardi-barbisan, A., Rodrigues, M., De camargo, J., Teran, E. & Barbisan, L. F. 2006. Lack of chemopreventive effects of ginger on colon carcinogenesis induced by 1, 2-dimethylhydrazine in rats. *Food and chemical Toxicology,* 44**,** 877-884.

Ding G. H, Naora K, Hayashibara M, Katagiri Y, Kano Y, Iwamoto K1991. Pharmacokinetics of [6]-gingerol after intravenous administration in rats. Chem Pharm Bull (Tokyo);39(6):1612–4.

Dixon B. 2003. Bacteria can't resist honey. Lancet Infect Dis.;3:116.

Dosunmu, M. I. & Johnson, E. C. 1995. Chemical evaluation of the nutritive value and changes in ascorbic acid content during storage of the fruit of 'bitter kola'(Garcinia kola). *Food chemistry,* 54**,** 67-71.

Dunford, C., Cooper, R. & Molan, P. 1999. Using honey as a dressing for infected skin lesions. *Nursing Times,* 96**,** 7-9.

Durand, D.O., Farid, B.M., Adolphe, A.H., Haziz,S.,Pacome,A.N., Hubert,A.S., Nafan,D., Chritine,N.,Francois, S.A., & Lamine,B.M. 2015. Phytochemical sceening and biologicalactivity of Garcinia kola (bark,leaves and seeds) collected in Benin. *African journal ofMicrobiology Research,* 9,28,1716-1727.

El-mahmood, A. & Ameh, J. 2007. In vitro antibacterial activity of Parkia biglobosa (Jacq.) root bark extract against some microorganisms associated with urinary tract infections. *African Journal of Biotechnology,* 6.

Egwuatu,C.C., Egwuatu, T.O., Iwuafor.A.A., Akujobi, C.C., Nnachi,A.U., & Aghanya,I.N. 2014. Time kill of crude extract of Garcinia kola seed on Methicillin-Rresistant *Staphylococcus aureus* from the Anterior Nares of Health care workers at Tertiary

Hospital in Nigeria. *Journal of Advances in Medical and pharmaceutical Sciences,* 8, 3.

El-sharaky, A., Newairy, A., Kamel, M. & Eweda, S. 2009. Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats. *Food and Chemical Toxicology,* 47**,** 1584-1590.

Eldershaw, T., Colquhoun, E., Dora, K., Peng, Z.-C. & Clark, M. 1992. Pungent principles of ginger (Zingiber officinale) are thermogenic in the perfused rat hindlimb. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity,* 16**,** 755-763.

Eleyinmi, A. F., Bressler, D. C., Amoo, I. A., Sporns, P. & Oshodi, A. A. 2006. Chemical composition of bitter cola (Garcinia kola) seed and hulls. *Polish journal of food and nutrition sciences,* 15**,** 395.

Engdal, S., Klepp, O. & Nilsen, O. G. 2009. Identification and exploration of herb-drug combinations used by cancer patients. *Integrative cancer therapies,* 8**,** 29-36.

Ensiyeh, J. & Sakineh, M.-A. C. 2009. Comparing ginger and vitamin B6 for the treatment of nausea and vomiting in pregnancy: a randomised controlled trial. *Midwifery,* 25**,** 649-653.

Ernst, E. & Schmidt, K. 2002. Health risks over the Internet: advice offered by "medical herbalists" to a pregnant woman. *Wiener Medizinische Wochenschrift,* 152**,** 190-192.

Esiegwu, A. & Udedibie, A. 2009. Growth performance of and microbial activities in broilers fed supplementary bitter kola (Garcinia kola). *Animal Production Research Advances,* 5.

Essien, E.U., G.J. Esenowo, and M.I. Akpanabiatu. 1995. Lipid composition of lesser known tropical seeds. *Plant Foods Hum. Nutr*. 48:135–140.

Ewnetu, Y., Lemma, W. & Birhane, N. 2013. Antibacterial effects of Apis mellifera and stingless bees honeys on susceptible and resistant strains of Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae in Gondar, Northwest Ethiopia. *BMC complementary and alternative medicine,* 13**,** 1.

Farombi, E., Tahnteng, J., Agboola, A., Nwankwo, J. & Emerole, G. 2000. Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron—a Garcinia kola seed extract. *Food and Chemical Toxicology,* 38**,** 535-541.

Fatope, M., Ibrahim, H. & Takeda, Y. 1993. Screening of higher plants reputed as pesticides using the brine shrimp lethality assay. *International Journal of pharmacognosy,* 31**,** 250-254.

Fauci, A. S. 2001. Infectious diseases: considerations for the 21st century. *Clinical Infectious Diseases,* 32**,** 675-685.

Ficker, C., M.L. Smith, K. Akpagana, M. Gbeassor, J. Zhang, 2003. Bioassay guided isolation andidentification of antifungal compounds from ginger. Phytother. Res., 17: 897-902.

French, V., Cooper, R. A. & Molan, P. C. 2005. The antibacterial activity of honey against coagulase-negative staphylococci. *Journal of Antimicrobial Chemotherapy,* 56**,** 228-231.

Fuhrman, B., Rosenblat, M., Hayek, T., Coleman, R. & Aviram, M. 2000. Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic, apolipoprotein E-deficient mice. *The Journal of nutrition,* 130**,** 1124-1131.

Funk, J. L., Frye, J. B., Oyarzo, J. N. & Timmermann, B. N. 2009. Comparative effects of two gingerol-containing zingiber officinale extracts on experimental rheumatoid arthritis⊥. *Journal of natural products,* 72**,** 403-407.

Gamil, S.G.Z., Samir, A.A., Abeer, M.A., Enas, S.K. 2016. Antimicrobial effect of honey and some herbal plant extracts against multidrug resistance bacteria isolated from patient in local riyadh hospital. *International Journal of advanced research*, 4, 283-291.

Garc, C.S & Bruc, D.A. 1993. Efficacy of ginger for nausea and vomiting, a systematic review of randomized clinical trials. Br. Journal Anesthes, 84, 367-371.

Ghayur, M. N., Gilani, A. H. & Janssen, L. J. 2008. Ginger attenuates acetylcholine-induced contraction and Ca2+ signalling in murine airway smooth muscle cells. *Canadian journal of physiology and pharmacology,* 86**,** 264-271.

Grange, J.M. and R.W. Darvey, 1990. Antibacterialproperties of propolis (bee glue). J. Roy. Soc.Grant K. L, Lutz R. B. 2000. Ginger. Am J Health Syst Pharm.;57(10):945–7.

Grøntved, A., Brask, T., Kambskard, J. & Hentzer, E. 1988. Ginger Root Against Seasickness: A Conctrolled Trial on the Open Sea. *Acta oto-laryngologica,* 105**,** 45-49.

Gull, I., Saeed, M., Shaukat, H., Aslam, S. M., Samra, Z. Q. & Athar, A. M. 2012. Inhibitory effect of Allium sativum and Zingiber officinale extracts on clinically important drug resistant pathogenic bacteria. *Annals of clinical microbiology and antimicrobials,* 11**,** 1.

Hailm, N.A. and M. Suharno, 2001. Teknik Mencangkok Royal Jelly, Lebah Madu *Apis mellifera* Ligustica dan Prospek bisnis. Kanisius Publisher, Yogyakarta.

Heckel, E. & Schlagdenhauffen, F. 1884. Some African kolas, in their botanical, chemical and therapeutical aspects. *Am Pharmacy,* 56**,** 81-177.

Hegazi, A. G. 2011. Antimicrobial activity of different egyptian honey as comparison of saudi arabia honey. *Journal microbiol.* 6(1), 488-495.

Hiba,A.H., Ayad,M.R.R., Basama, M.A.R & Bassan , A.R.H. 2015. Chemical composition and antimicrobial activity of crude extract isolated from Zingiber Officinale by different solvent. Pharmaceutica Analytica Acta,3,184.

http://www.onlinenigeria.com/map.gif accessed 1st july, 2016.

Hussain, R., Owegby, A., Parimoo, P. & Waterman, P. 1982. Kolanone, a novel polyisoprenylated benzophenone with antimicrobial properties from the fruit of Garcinia kola. *Planta Medica,* 44**,** 78-81.

Ibiblio, J.O. 1983. Some medicinal plants of Nigeria. Woody plants of Ghana. oxford University press, oxford, 146-147.

Ibekwe, H., Eteng, M. & Antigha, E. 2007. Proximate and Phytochemical composition of Garcinia kola and Vernonia amygdalina. *Global Journal of agricultural Sciences,* 6**,** 207-209.

Ippoushi, K., Azuma, K., Ito, H., Horie, H. & Higashio, H. 2003. [6]-Gingerol inhibits nitric oxide synthesis in activated J774. 1 mouse macrophages and prevents peroxynitrite-induced oxidation and nitration reactions. *Life sciences,* 73**,** 3427-3437.

Iris, F.F & Benzie, S.W. 2011. Herbal medicine : Biomolecular and Clinical Aspects. 2nd edittion by CRC press Tayor and Francis group, Boka Raton London New York.

Irvine, F. R. 1961. *Woody Plants of Ghana*. Oxford University press, Oxford, pp:146–147.

Iwasaki, Y., Morita, A., Iwasawa, T., Kobata, K., Sekiwa, Y., Morimitsu, Y., Kubota, K. & Watanabe, T. 2006. A nonpungent component of steamed ginger—[10]-shogaol—increases adrenaline secretion via the activation of TRPV1. *Nutritional neuroscience,* 9**,** 169-178.

Iwu, M.M. & Igboko, O. 1982. Flavonoid of Garcinia kola seeds. Journal Nat. prod., 45, 650-651.

Jagetia, G., Baliga, M. & Venkatesh, P. 2004. Ginger (Zingiber officinale Rosc.), a dietary supplement, protects mice against radiation-induced lethality: mechanism of action. *Cancer biotherapy & radiopharmaceuticals,* 19**,** 422-435.

Jiang, H., Xie, Z., Koo, H. J., Mclaughlin, S. P., Timmermann, B. N. & Gang, D. R. 2006. Metabolic profiling and phylogenetic analysis of medicinal Zingiber species: Tools for authentication of ginger (Zingiber officinale Rosc.). *Phytochemistry,* 67**,** 1673-1685.

Jiang, S. Z., Wang, N. S. & Mi, S. Q. 2008. Plasma pharmacokinetics and tissue distribution of [6]-gingerol in rats. *Biopharmaceutics & drug disposition,* 29**,** 529-537.

Jolad, S. D., Lantz, R. C., Chen, G. J., Bates, R. B. & Timmermann, B. N. 2005. Commercially processed dry ginger (Zingiber officinale): composition and effects on LPS-stimulated PGE 2 production. *Phytochemistry,* 66**,** 1614-1635.

Julie,I.,Shona,B., & Dee, A.C. 2011.The antibacterial activity of honey derived from Australian flora. Research Article.

Kabangu, K., Galeffi, C., Aonzo, E., Nicoletti, M. & Messana, I. 1987. A New Biflavanone from the Bark of Garcinia kola1. *Planta medica,* 53**,** 275-277.

Khan, F., Abadin, U. & Rauf, N. 2007. Honey; Nutritional and medical Value. *Medscape Today*.

Kianoush K.D, Ramin K, Saeideh E, Fatemeh S.R,Alaleh Z, and Saeede S. 2013. Antifungal and Anti-bacterial Synergistic Effects of Mixture of Honey and HerbalExtracts. Zahedan Journal of Research in Medical Sciences; 15(8): 30-33

Kim, H. W., Murakami, A., Nakamura, Y. & Ohigashi, H. 2002. Screening of edible Japanese plants for suppressive effects on phorbol ester-induced superoxide generation in differentiated HL-60 cells and AS52 cells. *Cancer letters,* 176**,** 7-16.

Kim, J.-K., Kim, Y., Na, K.-M., Surh, Y.-J. & Kim, T.-Y. 2007. [6]-Gingerol prevents UVB-induced ROS production and COX-2 expression in vitro and in vivo. *Free radical research,* 41**,** 603-614.

Koh, E. M., Kim, H. J., Kim, S., Choi, W. H., Choi, Y. H., Ryu, S. Y., Kim, Y. S., Koh, W. S. & Park, S.-Y. 2009. Modulation of macrophage functions by compounds isolated from Zingiber officinale. *Planta medica,* 75**,** 148-151.

Koo, K. L., Ammit, A. J., Tran, V. H., Duke, C. C. & Roufogalis, B. D. 2001. Gingerols and related analogues inhibit arachidonic acid-induced human platelet serotonin release and aggregation. *Thrombosis research,* 103**,** 387-397.

Kukate C.K. 2005. A Textbook of Practical Pharmacognosy. Vallabh Prakashan, Edition 5:105-111.

Kwakman, P. H., Te velde, A. A., De boer, L., Speijer, D., Vandenbroucke-grauls, C. M. & Zaat, S. A. 2010. How honey kills bacteria. *The FASEB Journal,* 24**,** 2576-2582.

Ladipo, D. 1995. Physiological/morphological growth rate and fruit/nut yields in Garcinia kola tree on acid soil of Onne, Port Harcourt. *ICRAF In-House Report*.

Levy, S. B. & Marshall, B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. *Nature medicine,* 10**,** S122-S129.

Lowery, T,H. & Richardson, K.S. 1987. *Mechanism and Theory in Organic Chemistry,* harper collins publishers 3rd edition.

Lusby, P., Coombes, A. & Wilkinson, J. 2002. Honey: a potent agent for wound healing? *Journal of Wound Ostomy & Continence Nursing,* 29**,** 295-300.

Lusby, P. E., Coombes, A. L. & Wilkinson, J. M. 2005. Bactericidal activity of different honeys against pathogenic bacteria. *Archives of medical research,* 36**,** 464-467.

Madubunyi,I.I. 2008. Antimicrobial avaluation of the differential solvent extract of Garcinia seeds. *International journal of pharmacognosy,*33,232-237.

Maier, L. S., Schwan, C., Schillinger, W., Minami, K., Schütt, U. & Pieske, B. 2000. Gingerol, isoproterenol and ouabain normalize impaired post-rest behavior but not force–frequency relation in failing human myocardium. *Cardiovascular research,* 45**,** 913-924.

Mallai, V. & Menon, T. 2007. Bactericidal activity of different types of honey against clinical and environmental isolates of Pseudomonas aeruginosa. *The Journal of Alternative and Complementary Medicine,* 13**,** 439-442.

Malmberg, C. & Maryott, A. 1956. Dielectric constant of water from 00 to 1000 C. *J Res Nat Bureau Stand,* 56**,** 1-8.

Manasa, D., Srinivas, P. & Sowbhagya, H. 2013. Enzyme-assisted extraction of bioactive compounds from ginger (Zingiber officinale Roscoe). *Food chemistry,* 139**,** 509-514.

Mandal, S., Debmandal, M., Pal, N. K. & Saha, K. 2010. Antibacterial activity of honey against clinical isolates of Escherichia coli, Pseudomonas aeruginosa and Salmonella enterica serovar Typhi. *Asian Pacific Journal of Tropical Medicine,* 3**,** 961-964.

Mandal, S., Pal, N. K., Chowdhury, I. H. & Debmandal, M. 2009. Antibacterial Activity of Ciprofloxacin and Trimethoprim, Alone and in Combination, Against Vibrio cholerae O 1 Biotype El Tor Serotype Ogawa Isolates. *Polish Journal of Microbiology,* 58**,** 57-60.

Marcus, D. M. & Suarez-almazor, M. E. 2001. Is there a role for ginger in the treatment of osteoarthritis? *Arthritis & Rheumatism,* 44**,** 2461-2462.

Matsuda, A., Wang, Z., Takahashi, S., Tokuda, T., Miura, N. & Hasegawa, J. 2009. Upregulation of mRNA of retinoid binding protein and fatty acid binding protein by cholesterol enriched-diet and effect of ginger on lipid metabolism. *Life sciences,* 84**,** 903-907.

Mavric, E., Wittmann, S., Barth, G. & Henle, T. 2008. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (Leptospermum scoparium) honeys from New Zealand. *Molecular nutrition & food research,* 52**,** 483-489.

Minghetti, P., Sosa, S., Cilurzo, F., Casiraghi, A., Alberti, E., Tubaro, A., Della loggia, R. & Montanari, L. 2007. Evaluation of the topical anti-inflammatory activity of ginger dry extracts from solutions and plasters. *Planta medica,* 73**,** 1525-1530.

Misirlioglu, A., Eroglu, S., Karacaoglan, N., Akan, M., Akoz, T. & Yildirim, S. 2003. Use of Honey as an Adjunct in the Healing of Split-Thickness Skin Graft Donor Site. *Dermatologic surgery,* 29**,** 168-172.

Molan, P. 2001. Why honey is effective as a medicine: 2. The scientific explanation of its effects. *Bee world,* 82**,** 22-40.

Molan, P. C. 1992. The antibacterial activity of honey: 1. The nature of the antibacterial activity. *Bee world,* 73**,** 5-28.

Mowrey D. B, Clayson D. E. 1982. Motion sickness, ginger, and psychophysics. Lancet.;1(8273):655–7.

Mullai V, Menon T. 2007. Bactericidal activity of different types of honey against clinical and environmental isolates of *Pseudomonas aeruginosa*. J Alternat Complement Med.;13:439–441.

Nakamura, H. & Yamamoto, T. 1983. The active part of the [6]-gingerol molecule in mutagenesis. *Mutation Research Letters,* 122**,** 87-94.

Nakazawa, T. & Ohsawa, K. 2002. Metabolism of [6]-gingerol in rats. *Life sciences,* 70**,** 2165-2175.

Natarajan, S., Williamson, D., Grey, J., Harding, K. & Cooper, R. 2001. Healing of an MRSA-colonized, hydroxyurea-induced leg ulcer with honey. *Journal of Dermatological Treatment,* 12**,** 33-36.

Nicoll, R. & Henein, M. Y. 2009. Ginger (Zingiber officinale Roscoe): a hot remedy for cardiovascular disease? *International journal of cardiology,* 131**,** 408-409.

Ntamag, C. N. 1997. *Spatial Distribution of Non Timber Forest Product Collection: A Case Study of South Cameroon*, Ntamag.

Nurtjahja-tjendraputra, E., Ammit, A. J., Roufogalis, B. D., Tran, V. H. & Duke, C. C. 2003. Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. *Thrombosis research,* 111**,** 259-265.

Ochei, J., &Kolhatkar, A (2008).Medical Laboratory Science: Theory and Practice. Tata McGraw Hill Publishing Company Limited, New Delhi, India.56,57,457,589,653-648,804,853-854.

Odebiyi, A. & Sofowora, E.A. 1978. Phytochemical screening of Nigeria medicinal plants. part II liyodia,403, 234-246.

Odebunmi, E., Oluwaniyi, O., Awolola, G. & Adediji, O. 2009. Proximate and nutritional composition of kola nut (Cola nitida), bitter cola (Garcinia cola) and alligator pepper (Afromomum melegueta). *African Journal of Biotechnology,* 8.

Ofor, M.O, Nwufo, M., Ogoke, I., Ngwuta, A., Ibeawuchi, I. & Duruigbo, C. 2004. Postharvest storage characteristics of bitter kola (Garcinia kola heckel.) in Imo State, Nigeria. *International Journal of Tropical Agriculture and Food Systems,* 3(3), 6-9.

Okwu, D. 2005. Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. *Int. J. Mol. Med. Adv. Sci,* 1**,** 375-381.

Okwu, D. & Ekeke, O. 2003. Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. *Global Journal of Pure and Applied Sciences,* 9**,** 235-238.

Omode, A. A., Fatoki, O. S. & Olaogun, K. 1995. Physicochemical properties of some underexploited and nonconventional oilseeds. *Journal of Agricultural and Food Chemistry,* 43**,** 2850-2853.

Omoya, F.O & Akharaiyi, F.C. 2011. Mixture of honey and ginger extract for antibacterial assessment on some clinical isolate. *International journal on pharmaceutical Biomedical Research,* 2, 39-47.

Onochie, C.F.A. and D.F. Stanfield. 1960. *Nigerian Trees*. Gov. Printer, Lagos, Nigeria. pp: 5-10.

Orie, N.N. and E.U. Ekon. 1993. The bronchodilator effect of *Garcinia kola*. *East Afri. Med. J.* 70(3):143–145.

Ozgoli, G., Goli, M. & Moattar, F. 2009a. Comparison of effects of ginger, mefenamic acid, and ibuprofen on pain in women with primary dysmenorrhea. *The journal of alternative and complementary medicine,* 15**,** 129-132.

Perez, C., Pauli, M. & Bazerque, P. 1990. An antibiotic assay by the agar well diffusion method. *Acta Biol Med Exp,* 15**,** 113-115.

Pongrojpaw, D., Somprasit, C. & Chanthasenanont, A. 2007. A randomized comparison of ginger and dimenhydrinate in the treatment of nausea and vomiting in pregnancy. *JOURNAL-MEDICAL ASSOCIATION OF THAILAND,* 90**,** 1703.

Portnoi, G., Chng, L.-A., Karimi-tabesh, L., Koren, G., Tan, M. P. & Einarson, A. 2003. Prospective comparative study of the safety and effectiveness of ginger for the treatment of nausea and vomiting in pregnancy. *American journal of obstetrics and gynecology,* 189**,** 1374-1377.

"Production/Crops for Ginger, World". Food and Agriculture Organization of the United Nations, Statistics Division. 2013. Retrieved 24 December 2015 accesssed 1st june 2016

Rajeswari, T., Venugopal, A., Viswanathan, C., Kishmu, L., Venil, C. & Sasikumar, J. 2010. Antibacterial activity of honey against Staphylococcus aureus from infected wounds. *Pharmacologyonline,* 1**,** 537-541.

Rohand,N.N., Alertia, E.M.T.,Christy, M.E., Agnes,M., Jane-francis,T.K.A., Lucy,M.N & Henry,N.L.2007.In-vitro antimicrobial activity of selected honeys on clinical isolates of Helicobacter pylori. *African Health Sciences journal*,7,4.

Roopal,V,P. & Patel,V.K. 2011. Antimicrobial activity of ginger and honey on isolates of extracted carious teeth during orthodontic treatment. *Asian pacific journal of Tropical Biomedicine,* 1,1,S58-S61.

Saad, B.A. & Steve, H.G. 2016. Antimicrobial effect of different types of honey on Staphylococcus aureus. Saudi journal of Biological Sciences.

Schmid, R., Schick, T., Steffen, R., Tschopp, A. & Wilk, T. 1994. Comparison of seven commonly used agents for prophylaxis of seasickness. *Journal of travel medicine,* 1**,** 203-206.

Schwertner, H. A., Rios, D. C. & Pascoe, J. E. 2006. Variation in concentration and labeling of ginger root dietary supplements. *Obstetrics & Gynecology,* 107**,** 1337-1343.

Sebiomo, A., Awofodu, A., Awosanya, A., Awotona, F. & Ajayi, A. 2011. Comparative studies of antibacterial effect of some antibiotics and ginger (Zingiber officinale) on two pathogenic bacteria. *Journal of Microbiology and Antimicrobials,* 3**,** 18-22.

Sherlock, O., Dolan, A., Athman, R., Power, A., Gethin, G., Cowman, S. & Humphreys, H. 2010. Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. *BMC complementary and alternative medicine,* 10**,** 1.

Shobana, S. & Naidu, K. A. 2000. Antioxidant activity of selected Indian spices. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA),* 62**,** 107-110.

Simon A, Traynor K, Santos K, Blaser G, Bode U, Molan P.2008 . Medical honey for wound care - still the 'Latest Resort' Evid Based Complement Alternat Med.

Smith, C., Crowther, C., Willson, K., Hotham, N. & Mcmillian, V. 2004. A randomized controlled trial of ginger to treat nausea and vomiting in pregnancy. *Obstetrics & Gynecology,* 103**,** 639-645.

Sofowora,A. 1982. Medicinal plants and traditional medicine in africa. John Wiley and Sons Ltd. 142-146.

Spinella, M. 2001. *The psychopharmacology of herbal medicine: plant drugs that alter mind, brain, and behavior*, MIT Press.

Sripramote, M. & Lekhyananda, N. 2003. A randomized comparison of ginger and vitamin B6 in the treatment of nausea and vomiting of pregnancy. *Journal of the Medical Association of Thailand= Chotmaihet thangphaet,* 86**,** 846-853.

Srivastava, K. & Mustafa, T. 1992. Ginger (Zingiber officinale) in rheumatism and musculoskeletal disorders. *Medical hypotheses,* 39**,** 342-348.

Stewart, J., Wood, M., Wood, C. & Mims, M. 1991. Effects of ginger on motion sickness susceptibility and gastric function. *Pharmacology,* 42**,** 111-120.

Surh, Y.-J. 1999. Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 428**,** 305-327.

Surh Y. J,& Lee S. S. 1994. Enzymic reduction of [6]-gingerol, a major pungent principle of ginger, in the cell-free preparation of rat liver. Life Sci.;54(19):L321–6.

Swapna L.A & Warangal, A.P. 2013. Antibacterial activity of organic honey. *Scholars journal of applied medical Sciences,1, 65-68.*

Tan, H. T., Rahman, R. A., Gan, S. H., Halim, A. S., Asma'hassan, S., Sulaiman, S. A. & Kirnpal-kaur, B. 2009. The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC complementary and alternative medicine,* 9**,** 1.

Taormina, P. J., Niemira, B. A. & Beuchat, L. R. 2001. Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. *International journal of food microbiology,* 69**,** 217-225.

Tjendraputra, E., Tran, V. H., Liu-brennan, D., Roufogalis, B. D. & Duke, C. C. 2001. Effect of ginger constituents and synthetic analogues on cyclooxygenase-2 enzyme in intact cells. *Bioorganic chemistry,* 29**,** 156-163.

Tonks, A., Cooper, R., Jones, K., Blair, S., Parton, J. & Tonks, A. 2003. Honey stimulates inflammatory cytokine production from monocytes. *Cytokine,* 21**,** 242-247.

Tonks, A., Cooper, R. A., Price, A., Molan, P. C. & Jones, K. 2001. Stimulation of TNF-α release in monocytes by honey. *Cytokine,* 14**,** 240-242.

Tripathi, S., Bruch, D. & Kittur, D. S. 2008. Ginger extract inhibits LPS induced macrophage activation and function. *BMC complementary and Alternative Medicine,* 8**,** 1.

Tripathi, S., Maier, K. G., Bruch, D. & Kittur, D. S. 2007. Effect of 6-gingerol on pro-inflammatory cytokine production and costimulatory molecule expression in murine peritoneal macrophages. *Journal of Surgical Research,* 138**,** 209-213.

Tsui, B., Dennehy, C. E. & Tsourounis, C. 2001. A survey of dietary supplement use during pregnancy at an academic medical center. *American journal of obstetrics and gynecology,* 185**,** 433-437.

Tumin, N., Halim, N., Shahjahan, M., Noor izani, N., Sattar, M. A., Khan, A. H. & Mohsin, S. 2005. Antibacterial activity of local Malaysian honey. *Malaysian Journal of Pharmaceutical Sciences,* 3**,** 1-10.

Ukaoma,A.A., Ukaoma, V.O., Okechukwu,R.I., & Iwuagwu,M. 2013. Phytochemical Screening and antibacterial property of Garcinia kola. *The journal of phytopharmacology(pharmacolognosy and phytomedicine research),* 2,3,34-38

Uruquiaga, I. and F. Leighton. 2000. Plant polyphenol antioxidants and oxidative stress. *Biological Research*. 33: 159–165.

US Food and Drug Administration. 1 September 2014. Retrieved 21 December 2014.

Uzondu,A.L.E.,Utoh-ndosa, U.A., & Anowi,C.F. 2010. Phytochemical screening and antimicrobial evaluation of N-hexane extract of Garcinia kola Heckel (Bitter Kola) seeds. *International journal of pharmacy*,6.

Uzondu,A., Lovet.E., Okafo.S., Eziuzo.2014. Extraction and formulation of a herbal product: The crude flavonoid extract of Garcinia kola heckel(Bitter kola) seeds into lozenges. *Eeropean journal of biomedical and pharmaceutical Sciences.*vol.1, 491-501.

Verma, S. & Bordia, A. 2001. Ginger, fat and fibrinolysis. *Indian journal of medical sciences,* 55**,** 83.

Verma, S., Singh, J., Khamesra, R. & Bordia, A. 1993. Effect of ginger on platelet aggregation in man. *The Indian journal of medical research,* 98**,** 240-242.

Verma, S., Singh, M., Jain, P. & Bordia, A. 2004. Protective effect of ginger, Zingiber officinale Rose on experimental atherosclerosis in rabbits. *Indian journal of experimental biology,* 42**,** 736-738.

Vutyavanich, T., Kraisarin, T. & Ruangsri, R.-A. 2001. Ginger for nausea and vomiting in pregnancy: randomized, double-masked, placebo-controlled trial. *Obstetrics & Gynecology,* 97**,** 577-582.

Wang, W., Li, C.-Y., Wen, X.-D., Li, P. & Qi, L.-W. 2009. Simultaneous determination of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol in rat plasma by liquid chromatography–mass spectrometry: application to pharmacokinetics. *Journal of Chromatography B,* 877**,** 671-679.

Weston, R. J. 2000. The contribution of catalase and other natural products to the antibacterial activity of honey: a review. *Food chemistry,* 71**,** 235-239.

"What are the benefits of ginger?". Medical News Today. 29 August 2014. Retrieved 11 November 2014 accessed 7th may 2016.

White, J. W., Subers, M. H. & Schepartz, A. I. 1963. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *Biochimica et Biophysica Acta (BBA)-Specialized Section on Enzymological Subjects,* 73**,** 57-70.

Wilkinson, J. M. 2000. Effect of ginger tea on the fetal development of Sprague-Dawley rats. *Reproductive Toxicology,* 14**,** 507-512.

Wilkinson J. M.2000b. What do we know about herbal morning sickness treatments? A literature survey. Midwifery.;16(3):224–8.

Wilkinson, J. M. & Cavanagh, H. M. 2005. Antibacterial activity of 13 honeys against Escherichia coli and Pseudomonas aeruginosa. *Journal of medicinal food,* 8**,** 100-103.

Willetts, K. E., Ekangaki, A. & Eden, J. A. 2003. Effect of a ginger extract on pregnancy-induced nausea: A randomised controlled trial. *Australian and New Zealand journal of obstetrics and gynaecology,* 43**,** 139-144.

Williamson, E.M., Okpako, D.G & Evan, F.J. 1996. Pharmacological methods in phytotherapy research. John wiley & Sons Ltd, 1,9-13.

Willix, D., Molan, P. & Harfoot, C. 1992. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. *Journal of Applied Bacteriology,* 73**,** 388-394.

Wu, K.-L., Rayner, C. K., Chuah, S.-K., Changchien, C.-S., Lu, S.-N., Chiu, Y.-C., Chiu, K.-W. & Lee, C.-M. 2008. Effects of ginger on gastric emptying and motility in healthy humans. *European journal of gastroenterology & hepatology,* 20**,** 436-440.

www.nutrition-and-you.com, accessed on 1st May, 2016

Yahaya,J.A.Y.,Umar,I.O.,Datshen,M.M, Egbunu,Z.K & Ameh,J. 2012. Combine antimicrobial effect of Ginger and honey on some human pathogens. *British journal of pharmocology and toxicology,3,237-239.*

Yalemwork, E.,Wossenseged, L. & Nega, B. 2014. Synergetic antimicrobial effects of mixtures of Ethiopia honeys and Ginger powder extracts on standard and Resistant Clinical Bacterial isolate.Evidence-Based complementary and alternative medicine.

Young, H.-Y., Liao, J.-C., Chang, Y.-S., Luo, Y.-L., Lu, M.-C. & Peng, W.-H. 2006. Synergistic effect of ginger and nifedipine on human platelet aggregation: a study in hypertensive patients and normal volunteers. *The American journal of Chinese medicine,* 34**,** 545-551.

Young, H.-Y., Luo, Y.-L., Cheng, H.-Y., Hsieh, W.-C., Liao, J.-C. & Peng, W.-H. 2005. Analgesic and anti-inflammatory activities of [6]-gingerol. *Journal of ethnopharmacology,* 96**,** 207-210.

Zahra, A., A. Manjieh and M. Mohsen, 2009. Inhibitory effect of ginger extract on Candida Albicans. Cancer Epidemiol Biomarkers Prev.;17(8):1930–6

Zick, S. M., Ruffin, M. T., Lee, J., Normolle, D. P., Siden, R., Alrawi, S. & Brenner, D. E. 2009. Phase II trial of encapsulated ginger as a treatment for chemotherapy-induced nausea and vomiting. *Supportive care in cancer,* 17**,** 563-572.

"Zingiber officinale information from NPGS/GRIN". ars-grin.gov. Retrieved 3 March 2008, accessed 7th may 2016.

"10 Health Benefits of Ginger Root". Retrieved 2 Feb 2016.

APPENDIX I

Ethical Approval

SPECIALIST HOSPITAL, SOKOTO

SULTAN ABUBAKAR ROAD,

P.M.B 2133, Sokoto, Nigeria

Tel: 060-232040/2383885/239839

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10th May, 2016

Okeh George R,
Department of Medical Microbiology,
Faculty of Medical Laboratory Science,
Usmanu Danfodiyo University,
Sokoto,

<u>**Re;- Ethical Clearance**</u>

I am directed to refer to your proposal dated 6th May, 2016 and to inform you that, the hospital management has approved your request to carry out a research on "**In vitro combination of antibacterial effects of *garcinia kola* (bitter kola), ginger and honey on some selected pathogens from specialist hospital Sokoto**".

All research programs should be carryout in line with the hospital regulations.

Copy of research program should be drop at the CMACs office.

HOD Surgery
P/s. attend the above request as approved by the management.
11/7/16

Thanks.

10/5/16

Muhammed Isah
Secretary clinical Services
For:- Chairman MAC.