INDIVIDUAL AND COMBINED ANTIMICROBIAL ACTIVITIES OF CRUDE EXTRACTS FROM HONEY, GARLIC AND GINGER AGAINST SELECTED HUMAN PATHOGENS

MSc THESIS

KAHSAY TEKA

MARCH 2017

HARAMAYA UNIVESITY, HARAMAYA

Individual and Combined Antimicrobial Activities of Crude Extracts from Honey, Garlic and Ginger against Selected Human Pathogens

A Thesis Submitted to the Department of Biology,
Postgraduate Program Directorate,
HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE IN BIOTECHNOLOGY

Kahsay Teka

March 2017
Haramaya University, Haramaya

HARAMAYA UNIVERSITY

Postgraduate Program Directorate

We hereby certify that we have read and evaluated this thesis entitled "Individual and combined Antimicrobial Activities of Crude Extracts from Honey, Garlic, and Ginger against Selected Human Pathogens" prepared under our guidance by Kahsay Teka. We recommend that it be submitted as fulfilling the thesis requirement.

Ameha Kebede (PhD)	_____	_____
Major Advisor	Signature	Date
Misrak Kebede (PhD)	_____	_____
Co-advisor	Signature	Date

As members of the Board of Examiners of the MSc Thesis Open Defense Examination, We certify that we have read and evaluated the thesis prepared by Kahsay Teka and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for Master of Science Degree in Biotechnology

_____	_____	_____
Chairperson	Signature	Date
_____	_____	_____
Internal examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date

Final approval and acceptance of the Thesis is contingent upon the submission of its final copy to the Council of Graduated Studies (CGS) through the candidate's Department Graduate Committee (DGC)

DEDICATION

This thesis manuscript is dedicated to my father Teka Abrha and my beloved mother Tsehaynesh Berihun for nursing me with love and for their commitment and sacrifice that made me to be what I am today.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this thesis is my own work. I have followed all the ethical and technical principles of scholarship in preparation, data collection, data analysis and compilation of this thesis. Any scholarly matter that is included in the thesis has been recognized through citation.

This thesis is submitted in partial fulfillment of the requirements for a Master of Science degree in Biotechnology Program, Biology Department, at the Haramaya University. The thesis is deposited in Haramaya University library and made available to borrowers under the rules of the library. I solemnly declare that this thesis has not been submitted to any other institution anywhere for the award of degree, diploma or certificate.

Brief quotations from this thesis may be made without special permission provided that accurate and complete acknowledgment of the source is made. Request for permission for extended quotations from or reproduction of this thesis in whole in part may be granted by the head of the department when in his or her judgment the proposed use of the material is in interest of scholarship. In all other instances, however, permission must be obtained from the author of the thesis.

Name: …….. Signature ……..

Date: …….

School/Department: ……

BIOGRAPHICAL SKECTCH

The author was born in Hashengie, South Tigray, Ethiopia, from his father Teka Abrha and his mother Tsehaynesh Berihun in February 21, 1987. He attended his elementary school at Hashengie Primary School from 1997-2002. Then, he attended his secondary and preparatory school at Korem Comprehensive Secondary School. After successful completion of his preparatory school in 2006, he joined University of Gondar in December 2007 and graduated with Bachelor of Science in Medical Laboratory Technology in July 2009.

After graduation, he has been working since October 2010 as a Medical Laboratory Professional at Haramaya University Higher Health Center. Then he joined the regular program of the Department of Biology, Haramaya University, in 2014 academic year to pursue MSc degree in Biotechnology.

ACKNOWLEDGEMENTS

First of all, I would like to express my most sincere gratitude to my advisors Dr. Ameha Kebede and Dr. Misrak Kebede who have helped and supported me from the beginning of the proposal development up to the completion of this thesis. Without their support, constructive suggestion and encouragement, the completion of this thesis would not have been possible. I would like also to thank the Department of Biology and College of Natural and Computational Sciences as well as the Postgraduate Program Directorate of Haramaya University for allowing me to attend this program and their encouragements and Ethiopian Public Health Institute for providing me the test pathogens I have used in my study.

It is also worthy to thank Estifanos Hawaz, College of Natural and Computational Sciences and Dadi Marami, College of Medical and Health Sciences, for providing me with growth media required for my research work. I am also thankful to the laboratory staffs of Biology Department and Central Laboratory of Haramaya University for their support in my research work. Special thanks are extended to my friend Yimeslal Atinafu for his support and for being with me throughout the laboratory work.

I would like to extend my great gratitude to my workmates of Haramaya University Health Center staffs, without whom it would have been impossible to finish the course work in time. I also express my thanks to Wubetu Belay and all my friends for their support and encouragements during my study period.

I especially need to thank my uncle, Molla Berihun, who took the initiative to help me continue my study and my parents who have been supporting and encouraging me over the years for my dream to become true.

ACRONYMS AND ABBREVIATIONS

ATCC	American Type Culture Collection
DMSO	Dimethyl Sulphoxide
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
MRSA	Methicillin Resistant *Staphylococcus auerus*
OD	Optical Density
WHO	World Health Organization
ZOI	Zone of Inhibition

TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure

Page

LIST OF TABLES IN THE APPENDIX

Appendix Table **Page**

Individual and Combined Antimicrobial Activities of Crude Extracts from Honey, Garlic and Ginger against Selected Human Pathogens

ABSTRACT

Concerns about disease causing bacteria are now ever increasing. The common and immediate action to these disease causing microbes is the use of antibiotics. However, due to the challenges of drug-resistance in bacteria and the problems of affordability of the drugs, the search for new compounds from plant and animal sources that act against the pathogens has become crucial in many countries. The aim of this study was to investigate the possibility of using honey, garlic and ginger as source of antimicrobial sources to alleviate different ailments. Crude extracts of honey, garlic and ginger were obtained using distilled water, methanol (98%), and ethanol (97%) as extraction solvents. The antimicrobial activities of these crude extracts and their combinations were evaluated using disc diffusion method against Shigella boydii, Staphylococcus auerus, Salmonella Tyhimurium, Pseudomonas aeruginosa and Klebsiella pneumoniae. The MIC of crude extracts with the highest zone of inhibition was determined using

the broth dilution method. The negative tubes from the MIC experiments were then sub-cultured into fresh MHA for MBC determination. The antimicrobial activities were performed at concentration of 10 mg/disc against the test pathogens. The results revealed that aqueous and alcoholic extracts of garlic had inhibitory activities against all tested pathogens except P. aeruginosa. Among the crude extracts, ethanolic crude extract of garlic showed the widest zone of inhibition (14 mm) against Salmonella Typhimurium despite the fact that it was resistant to the standard antibiotics used in this study. The MIC of the crude extracts was in the range of 3.90–15.625 mg/ml and the MBC was in the range of 15.625–62.5 mg/ml. In conclusion, the present study indicated that all extracts, except the aqueous extract of ginger showed varying inhibitory activities against the test pathogens at different concentrations and when using different extraction solvents. Further in vivo investigations and phytochemical screening are needed for the future implementation of these medicinal remedies.

***KEYWORDS**: Allium sativum, Apis mellifera, Broth dilutions, Crude extract, Disc diffusion, Human pathogens, MIC, Zingiber officinale*

1. INTRODUCTION

Infectious diseases represent an important cause of morbidity and mortality worldwide (Fauci, 2001). In developing countries especially in Africa, large number of people die daily of preventable and curable diseases because of lack of even simple health care. Despite the enormous advances made in health care during the last century, infectious diseases still account for 45% of deaths in low-income countries and for 26% of annual deaths worldwide (Morens *et al*., 2008). Chemotherapy of infected individuals with antimicrobial drugs is one of the widely used strategies for the control of infectious diseases and in reducing the global burden of infectious diseases (Mulvey *et al*., 2011). Nevertheless, affordability and accessibility of newer pharmaceutical antimicrobials have become issues of major concern in developing countries, especially in rural areas. Furthermore, as resistant microbes develop and spread, the effectiveness of the drugs continues to diminish (WHO, 1999) and this is becoming a challenge to the global infectious disease control program (Shears, 2000).

The impact of bacterial diseases is particularly significant in Africa where drugs are limited and the emergence of drug resistance has made many of the currently available drugs ineffective (Madureira *et al*., 2012). This is a serious and growing problem of public health representing a global threat and makes more difficult to treat infectious diseases. Additionally, the repeated uses of antibiotics have their own side effects and complications like susceptibility to fungal infections and gastrointestinal tract disturbances, which usually result in discontinuation of the treatment (Zakia *et al*., 2014). This has resulted in the search for safe, cheaper and more effective sources of natural products from plants and some insects that act as potential sources of alternative antimicrobial agents that can be used for treatment of various ailments (Cowan, 1999) and urgent need for new antibiotics (Gootz, 2010).

Antimicrobial agents are substances that are known to have bacteriostatic or bactericidal effects on microorganisms and used either as a means of control or prevention or cure of microbial diseases. These antimicrobial agents are synthesized chemotherapeutic substances obtained primarily from microorganisms, plants and some animal products. Plants have been used as source of medicine for thousands of years, playing a crucial role in drug discovery and development and

act generally to stimulate and supplement the body's healing forces, and are the natural foods for human beings (Fabiola *et al*., 2003). The importance of natural products is particularly evident in the area of infectious diseases, where over 60% of antimicrobial agents are of natural origin (Newman and Cragg, 2007). The large number of natural product-derived antibiotics may be due, in part, to the evolution of secondary metabolites like alkaloids, flavonoids, tannins and phenolic compounds (Edeoga *et al*., 2005).

During the last decades, use of traditional medicine to treat infectious diseases has expanded globally and gained popularity. With the tremendous expansion in the use of traditional medicine worldwide, safety and efficacy as well as quality control of herbal medicines and traditional procedure-based therapies have become important concerns for both health authorities and the public. According to WHO (2000) survey, about 70–80% of the world population relies on non-conventional medicine mainly of herbal sources in their primary healthcare, and 80% of people living in Sub-Saharan Africa are almost completely dependent on folk medical practices for their primary healthcare needs, and plants are known to play an indispensable role in traditional medicine (WHO, 2002). In many areas, especially in the tropics, several species of medicinal plants offer access to safe and effective products for use in the prevention and treatment of various illnesses. Medicinal plants are useful not only in the traditional system of medical care at the local level but also in the production of modern medicines (Tefere, 1996). At the moment, there is a global interest to go for the accumulated knowledge of traditional medicine, and therefore, research is being carried out in many countries with the stated aim of increasing the contributions of traditional medicine to the welfare of the human population (Demissew and Dagne, 2001).

In Ethiopia, traditional medicines continue to be an important segment of primary health care to the majority of rural populations, and as a result a number of plants have been documented to be used as sources of antimicrobial agents in folkloric practices (Araya *et al*., 2015; Abraha *et al*., 2013; Giday and Ameni, 2003). This wide usage of traditional remedies among the population of Ethiopia could be in part attributed to their probable efficacies against some diseases and their accessibility and affordability compared to allopathic medicines. Despite the wide usage,

information regarding *in vivo* or *in vitro* efficacies of Ethiopian traditional medicines against different infectious pathogens is very limited. Studies on a number of plants and substances originating from these plants could provide information about the fact that they could be used as source of new antimicrobial agents. Among these, honey which is produced by honeybees from different flowering plants, garlic and ginger are commonly used as remedies to treat different ailments (Berhanu, 2013). Besides, there is a habit of using these remedies individually and/or in combinations to treat different illnesses involving drug resistant microorganisms based on the concept of the effectiveness of combination therapy (Eja *et al*., 2011). Recent years have witnessed an increasing interest among scientific institutions including biological and major pharmaceutical research institutions in the use of medicinal plant parts and/or their active ingredients in health products. In the context of countries like Ethiopia, the prohibitively expensive cost of antibiotics and the emergence of antibiotic resistance of bacterial diseases call for the search in alternative agents with possible antibacterial effects from natural products. Such pharmacologically active agents may be obtained through screening from traditionally claimed medicinal plants for their possible antibacterial effects (Ahmed *et al*., 2007). Therefore, the aim of this study was to investigate the possibility of using honey, garlic, and ginger as sources of antimicrobial substances that alleviate problems of respiratory, wound and gastrointestinal infections.

General Objective;

The general objective of this study is to determine the amount of crude extracts from honey, garlic and ginger and their antimicrobial activities against selected human pathogens.

Specific Objectives;

- To compare the amount of crude extracts that could be obtained from honey, garlic and ginger using water, ethanol and methanol.
- To determine the individual and combination antimicrobial activities of honey, ginger and garlic extracts against *Shigella boydii, Staphylococcus aureus, Salmonella* Typhimurium*, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

- To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract with the highest zone of inhibition against the selected test pathogens against *Shigella boydii, Staphylococcus aureus, Salmonella* Typhimurium*, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

2. LITERATURE REVIEW

2.1. Antimicrobial Substances

Use of substances with antimicrobial properties is known to have been common practice for at least 2000 years. Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infection (Wainwright, 1989).

Antimicrobial substances are any substances of natural, semisynthetic or synthetic origin that kills or inhibits the growth of microorganisms but causes little or no damage to the host. Antimicrobial medicines can be primarily grouped according to the microorganisms they act against. The main classes of antimicrobial agents are disinfectants nonselective antimicrobials such as bleach, which kill a wide range of microbes on non-living surfaces to prevent the spread of illness; antiseptics which are applied to living tissue and help reduce infection during medical process and antibiotics which destroy microorganisms within the body. They are obtained primarily from microorganisms, plants and some animal products (Fabiola *et al*., 2003).

2.2. Sources of Antimicrobial Substances and Yields Obtained

Antimicrobial substances are substances known to have inhibitory effect on microorganisms and they can be obtained from different sources. There are numerous antimicrobial substances of animal and their products origin which have often evolved as host defense mechanisms. These include lipids, lysozymes, lactoperoxidase and defensins which have antimicrobial activity against a wide range of microorganisms. Many of the antimicrobial agents inherent to animals are in the form of antimicrobial peptides (Bibel*et al*., 1989). Other sources of antimicrobial substances are

microorganisms which produce many compounds that are active against other microbes, which can be harnessed to inhibit the growth of potential spoilage or pathogenic microorganisms. These include fermentation end products such as organic acids, hydrogen peroxide, and diacetyl, in addition to bacteriocins and other compounds (Daeschel, 1989).

There are also antimicrobial substances of plant origin. Edible medicinal plants and their derived essential oils contain a large number of secondary metabolites and other compounds that are known to inhibit the growth of bacteria, yeast, and molds (Burt and Reinders, 2003; Chorianopoulos *et al*., 2008). The antimicrobial compounds in plant materials are commonly extracted from their leaves, flowers, clove, bulbs, seeds, rhizomes, fruits and from other parts of plants (Gutierrez *et al*., 2008).

The amounts of crude extracts obtained are different according the solvents used. Some of them dissolve in organic solvent while others do not (Cowan, 1999). This leads researchers to use different solvents for extraction of bioactive molecules from animal and plant sources which are claimed to have medicinal importance. In this regard honey yield percent of the extract varies with respect to solvents used. Mohamed *et al*. (2016) reported that the percentage yields of honey were 64% and 36.7% using ethanol and methanol, respectively, as extraction solvents. Kalia *et al*. (2012) also reported the percentage yields of honeybee propolis using ethanol, methanol and water were 57.2%, 32.83% and 21.2%, respectively. With respect to garlic the percentage yield was 56% using water as extraction solvent and 60% using ethanol (Ekweney and Elegalan, 2005). The yield percent of garlic in the study conducted by El-Hamidi and El-Shami (2015) was 71.73% 57.26% and 21.97% using water, methanol/water (1:1) and methanol, respectively, while the yield percent using acetone and hexane was poor. Iqbal and Bhanger (2007) also reported that the percentage yield of garlic was 23.15% using methanol. Ekweney and Elegalan (2005) reported the percentage yields of ginger using different solvent were different. In his report the percentage yields of ginger were 70% and 78% with extraction solvents of water and ethanol, respectively, while Yassen *et al*. (2016) reported the percentage yields of ginger using water, methanol and ethanol were 32%, 4.1% and 3.4%, respectively. Yisehak *et al*. (2014) also reported that percentage yield of ginger was 1.02% using methanol as a solvent.

2.2.1. Honey and its antimicrobial activities

Honey is the natural substance produced when the nectar (floral) and sweet deposits from plants (non-floral) are gathered, modified and stored in the honeycombs by honeybees with carbohydrates constituting about 95 to 97% of the dry weight of honey (Namias, 2003). Fructose and glucose are the most predominant sugars present and responsible for most of the physical and nutritional characteristics of honey (Alvarez-Saurez *et al*., 2009). Common *Apinae* honey bee (*Apis mellifera*) honey and stingless honeybees honey are the two types of honeys found in the world. In Ethiopia, *Apinae* honeybees (*Apis mellifera*) are mostly domestic unlike the wild stingless honeybees (Yalemwork *et al*., 2013). Honey produced by *Apis mellifera* is one of the oldest traditional medicines considered to be important in the treatment of several human ailments (Manisha and Shyamapada , 2011).

2.2.1.1. Chemical composition of honey

Honey is essentially a highly concentrated water solution of two sugars, dextrose and levulose, with small amounts of at least 22 other more complex sugars. Many other substances also occur in honey, but the sugars are by far the major components. The principal physical characteristics and behavior of honey are due to its sugars, but the minor constituents such as flavoring materials, pigments, acids, and minerals are largely responsible for the differences among individual honey types. In addition, according to Subrahmanyam and Jibril (2001), honey includes hydrogen-peroxide, flavonoids and phenolic acids plus many other unidentified components. Furthermore, the chemical composition of honey is said to comprise of tetracycline derivatives, volatile compounds including fatty acids, lipids, amylase, ascorbic acid, peroxidase, glucose and fructose. Its antimicrobial activities are attributed to the presence of these compounds together with high osmolarity, low pH (3.6-3.7), presence of phenol, peroxidase, and the presence of tetracycline derivatives of fatty acids (Manyi-Loh *et al*., 2010; Ndip *et al*, 2007).

The volatile compounds found in honey include alcohols, ketones, aldehydes, acids, esters and terpenes. Phenolic acids and flavonoids contribute significantly to the therapeutic capacity of honey which varies greatly depending on the floral source (Gheldof, 2002). Apart from these

compounds, honey also contains several vitamins, including B complex and vitamin C, ascorbic acid, pantothenic acid, niacin and riboflavin together with a lot of minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc (Ajibola *et al*., 2012). It also contains other bioactive substances such as organic acids, carotenoid-derived compounds, nitric oxide (NO), amino acids and proteins (Arriaga *et al*., 2011; Beretta *et al*., 2010).

2.2.1.2. Antimicrobial activities of honey

Honey is one of the potent antimicrobial agents used and several authors reported that different honeys vary substantially in the potency of their antibacterial activity, which varies with the plant source and its antimicrobial activities may range from concentrations of 3% to 50% and higher (Lusby *et al*., 2005). The bactericidal effect of honey is reported to be dependent on concentration of honey used and the nature of the bacteria (Basualdo *et al*., 2007; 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). Molan and Cooper (2000) also reported that the difference in antimicrobial potency among the different honeys depends on its geographical, seasonal and botanical source as well as harvesting, processing and storage conditions. The antibacterial nature of honey relies on various factors working either singularly or synergistically.

The major antibacterial effect, however, was reported due to hydrogen peroxide (Temaru *et al*., 2007). But non-peroxidase manuka honey was tested against seven species of bacteria and was found to have MIC that range from 1.8% to 10.8% (v/v) (Willix *et al*., 1992). This result, therefore, indicated that the major antimicrobial effect of honey may not be due to hydrogen peroxide. This may be related to the low pH level of honey (between 3.2 and 4.5) which is low to be inhibitory for many bacteria (Haniyeh *et al*., 2010) and its high sugar content (high osmolarity) that is enough to hinder the growth of microbes. Vallianou *et al*. (2014) in his review also stated that honey has antibacterial and wound healing activity, anti-inflammatory effect and antioxidant activities. In addition, the antibacterial activity of honey is highly complex due to the involvement of multiple compounds and due to the large variation in the concentrations of these compounds among honeys (Kwakman *et al*., 2012).

Studies on honey produced by honeybees (*Apis meliffera*) have shown that honey has antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Citrobacter ferundi, Streptococcus faecalis, Shigella flexineri,* and *Salmonella* Typhi (Nzeako and Hamdi, 2000). It completely inhibits major wound infection pathogens including *Streptococcus pyogenes and S. aureus* (Kingsley, 2001).

Yalemwork *et al*. (2013) a study on conducted antibacterial effects of *Apis mellifera* and stingless bees honeys on susceptible and resistant strains of *E. coli, S. aureus* and *Klebsiella pneumoniae* and found highest inhibition (27mm) produced by stingless bees honey on both susceptible *E. coli* and *S. aureus*. The level of inhibition (Mean ± SD) was 22.27±3.78, 21±2.69 and 18±2.31 for stingless honey bees honey, white honey and yellow honey respectively on standard *S. aureus, S. aureus* MRSA (Methicillin resistance *Staphylococcus aureus*), *E. coli isolate, E. coli* R (Resistant) and *K. peumoniae* (R) at 50% concentration (v/v). Plus to this the inhibitions produced by singless Gojam honey and *Apis mellifera* white Tigray honey on *S. aureus* (25mm, 27 mm) and *E. coli* (22mm, 26mm) respectively at 50% concentration (v/v) was greater than inhibitions produced by the standard antibiotics used in the test. Selcuk and Nevin (2002) also reported honey inhibits the growth of *E.coli, S. aureus* and *K. pneumoniae* at a concentration of 50% and above while no activity was observed at a concentration less than 50%. Manisha and Shyamapada (2011) also reported honey did not exhibit antimicrobial activitiey against *Pseudomonas* and *E.coli* at lower concentration but they do have at higher concentrations. Stingless bees Tazma honey have 6.25% (6.25 mg/ml) MIC value for 80% of the test microorganisms (Yalemwork *et al*., 2013) which is almost similar with Andargachew *et al*. (2004) who reported MIC values of *Apis mellifera* honey were 6.25% for 90% of the test organisms and all the honeys were found to have 12.5% (12.5 mg/ml) MBC (Yalemwork *et al*., 2013) and this did not agree with Andargachew *et al*. (2004).

A study by Molan (1992) indicated that the concentration of honey (% V/V) against various strains of bacteria which cause gastroenteritis, the MIC and MBC were found to be in the range of 7-10%. A study by Mogessie Ashenafi (1994) reported that 'tazma' honey produced by stingless bee (*Apis mellipodae*) was found to be effective against some food-borne pathogens of humans. Growth inhibition on *Salmonella* Typhimurium, *Shigella enteritidis* and *E.coli* were

noted at 15 and 20% concentration, while a more marked growth retardation and inhibition on *B. cereus* and *S. aureus* were observed at concentrations of 10%.

Another study by Mohapatra *et al*. (2010) who investigated the antibacterial activity of methanol, ethanol, and ethyl acetate extracts of raw and processed honey was tested against *S. aureus, B. subtilis, B.cereus, Enterococcus faecalis, Micrococcus luteus, E. coli, P. aeruginosa,* and *S.*Typhi. Both types of honey showed antibacterial activity against tested organisms with the zone of inhibition ranging from 6.94 to 37.94 mm, while *E. coli, S.* Typhi*,* and *P. aeruginosa* showed that susceptibility towards all the extracts with zone of inhibition ranged between 13.09 to 37.94 mm. The methanol extract showed more potent activity than other organic extracts. The broth micro-dilution assay gave MIC of 625µg/ml, while the MBC ranges between 625µg/ml - 2500µg/ml. Basualdo *et al*. (2007) also revealed the same results in which *S.* Typhi*, P.aeruginosa* and *E. coli* were significantly inhibited with the range of inhibition between 13.94mm and 37.94mm. On the other hand Akinnibosun and Itedjere (2013) reported that honey at a concentration of 75% and below did not exhibit antimicrobial activities against *staphylococcus aureus* and limited effect(2, 4 mm) on *K. pneumoniae* and *salmonella* species at a concentration of 25 and 50%.

These results have indicated the potential of honeys as therapeutic agents to treat both susceptible and drug resistant bacteria. This variation in the activities of different honey might be due to the differences in the species of bees, which in turn results in difference in the production and type of honey and the differences in the test methods and test organisms (Mohapatra *et al*., 2010; Akinnibosun and Itedjere, 2013; Yalemwork *et al*., 2012).

2.2.2. Garlic and its antimicrobial activities

Garlic (*Allium sativum*) in the family Liliaceae is a bulb-forming plant and world-wide known, as dietary and medicinal purposes (Daka, 2011; Palaksha *et al*., 2010). Other members of the garlic family include *Allium cepa* (onion), *Allium ascalanicum* (shallot) and *Allium porrum* (Heeks). Of all the *Allium* species, garlic is the most important (Alli *et al*., 2011) and *Allium sativum* is

broadly classified into two sub varieties *ophioscordon* (hard neck garlic) and *sativum* (soft neck garlic) (Shobana *et al*., 2009).

Garlic has a long folklore history as a treatment for cold, cough and asthma and is reported to strengthen the immune system (Borek, 2001). It has many medicinal effects such as anti-inflammatory (Baek *et al*., 2001) and antibacterial activity (Ekweney and Elegalan, 2005). Harris *et al*. (2001) on his review also indicated that principals from garlic have been shown to have antibacterial, antifungal, antiviral and antiprotozoal activities. In detail, garlic has very strong antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *Staphylococcus epidermidis*, *S.* Typhi and various yeasts (Victor and Igeleke, 2012). The antimicrobial potency of garlic has been attributed to its ability to inhibit toxin production and expression of enzymes for pathogenesis (Iwalokun *et al*., 2004). This shows garlic has a broad spectrum of activity and it provides scientific basis for its utilization in traditional and folk medicine (Meriga *et al*., 2012).

2.2.2.1. Chemical composition of garlic

The most important chemical compounds of garlic thought to be responsible for antimicrobial activity are the organosulphur compound including allicin (Hovana *et al*., 2011) which is formed when the garlic is crushed. Allicin has even been noted to act synergistically with antibiotics and enhance the antimicrobial activity of antibiotics against the microbes (Khodavandi *et al*., 2010) and to fight agents of nosocomial infections that are so prevalent in hospitals (Abubakar,2009). Garlic is also rich in anionic components such as nitrates, chlorides and sulfates (Astal, 2004) as well as other phytochemical compounds like alkaloids, saponins alliin, ajoene, flavonoids, tannins and non sulphur-containing compounds including vitamin B, proteins, carbohydrate, glycoside and minerals which may contribute for its antimicrobial activity (Akintobi *et al*., 2013 and Garba *et al*., 2013).

2.2.2.2. Antimicrobial activities of garlic

Garlic has been considered to be an excellent medical remedy and a natural antimicrobial drug that can be considered as an alternative form of treatment for pathogenic infections. The antimicrobial effects of fresh aqueous garlic extract and dried aqueous garlic extract against *S.* Typhi was

studied. Antibacterial activity of fresh aqueous garlic extract and dried aqueous garlic extract was characterized by inhibition zones of 5-29 mm and 5-19 mm, respectively with fresh aqueous garlic extract giving a higher sensitivity against the tested isolate. Antimicrobial activity was exhibited when garlic extract was added into agar well at concentration of 0.25-0.8mg and growth inhibition was observed increased with increase in extract concentrations (Yabaya *et al*., 2010). A study by Iwalokun *et al*. (2004) revealed that the antimicrobial effects of aqueous garlic extract against multidrug-resistant gram-positive and gram-negative bacterial isolates including *S. aureus, S. epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae, S. Typhi, P. aeruginosa, E. coli, Shigella* species, *Proteus* species and *Candida* species (27.4±3.7 mm) had better inhibition zone of 20.2-22.7 mm for gram positives and 19.8-24.5 mm for gram negatives. This agrees with the study by Gull *et al*. (2012).

According Garba *et al*. (2013) who studied phytochemical and antibacterial properties of garlic extracts; the antibacterial potency of aqueous and methanol extracts of garlic was determined against *E. coli, S. aureus* and *P. aeruginosa* by agar well diffusion method. The aqueous and methanol extract of garlic were observed to be more potent against *E. coli* with maximum zone of growth inhibition of 21.5 mm and 24.0 mm at 200 mg/ml, respectively and 20 mm and 23 mm for *S. aureus*; 21 mm and 20 mm for *P. aeruginosa*. The aqueous extract of this finding agrees with the study of Gull *et al*. (2012) but it is in difference with methanol extract antimicrobial activities (11-12 mm). Garba *et al*. (2013) also evaluated the MIC and MBC value of garlic extracts and MIC values of garlic aqueous and methanolic extract were respectively, 100mg/ml and 50 mg/ml for *E. coli*, 200mg/ml and 100mg/ml for *S. aureus* and 200mg/ml and 200mg/ml for *P. aeruginosa*, while the MBC values were, 300mg/ml and 250mg/ml for *P. aeruginosa*, 300mg/ml and 200mg/ml for *S. aureus* and 200mg/ml for both aqueous and methanol extract for *E. coli*, respectively. Additionally according the study by Tagoe and Gbadago (2009) MIC's of garlic aqueous extracts on *Salmonella*, *Shigella* and *B. cereus* were 150mg/ml, 50mg/ml and 100mg/ml respectively. Contrary to this Iwalokun *et al*. (2004) reported MIC in the range of 15.6-48.3 mg/ml against *Salmonella* Typhi*, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Haemophilus influenzae, Proteus mirabilis, Streptococcus pneumoniae, Streptococcus pyogenes* and *candida* species. Other authors also reported MIC value of 0.05 mg/ml to 1.0

mg/ml (Gull *et al*., 2012) against *Salmonella* Typhi, *Staphylococcus aureus*, *Pseudomonas aeruginosa* as well as *Klebsiella pneumoniae* and 0.5-32 mg/ml (Groppo *et al*., 2007) against *streptococci*. Additionally, Nanasombat and Lohasupthawee (2005) reported MIC values of ethanolic extracts of garlic 41.7 mg/ml and 166.7 mg/ml against *Salmonella* Typhimurium and *Klebsilella Pneumoniae*. Abubakar (2009) also reported MIC and MBC of aqueous and ethanolic extracts of garlic against *S. aureus* and *P. aeruginosa*. It was found that the MIC values were 50 mg/ml, 75 mg/ml against *S.aureus* and 125 mg/ml; 150 mg/ml against *P. aeruginosa* using water and ethanol as extraction solvents respectively and their MBC were at higher concentration than their MIC.

Shobana *et al*. (2009) also conducted antibacterial activity of garlic varieties (*Ophioscordon* and *sativum*) on enteric pathogens such as *E. coli, Proteus mirabilis, S.* Typhi*, Shigella flexneri* and *Enterobacter aerogenes*. Aqueous extracts of both varieties exhibit anti-bacterial activities at higher concentration except to *Enterobacter aerogenes*. Ethanolic extract of *A. sativum* was found to be highly effective against all the bacteria tested from 200-500 µg concentration, the range of inhibition was 14-24 mm for *E.coli*, 20-30 mm for *P.mirabilis*, 20-28 mm for *Salmonella* Typhi and 22-30 mm for *Shigella flexneri*, and the inhibition zone increases as the concentration increases.

In contrast the crude extracts of garlic and ginger applied singly and in combination did not exhibit any in vitro inhibition on the growth of *S. aureus, Bacillus* species*, Escherichia coli* and *Salmonella* species. However with lime they inhibit *Bacillus* species*, Salmonella* species *and E. coli. Salmonella* was only slightly inhibited by lime (singly) and a mixture of aqueous extracts of garlic, ginger, and lime-juices (Onyeagba *et al*., 2004). Nanasombat and Lohasupthawee (2005) also stated ethanolic extract of garlic did not exhibit inhibitory effect on salmonellae and other enterobacteria using disc diffusion method.

2.2.3. Ginger and its antimicrobial activities

Ginger belongs to Zingiberaceae family. The zingiberaceous plants have strong aromatic and medicinal properties and are characterized by their tuberous or non-tuberous rhizomes (Chen,

2008). Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. It is also "Generally Recognized as Safe" (GRAS) by the United State food and drug administration (ICMR Bulletin, 2003). The most well-known member of Zingiber (ginger) is *Zingiber officinale*, and in many parts of the world, it has medicinal and culinary values.

2.2.3.1. Chemical composition of ginger

Composition of Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fiber and 12.3% carbohydrates. The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C and it has several ethno-medicinal and nutritional values as spice and flavoring agents in Ethiopia and elsewhere (Kumar *et al*., 2011). Recently ginger is extensively studied for its medicinal properties by advanced scientific techniques and a variety of bioactive compounds such as tannins, flavonoid, glycosides, essential oils, furostanol, spirostanol, saponins, phytosterols, amides, alkaloids have been isolated from the different parts of the plant which were analyzed pharmacologically (Otunola *et al*., 2011; Sasidharan and Menon, 2010). It is also reported that sesquiterpenoids are the main component of ginger which attributes its antibacterial activity (Gull *et al*., 2012; Malu *et al*., 2008). Though the volatile oil gingerol and other pungent principals give ginger its pungent aroma, its antimicrobial effect is also believed to come from its aromatic and absorbent properties (Portoi *et al*., 2003).

2.2.3.2. Antimicrobial and other medicinal values of ginger

Traditionally ginger is reported to treat nausea, vomiting, asthma, cough, shiver, inflammation, loss of appetite, constipation, indigestion, arthritis, cramps, rheumatism, sore throats, muscular aches, pains, hypertension, fever and infectious diseases in different parts of the world (Carrasco *et al*., 2009; Ernst and Pittler, 2008). Similarly ginger is used, traditionally, for treating common cold, stomachache, cough, fever, and influenza in Ethiopia (Shenkute, 2008) and reported to have antibacterial, anti-oxidant, anti-protozoan, anti-fungal, anti-rhinoviral, anti-inflammatory, anti-insecticidal activity (Ficker *et al*., 2003). Rahmani *et al*. (2014) on his review also indicated

that ginger has antimicrobial, anti-diabetic activities and gastro, hepato and neuroprotective effect as well as effects on eye, migraine and osteoarthritis.

Gull *et al*. (2012) reported the inhibitory effects of *A. sativum* and *Z. officinale* extracts on drug resistant *E. coli, P. aeruginosa, B. subtilis, S. aureus, K. pneumoniae, Shigella sonnei, Staphylococcus epidermidis* and *S.* Typhi, in which ginger methanol and ethanol extracts were demonstrated to be more effective against all tested bacterial strains (in the range of 11- 15 mm) than ginger aqueous extracts (11-13 mm). *E. coli* and *Shigella* were also more susceptible to the ginger extracts. *E. coli* showed maximum susceptibility to ethanol extracts of ginger (15 mm) while *Shigella* showed maximum susceptibility to both methanol and ethanol extract of ginger (15 mm). Better inhibitions were found by Yalemwork *et al*. (2014) who evaluated the antimicrobial effects of mixtures of Ethiopian honeys and ginger powder extracts on *S. aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *S. aureus* (MRSA), *E. coli* (R), and *K. pneumoniae* (R) in which the ginger extract inhibits the growth of these microorganisms in the range of 15.5- 23 mm and the inhibition increases as mixed with honey (22.5- 28.25 mm). As per Yalemwork *et al*. (2014) the MIC of ginger extract was 6.25% for clinical isolates and 12.25% for resistant strains of tested microorganisms. Nikolić *et al*. (2014) on his study also indicated the MIC values of ethanolic extract of ginger were in the range from 0.0024 mg/ml to 20 mg/ml, while the MBC values were in the range from 0.156 mg/ml to 20 mg/ml. In contrast Tagoe and Gbadago (2009) stated that aqueous extract of ginger had no effect on *E. coli*, *Salmonella* species, *Shigella* species and *Bacillus cereus* between the concentrations of 50-500mg/ml used for experiment. Zakia *et al*. (2014) also reported ginger has not inhibitory effect on *Staphylococcus aureus* and *E.coli*.

In the study by Sebiomo *et al*. (2011), there was no significant difference in the effects of both water and ethanol extract of ginger on the zone of inhibition of the *S. aureus* and *S. pyogenes*, while the concentration of the plant extract (water and ethanol) had significant effect on the zone of inhibition of both organisms. Ginger leaf and root had the lowest zone of inhibition of 10 mm on *S. aureus*, but it had 18 and 19 mm inhibition zone on *S. pyogene* (ginger leaf and root respectively) at 200 mg/ml concentration of the water extract and it increased significantly as the concentration increased to 1000 mg/ml concentration with the highest zone of inhibition of 30 and

32 mm on *S. aureus*, 25 and 28 mm on *S. pyogenes* of both the ginger leaf and root water extract. On another study *K. pneumoniae* (12mm, 22mm) and *S. aureus* (14 mm, 24mm) were inhibited by aqueous extract of ginger at a concentration of 0.3 and 0.4 mg/ml respectively (Ahmed *et al*., 2012). The ethanol extract had a zone of inhibition of 20 and 21 mm on *S. pyogenes* at a concentration of 200 mg/ml and 30 mm at concentration of 1000 mg/ml of ginger leaf and ginger root, respectively (Sebiomo *et al*., 2011). In contrast to this ethanolic extract of ginger had not inhibitory effect on *K. pneumoniae* and *Salmonella* Typhimurium using disc diffusion method (Nanasombat and Lohasupthawee, 2005) and Hasan *et al*. (2012) reported methanol extract of ginger did not exhibit inhibitory effect on *K. pneumoniae* and *Psedomonas* species below 25 mg/ml and S. aeurus at a concentraton of 12.5 mf/ml and lower while n-hexane extracts of ginger did not have inhibitory effect against *K. pneumoniae* at 50 mg/ml and lower concentration.

Akintobi *et al.* (2013) had also investigated the antimicrobial activities of ginger extracts on six pathogenic bacteria (*P. aeruginosa, S. aureus, Proteus mirabilis, E. coli, B. subtilis and S.* Typhi) using the agar well diffusion method and found that ethanol extract of *Zingiber officinale* produced the highest zone of inhibition on *Proteus mirabilis* (17mm), while observing at the same time relatively lower inhibitory effect on *S.* Typhi (10mm), *S. aureus* (13mm), and *P. aeruginosa* (14mm) and no inhibitory effect on *E. coli* and *B. subtilis*. The result with *E. coli* was in contrast with the finding of Omoya and Akharaiyi (2012) in which ginger ethanol extract had inhibitory effect on *E. coli* (18 mm) which was the same as the findings of Ahmed El Sayed and Aly (2014) who also reported that organic solvent extract of ginger resulted in inhibition zones of 18 mm for *E. coli, P. aeruginosa* and *Shigella dysenteriae*, 15 mm for *K. pneumoniae* and 11 mm for *S. aureus*. Whereas water extract of *Zingiber officinale* produced the highest zone of inhibition on *S.* Typhi (13mm), *S. aureus* (9mm) and *P. mirabilis* (11mm) but had no effect on *E. coli , B. subtilis and P. aeruginosa* (Akintobi *et al.,*2013).

The antibacterial effect of ginger and garlic extracts on some pathogenic bacteria isolated from patients with otitis media (*S. aureus, Staphylococcus epidermidis, K. pneumoniae, S. pyogenes, P. aeruginosa* and *Proteus mirabilis*) have been evaluated by Abdulzahra and Mohammed (2014) and the susceptibility experiment depicted that ethanolic extract of garlic and ginger (each alone and in combination) showed more inhibitory effect than aqueous extract and also the combination

of ethanolic extract of both ginger and garlic resulted in inhibitory effect greater than each extract alone. The inhibition zone of ginger ethanolic extract was in the range of 13-16 mm with *S. aureus* beingthe most inhibited (16 mm) while the inhibition zones produced by a mixture of ethanolic extract of ginger and garlic ranged from 15-23 mm and K. *pneumoniae* was the most inhibited, while S. *pyogens* was neither inhibited by any of the extracts nor by their mixture.

2.2.4. Antimicrobial activities of combinations of extracts of honey, garlic, ginger and others

In modern medicine interest in the application of honey for the treatment of infections has increased and advancement in industrial production and processing of honey especially in food and drug industries introduced fortification of honey with different fortifying agents known to possess certain individual medicinal value. The fortifying agents in honey (ginger, garlic and lemon) may act in synergy with honey and thus enhance the property of honey's antibacterial effect. Many authors have performed the antimicrobial activities of traditional medicines in combination with other herbal remedies or antibiotics like honey with onion (Saad and mona, 2012); honey with bovine milk (Al-Jabri *et al*., 2005) and honey with gentamicin (Al-Jabri *et al*., 2005a).

Antimicrobial effects of mixtures of Ethiopian honeys and ginger powder extracts on standard and resistant clinical bacteria isolates was evaluated by Yalemwork *et al*. (2014) and the comparison of the antimicrobial agents has shown that honey-ginger extract mixtures produced the highest mean inhibition (25.62±2.55 mm) for the total test organisms compared to the use of honeys (21.63±3.30 mm) or ginger extracts (19.23mm±3.42 mm) individually. The range of inhibitions produced by honey-ginger extract mixtures on the susceptible bacteria isolates (26–30 mm) and resistant clinical isolates (19–27 mm) was also greater than the antibiotic discs (Methicillin, Amoxicillin, and Penicillin) used in the study. This finding is almost similar with Omoya and Akharaiyi (2011) finding who conducted the antimicrobial activities of honey, ginger methanol and ethanol extracts and the mixture of honey and ginger extracts using agar diffusion method. The MBC for honeys, ginger extracts, and honey-ginger extract mixtures was 12.5% (12.5 mg/mL) for all test organisms while the MIC of were in the range of 6.25-12.5% (Yalemwork *et*

al., 2014). Premkishore *et al*. (2013) also investigated the effect of honey and aqueous extract of ginger against *Streptococcus mutans* and the mean diameter of zone of inhibition was found to be 27±0.5 mm for honey and 23±0.5 mm for ginger extract as compared to Gentamycin with zone of inhibition of 23±0 mm.

Isiaka *et al*. (2015) also conducted the antibacterial activities of various honey samples (honey fortified with ginger, honey fortified with lemon, unfortified honey and natural honey) against clinical bacterial isolates (*Escherichia coli* and *Salmonella* Typhi) at different concentration (20, 40, 60, 80 and 100%) and found varying antibacterial activities against the tested microbes. The inhibition zone ranges from 13-31mm for *S.* Typhi, 10-32.5 for *E. coli*. Plus to this the antimicrobial activity increases with the increment of concentration. In comparing the antibacterial activities of fortified and unfortified honey tested on the two clinical bacterial isolates, honey fortified with lemon shows higher antibacterial activity with the zones of inhibition of 25, 28, 29.5, 30 and 31 mm at the concentrations of 20, 40, 60, 80 and 100 % (v/v) respectively. This correlates with the findings of Adeshina *et al*. (2014). Yahaya *et al*. (2012) also reported that *S.* Typhi, *S. dysenteriae*, *E. coli* and *C. albicans* were inhibited by the combined effect ginger and honey at the concentration of 30% and above.

Additionally, Saad and Mona (2013) tested the antimicrobial activities of garlic juice, honey and honey garlic mixture against sensitive and resistant microbial species like *P. aeruginosa*, *E. coli* and *C. albicans* using broth dilution method. It was revealed that garlic-honey mixtures (1/1 v, 4/1 v, 1/4 v) at all concentrations of 100%, 50%, 20%, and 10% were significantly have the best antimicrobial effect on all tested organisms without any growth and this effect was significantly has stronger antimicrobial activity on all tested organisms than garlic alone and honey alone which agreed with Saad and Mona (2012), who found that a combination of onion juice suspended in honey inhibits the growth of some microbes showing stronger effect than that of observed by honey alone or onion juice alone. Also agree with, Omoya and Akharaiyi (2011) who proved that there is a synergistic effect of antimicrobial activity from the combination of ginger and honey against isolates from carious teeth and on some clinical isolates. However, Saad and Mona (2013) also reported that honey at a concentration less than 20% did not had inhibitory effect against *Pseudomonas* and *C. albicans* using dilution method. Berhanu (2013) also investigated the

combined effect of Tazma honey and garlic juice against standard and clinical isolated bacteria. The diameter of inhibition zone was in the ranged of 18±1-35±1 for mixture of tazma honey and garlic juice while the MIC and MBC was found to be in the range of 6.25-12.5% for garlic juice, stingless bees honey and their mixture.

3. MATERIALS AND METHODS

3.1. Description of the Study Areas

This study was conducted at Haramaya University which is located about 18km and 508 km west of Harar and East of Addis Ababa, respectively, at a latitude of 9º26'N, longitude of 42º03'E and an altitude of 1980 m.a.s.l. Specifically, the preparation, extraction and determination of antibacterial activities of all crude extracts were carried out at Microbiology Laboratory of the Department of Biology, Haramaya University.

The plant materials used as source of antimicrobials, i.e. garlic cloves and ginger rhizomes were, however, purchased at Harar, the capital city of Harari Region, which is located 526 km away from Addis Ababa towards the East. Geographically Harar lies at a latitude and longitude of 9° 15' 00" N and 42° 10' 00" E, respectively, and its elevation is 1639 m. The mean annual temperature of the area varies from 10 to 26 ^{0}C with annual rainfall between 700-900 mm. Whereas honey was purchased from Mertule Maryam, East Gojam, Amhara, Ethiopia located at a latitude and longitude of 10° 50' 0" N and 38° 16' 0" E, respectively, with an elevation of 2650 m.a.s.l. The mean annual temperature ranges from 22.5 ^{0}C to 25 ^{0}C and the average annual rainfall of the area ranges from (941 - 1203 mm).

3.2. Sources of Antimicrobial Substances and Test Pathogens

In this study red *Apis mellifera* honey, Garlic (*Allium sativum*) cloves, Ginger (*Zingiber officinale*) rhizomes were used as source of antimicrobial substances. Red *Apis mellifera* honey was purchased from Mertule Maryam (East Gojam, North West Ethiopia). Garlic and ginger were purchased from Deker local market (Harar, Eastern Ethiopia).

Five test pathogens namely, *Shigella boydii ATCC 9207, Staphylococcus aureus ATCC 25923,Salmonell*a Typhimurium ATCC 13311, *Pseudomonas aeruginosa ATCC 27853* and *Klebsiella pneumoniae ATCC 700603*, which are commonly involved in causing respiratory tract,

gastrointestinal tract, urinary tract and wound infections were collected from Ethiopian Public Health Institute culture collection center, Addis Ababa.

3.3. Experimental Design and Study Period

Crude extracts of honey, garlic and ginger to evaluate their antimicrobial activities were extracted using water, methanol and ethanol as extraction solvents. Twenty one (21) treatments (three individual and 4 combinations using three solvents) in triplicate were used against each test pathogen. These crude extracts were aqueous, methanol and ethanol extracts of honey, garlic and ginger alone and their combinations namely honey-garlic, honey-ginger, garlic ginger and honey-garlic-ginger. Antimicrobial activities of the crude extracts were determined by disc diffusion method using Muller Hinton Agar (MHA) at Haramaya University Microbiology Laboratory. Then the MICs of the crude extracts with highest zone of inhibitions against the selected pathogens were determined using the broth dilution method. Finally MBC was determined by taking aliquots from test tubes of the MIC determination set in which no growth were detected and making streaks on to freshly prepared MHA. The study was carried out from September – December, 2016.

3.4. Extraction of Antimicrobial Agents

3.4.1. Preparation of crude extract from honey

The preparation of crude extracts of honey was done using three different solvents (water, methanol and ethanol). Forty (40) grams of honey were placed into separate flasks containing 100 ml of sterile distilled water, methanol and ethanol. Then the solution was mixed well by vortexing and the supernatant was collected from each test tube by filtrations (Mohapatra *et al*., 2010). The resulting supernatant of the alcoholic extracts was evaporated to dryness in rotary evaporator while that of crude extract obtained using water was subjected to water bath in order to get concentrated crude extracts. Then it was stored at 4^0C until use.

3.4.2. Preparation of crude extracts from garlic and ginger

Three types of crude extracts namely aqueous, methanol and ethanol extract from garlic and ginger were prepared separately. The fresh garlic cloves and ginger rhizomes were washed, peeled, sliced and air dried for 14 days (Tagoe and Gbadago, 2009) and they were subjected to an electric blender separately to get fine powder. Sixty (60) grams of the grounded material (garlic and ginger) were soaked in 300 ml of distilled water, methanol and ethanol and allowed to stand for 72 h (Onyeagba *et al*., 2004) with shaking at 120 rpm (Gull *et al*., 2012). Then they were filtered separately using WhatmanNo1 filter paper to obtain the corresponding extracts (Onyeagba *et al*., 2004). The resulting filtrate of methanol and ethanol were concentrated using rotary evaporator at 50 ^{0}C while the aqueous extracts of garlic and ginger were subjected to water bath. The extracts were stored at 4 ^{0}C until use.

3.5. Media Preparation and Sterilization

The following media namely, Muller Hinton Agar (Oxoid, UK) for determination of antimicrobial activity and MBC, Nutrient Agar (Oxoid, UK) for sub culturing of the collected pathogens and Nutrient broth (Oxoid, UK) for determining MIC was used in this study. These media were prepared and sterilized according to the manufacturer's instruction.

3.6. Preparation of 0.5 McFarland Standards

In this study, 0.5mL of 1.175% $BaCl_2$ (w/v) (1.175mg in 100ml distilled water) was added to

99.5mL of 1% H_2SO_4 (v/v) (1ml H_2SO_4 in 99 ml distilled water) with constant stirring to make 0.5 McFarland Standards. The standard was distributed into a test tube of the same size for color comparison of the test inoculum.

3.7. Preparation of Stock Solutions and Antimicrobial Discs

Discs of 6 mm in diameter were punched out from filter paper with the aid of a paper punch and placed in petri dish. The discs were then be sterilized by autoclaving at 121^0C for 15 min and allowed to cool before use (Karuppiah and Rajaram, 2012). Stock solution of each extracts were prepared by dissolving 4 gram of the crude extracts in appropriate reconstituting solutions namely sterile water for crude aqueous extracts and DMSO for crude alcoholic extracts to give a concentration of 1g/ml. Finally 10µl from each stock solution was added to the filter paper discs for antimicrobial activity testing (Yassen *et al*., 2016; Okigbo and Mmeka, 2008).

3.8. Determination of Antimicrobial Activities of Test Samples

The disc diffusion method was employed to determine the antimicrobial activities of the extracts (Rashmi *et al*., 2011). 3-5 colonies of the test organisms were aseptically inoculated into physiological saline(0.85%) solution and the turbidity adjusted to 0.5 McFarland standards (a concentration of $1.5x10^8$ CFU/ml) (Andrews, 2005). Then the prepared antimicrobial discs were placed over the media which was inoculated with bacterial suspension by spreading method with the aid of cotton swab and pressed slightly along with a control. Discs containing 30µg of ceftriaxone and cefotaxime were used as positive control in all tests while discs with water and DMSO were used as negative controls. The plates were incubated for 24 hours at 37^0C. Antimicrobial activities were determined by measuring the diameters of inhibition zones using a ruler. Equal volumes of the extracts were mixed thoroughly by measuring 1ml from their respective stock solutions for the determination of combined antimicrobial activities of the crude extracts against the test pathogens. All experiments were carried out in triplicate and the averages diameter of zone of inhibition was recorded for further analysis (Rashmi *et al*., 2011).

3.9. Determination of Minimum inhibitory concentration and Minimum Bactericidal Concentration

Minimum inhibitory concentrations of crude extracts with the highest zone of inhibition against each test pathogens were determined by preparing various concentrations (500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml, 7.81mg/ml and 3.90mg/ml) of the crude extracts using serial dilutions kept in test tubes containing 2 ml of nutrient broth. These test tubes were then inoculated with standardized inocula of 0.1 ml of the test pathogens (Taura *et al*., 2014) and incubated at 37°C for 24 h. Microbial growth was detected by inspecting the microbial growth visually and soon after measuring optical density (OD) at 500 nm (Andrews, 2005). The OD was compared with the tube without bacterial inoculum. The lowest concentration at which the bacteria do not show visible growth was recorded as the MIC of a test sample. In addition to this for crude extracts with no inhibition zone at a concentration of 10 mg/disc broth dilution method was employed by preparing different concentration of the crude extracts for further evaluation of their activities. Dilutions showing no visible growth in the MIC determination test tubes were used as source of inoculum for the freshly prepared MHA plates containing no crude extracts. After inoculation, the plates were incubated at 37 °C for 24 h. The lowest concentration of the extract with no visible colony growth on the MHA plate after incubation was recorded as the minimum bactericidal concentration (MBC) (Patel *et al*., 2011).

3.10. Data Analysis

The antibacterial activities (diameter of zone of inhibitions) of crude extracts of honey, garlic, ginger and their combinations (mean ± Standard deviation (SD)) were compared using descriptive statistics. All statistical analyses (mean and SD) have been performed using the statistical package for the social sciences (SPSS) version 16. Comparison of honey extracts, garlic extracts, ginger extracts and their combinations for their mean zone of inhibitions were done using one way analysis of variance (ANOVA). Mean zone of inhibitions of the different crude extracts of honey, garlic, ginger and their combinations were considered significantly different at $p < 0.05$.

4. RESULTS AND DISCUSSIONS

4.1. Determination of the Amount of Crude Extracts Recovered from Honey, Garlic and Ginger

After extraction procedure, the crude aqueous, methanolic and ethanolic extract of honey, garlic(*Allium sativum*) and ginger (*Zingiber officinale*) were obtained and weighed. Crude extracts of honey, garlic and ginger recovered were summarized in Table 1.

Table 1. Yields of extracts with respect to solvents

Antimicrobial agent used	Parts used	Solvent used	Amount used (g)	Yield (g)	Yield (%)

Honey of honeybee	Pure honey	Water	40	18.55	46.37
		Methanol	40	17.00	42.50
		Ethanol	40	15.69	39.22
Garlic	Clove/bulb	Water	60	16.11	26.85
		Methanol	60	15.51	25.85
		Ethanol	60	15.21	25.35
Ginger	Rhizome	Water	60	17.81	29.68
		Methanol	60	17.95	29.92
		Ethanol	60	17.91	29.85

Table 1 demonstrates that the percentages of yield for honey were 46.37, 42.50 and 39.22% when using distilled water, methanol and ethanol as extraction solvents, respectively. The percentage yield obtained in this study using ethanol was lower than the yield reported by Mohamed *et al*. (2016) while the percentage yield using methanol was almost similar to the present study. In the case of garlic and ginger the percentage yields ranged from 25.35-26.85% and 29.68-29.92%, respectively (Table 1). The percentage yield of garlic in this study was lower as compared to those reported by Ekweney and Elegalan (2005) using methanol and ethanol as extraction solvent as well as El-Hamidi and El-Shami (2015) using water as extraction solvent. However, the results in the present study were in close agreement with those of El-Hamidi and El-Shami (2015) and Iqbal and Bhanger (2007) in which methanol was used as extraction solvent. Generally the percentage yield of garlic was relatively less than the percentage yield of honey and ginger, which seemed that garlic powder is somewhat hard in dissolving and forming solutions. With regard to ginger the percentage yields found in the present study were also lower than the percentage yield reported by Ekweney and Elegalan (2005) and percentage yield of aqueous extract of ginger were in agreement with the report by Yassen *et al*. (2016) whereas methanolic and ethanolic extracts

yield percent were higher compared to those reported by Yassen *et al*., 2016 and Yisehak *et al*., 2014. The variation in the amount of crude extracts recovered using methanol and ethanol as extraction solvents could be due to differences in the concentrations of the alcohols used. Generally as indicated in Table 1 the percentage yield of honey and garlic using water were higher than the alcoholic extracts while the percentage yield of ginger using water was somewhat lower than the alcoholic extracts. This difference could be the ability of the solvents to dissolve the powder garlic and ginger as well as the honey.

4.2. Determination of Antimicrobial Activities of the Crude Extracts using the Disc Diffusion and Broth Dilution Methods

A total of 9 crude extracts (aqueous, methanol and ethanol) of honey, garlic and ginger were obtained and prepared for determining their antimicrobial activities against the test pathogens. These crude extracts as well as their combinations were tested against five test pathogens using disc diffusion method and the results are summarized in Table 2, Table 4 and Table 6. The negative controls used in this were sterile water and DMSO and they did not exhibit inhibitory effect against the test pathogens.

4.2.1. Antimicrobial activities of honey

The antimicrobial activities of the aqueous, methanolic and ethanolic extracts of honey against *Shigella boydii, Staphylococcus aureus ATCC, Salmonella* Typhimurium*, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are shown in Table 2. As indicated in the table, the aqueous, methanol and ethanol crude extracts did not show any inhibitory effect on the test pathogens at a concentration of 10 mg/disc. However, at the same concentration, the ethanolic extract of honey had an inhibitory activity only against *Pseudomonas aeruginosa* which resulted in a zone of inhibition of 7.33±0.58 mm. Contrary to this result, Selcuk and Nevin (2002) and Manisha and Shyamapada (2011) reported that honey did not exhibit antimicrobial activity against *Pseudomonas aeruginosa* and *E. coli*. In addition, Akinnibosun and Itedjere (2013) had reported that honey at a concentration of 75% and below did not exhibit antimicrobial activities against *Staphylococcus aureus*. In contrast many researchers had shown that honey has antimicrobial

activities against different human pathogens (Yalemwork *et al*., 2013; Mohapatra *et al*., 2010; Berhanu (2013) and Basualdo *et al*., 2007). Such variations in activity of honey might be due to the differences in the types of honey tested, which in turn results from differences in the producer species of bees and the floral source of the honey, geographical location, seasonal variation, harvesting and storage conditions as well as the differences in the methodology employed.

Table 2. Antimicrobial activities of crude extracts of honey and antibiotics against the test pathogens using the disc diffusion method (Mean ± SD, n=3)

Test pathogen	Zone of Inhibition (mm)				
	Crude extracts of the different solvents at 10 mg/disc concentration			Antibiotics (30µg)	
	Water	Methanol	Ethanol	Ceftriaxone	Cefotaxime
Shigella boydii	0.00	0.00	0.00	17.67±0.58	21±1.00
S. aureus	0.00	0.00	0.00	14.67±1.53	14.67±0.58
*S.*Typhimurium	0.00	0.00	0.00	0.00	0.00
P. aeruginosa	0.00	0.00	7.33 ± 0.58^{Aa}	13.67±0.58	14±1.00
K. pneumoniae	0.00	0.00	0.00	14±1.00	13±1.00

n= number of experimental replicates, SD= standard deviation; means of crude extracts with the same letter (upper case) in the same row and lower case in the column are not significantly different.

As shown in Table 3, the aqueous extracts of honey had the minimum inhibitory activities at a concentration of 250mg/ml against *Staphylococcus aureus* and *Klebsiella pneumoniae* and at 125mg/ml against the other test pathogens used in this study. The MIC of methanol extracts of honey against all test pathogens was 125 mg/ml. Likewise, the ethanol crude extracts of honey were inhibitory at a minimum concentration of 125 mg/ml honey extract against *Shigella boydii*, *Staphylococcus aureus*, and *Salmonella* Typhimurium. This indicates that the inhibitory effect of honey is concentration dependent. Basualdo *et al*. (2007) and Adeleke *et al*. (2006) also reported that the inhibitory activity of honey is dependent on the concentration and the type of honey. As indicated in Table 3, methanolic and ethanolic extracts of honey have better inhibitory activities

than aqueous extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The possible reason for this variation is probably due to the ability of the solvents to dissolve the bioactive molecules that have antimicrobial activities against these microbes.

Table 3. Minimum inhibitory concentration of crude extract of honey against test pathogens

Test pathogen	Minimum inhibitory concentration (mg/ml) of the crude extract		
	Aqueous extract	Methanolic extract	Ethanolic extracts
Shigella boydii	125	125	125
Staphylococcus aureus	250	125	125
Salmonella Typhimurium	125	125	125
Pseudomonas aeruginosa	125	125	*
Klebsiella pneumoniae	250	125	125

* indicates MIC was not performed because it had antimicrobial activities using disc diffusion.

4.2.2. Antimicrobial activities of garlic

The results of the *in vitro* antimicrobial activities of garlic crude extracts using water, methanol and ethanol as extraction solvent indicates that garlic has varying antimicrobial activities against the test pathogens used in this study. As shown in Table 4, all crude extracts of garlic showed inhibitory activities against the test pathogens except *Pseudomonas aeruginosa*. The ranges of ZOI of the crude extracts of garlic were 10-13, 10.67-12.33 and 11-14 mm for aqueous, methanolic and ethanolic extracts respectively. Generally the ethanolic crude extract of garlic was found to have better inhibitory activity against the test pathogens in this study (Table 4) which is in agreement with Shobana *et al*. (2009). The ZOI of methanolic crude extract of garlic aginst *Shigella boydii* ($p=0.027$) and *Staphylococcus auerus* ($p=0.006$) was significantly lower than aqueous and ethanolic crude extracts. In addition, the antimicrobial activities of garlic using different solvents were significantly different against *Salmonella* Typhimurium ($p=0.036$) but they were not significantly different against *K. pneumoniae* ($p=0.252$). The ZOIs found in this study were in close proximity with those of Yabaya *et al*. (2010). In contrast, Garba *et al*. (2013), Iwalokun *et al*. (2004) and Gull *et al*. (2012) reported higher ZOI all including *Pseudomonas*

aeruginosa. Whereas Nanasombat and Lohasupthawee (2005), using the disc diffusion method, reported that ethanolic extract of garlic did not exhibit inhibitory activities against *Salmonella* species and other enterobacteria. Onyeagba *et al*. (2004) also reported garlic had no antimicrobial activity against *S. auerus, Bacillus* species, *E. coli* and *Salmonella* species.

Table 4. Antimicrobial activities of crude extracts of garlic and antibiotics against test pathogens (Mean ± SD, n=3)

Test pathogen	Zone of Inhibition (mm)				
	Crude extracts of the different solvents at 10 mg/disc concentration			Antibiotics(30µg)	
	Water	Methanol	Ethanol	Ceftriaxone	Cefotaxime
Shigella boydii	13 ± 1.00^{Aa}	11 ± 0.00^{Bb}	12.33 ± 0.58^{Ab}	17.67±0.58	21±1.00
Staphylococcus aureus	13 ± 1.00^{Aa}	10.67 ± 0.58^{Bb}	13.67 ± 0.58^{Aa}	14.67±1.53	14.67±0.58
Salmonella Typhimurium	11 ± 1.00^{Cb}	12.33 ± 1.53^{Ba}	14 ± 0.00^{Aa}	0.00	0.00
Pseudomonas aeruginosa	0.00	0.00	0.00	13.67±0.58	14±1.00
Klebsiella pneumoniae	10 ± 1.00^{Ab}	10.67 ± 0.58^{Ab}	11 ± 0.00^{Ab}	14±1.00	13±1.00

n= number of experimental replicates, SD= standard deviation; means of the crude extracts with the same letters (upper cases) in the same row and lower cases in the same column are not significantly different.

As indicated in Table 4 garlic did not exhibit antimicrobial activity against *Pseudomonas aeruginosa*. The antimicrobial activity of garlic against *Pseudomonas aeruginosa* was further investigated using the broth dilution method by increasing the concentration of the crude extract.

Table 5. Minimum inhibitory concentration of crude extract of garlic against *Pseudomonas aeruginosa*

Crude extract of garlic	Minimum inhibitory concentration (mg/ml)of the crude extract
Aqueous extract	250
Methanolic extract	250
Ethanolic extracts	125

As it was in Table 5 the aqueous and methanolic extracts of garlic had inhibitory activities at a concentration of 250mg/ml and above while the ethanolic extract of garlic had activities at 125 mg/ml and above (Table 5). Here it is clear that the antimicrobial activities of crude extracts of garlic against *Pseudomonas aeruginosa* depends on concentration and the solvent used for extraction.

4.2.3. Antimicrobial activities of ginger

The antimicrobial activities of the aqueous, methanolic, and ethanolic extracts of ginger were evaluated against the test pathogens and the results are shown in Table 6. The data indicate that the aqueous and methanol extracts of ginger had no inhibitory activity at 10mg/disc concentration against all test pathogens. The only observed ZOIs were obtained when the ethanol extract of ginger was employed at the same concentration (i.e. 10mg/disc) against *Salmonella* Typhimurium and *Pseudomonas aeuroginosa* with ZOIs of 9.67±0.58 mm and 8.33±0.58 mm, respectively (Table 6). It was found that all ginger extracts did not exhibit antimicrobial activities against the other test pathogens at a concentration of 10 mg/disc (Table 6) except the ethanolic extract which do have inhibitory effect against *Salmonella* Typhimurium and *Pseudomonas aeuroginosa*. Likewise Zakia *et al*. (2014) and Nanasombat and Lohasupthawee (2005) reported that ginger extracts did not exhibit antimicrobial activities. Akintobi *et al*. (2013) also reported that aqueous extract of ginger did not have inhibitory effect against *Pseudomonas aeruginosa* while the ethanolic extract did. This finding is in agreement with the present study. However, Yalemwork *et*

al. (2014), Akintobi *et al*. (2013) and Gull *et al*. (2012) had shown that ginger extracts exhibit antimicrobial activities against different human pathogens.

Table 6. Antimicrobial activities of crude extract of ginger and antibiotics against the test pathogens using the disc diffusion method (Mean ± SD, n=3)

Test pathogen	Zone of Inhibition				
	Crude extracts obtained using different solvents at 10 mg/disc			Antibiotics(30µg)	
	Water	Methanol	Ethanol	Ceftriaxone	Cefotaxime
Shigella boydii	0.00	0.00	0.00	17.67±0.58	21±1.00
S. aureus	0.00	0.00	0.00	14.67±1.53	14.67±0.58
S. Typhimurium	0.00	0.00	9.67±0.58^{Aa}	0.00	0.00
P. aeruginosa	0.00	0.00	8.33±0.58^{Aa}	13.67±0.58	14±1.00
K. pneumoniae	0.00	0.00	0.00	14±1.00	13±1.00

n= number of experimental replicates, SD= standard deviation; means of the crude extracts with the same letter (upper case) in the same row and lower case in the same column are not significantly different

In the broth dilution method ginger aqueous extract also did not exhibit inhibitory activity against any of the pathogens at concentrations of 31.25-500 mg/ml; a result which is in agreement with the report of Tagoe and Gbadago (2009). In contrast, the methanolic extracts had inhibitory activities at a concentration of 125 mg/ml and above against *Shigella boydii;* 250 mg/ml and above against other test pathogens (Table 7). In addition to this ethanolic extracts had inhibitory effect at a concentration of 125 mg/ml against *Shigella boydii* and *Klebsilla pneumoniae*; and at a minimum concentration of 250mg/ml against *Staphylococcus aureus*. On the basis of these results, it is possible to say that the antimicrobial activities of ginger extracts vary depending on the methodology employed, the solvent used for extraction of the bioactive compounds and the microorganisms tested as well as the concentration of the crude extracts. Hasan *et al*. (2012) also

reported that the inhibitory effect of ginger is dependent on concentration and the solvents used for extraction.

Table 7. Minimum inhibitory concentration of crude extracts of ginger against the test pathogens

Test pathogen	Minimum inhibitory concentration (mg/ml) of the crude extract		
	Aqueous extract	Methanolic extract	Ethanolic extracts
Shigella boydii	NA	125	125
Staphylococcus aureus	NA	250	250
Salmonella Typhimurium	NA	250	
Pseudomonas aeruginosa	NA	250	
Klebsiella pneumoniae	NA	250	125

NA= No Activity, NA= No inhibitory activity was observed at concentrations ranging from 31.25-500mg/ml of the crude extract

Generally the antimicrobial activities of crude extracts of honey, garlic and ginger varies differently. The main factories for the variation could be the type and composition of the extracts, the amount of crude extract used, and the type of microorganism against which the extract has been applied (Gull et al., 2012). The solvents which are used for extraction of the bioactive materials also affect the antimicrobial activities of the extracts (Niranjan et al., 2013). In addition to this the antimicrobial activities of crude extracts also depends on the test pathogens. As revealed in this study the crude extracts of the same solvent showed different ZOI against different pathogens (Figure 1). This could be probably associated with the nature of bacteria like cell wall, the level of permeability of the cell wall of the bacteria and the degree of the bioactive molecules to penetrate the cell wall of the bacteria.

AEH/MEH/EEH- Aqueous/Methanol/Ethanol extract of honey, GA-Garlic and GI-Ginger

Figure 1. Comparison of Zone of inhibitions by crude extracts of honey, garlic and ginger using the three solvents.

4.2.4. Antimicrobial activities of combined crude extracts of honey, garlic and ginger

In addition to individual evaluation of the activities of aqueous, methanolic and ethanolic crude extracts of honey, garlic and ginger, their combined antimicrobial activities against the test pathogens were also evaluated. In most cases it was obtained that combination of crude extracts (crude extracts that showed ZOI individual and those which did not) exhibits antimicrobial activities with decrement in ZOI at a concentration of 10mg/disc using disc diffusion method (Table 8). Though the reason for the decrement in ZOI was unclear it is probably due to the decrement of concentration.

Table 8. Antimicrobial activities of combination of crude extracts and antibiotics against the test pathogens (Mean ± SD, n=3)

Crude extracts	Solvents	Zone of inhibition (mean ± SD) of the crude extracts at 10 mg/disc and standard antibiotics (30µg)				
		Shigella boydii	*S. aureus*	*Salmonella* Typhimuriu	*P. aeruginosa*	*Klebsiella pneumonia*
Honey-garlic	Water	10 ±1.00^{A}	10.67±0.58^{A}	8±1.00^{B}	0.00	7.33±0.58^{A}
	Methanol	9±1.00^{A}	9.67±1.15^{A}	9±1.00^{B}	0.00	8±1.00^{A}
	Ethanol	9±1.00^{A}	10±1.00^{A}	10.33±0.58 A	9±1.00^{A}	8.67±0.58^{A}
Honey-ginger	Water	0.00	0.00	0.00	0.00	0.00
	Methanol	0.00	0.00	0.00	0.00	0.00
	Ethanol	0.00	0.00	0.00	8.33±0.58 A	0.00
Garlic-ginger	Water	11±1.00^{A}	10±1.00^{B}	8±0.00^{B}	0.00	8±1.00^{A}
	Methanol	7.33± 0.58^{B}	8.67± 0.58^{B}	8.67±1.53^{B}	0.00	7.33±0.58^{A}
	Ethanol	9±1.00^{B}	10.67±0.58^{A}	10.33±0.58 A	8±0.00	7.67±0.58^{A}
Honey-garlic-ginger	Water	10 ±1.00^{A}	8±1.00^{A}	8±1.00^{A}	0.00	8.67±0.58^{A}

	Methanol	0.00	0.00	7±0.00^{A}	0.00	0.00
	Ethanol	8.67±0.58^{A}	8±1.00^{A}	8.33±0.58^{A}	7.67±0.58 A	8±0.00^{A}
Antibiotics	Ceft	17.67±0.58	14.67±1.53	0.00	13.67±0.58	14±1.00
	Cefo	21±1.00	14.67±0.58	0.00	14±1.00	13±1.00

n= number of experimental replicates, SD= standard deviation, Ceft=ceftriaxone, Cefo= Cefotaxime; means with the same letter in the same column of each combined crude extracts are not significantly different

As compared to the ZOI created by crude extract of garlic individually against the test pathogens, the ZOI by the combined crude extracts were decreased against *Shigella boydii, Staphylococcus aureus*, *Salmonella* Typhimurium and *Klebsiella pneumoniae* at a concentration of 10 mg/disc using the disc diffusion method (Table 4, Table 8) The antimicrobial activities of combined crude extracts of honey-garlic were not significantly different against *Shigella boydii*, *Staphylococcus aureus*, *Salmonella* Typhimurium and *Klebsiella pneumoniae* ($p>0.05$). Contrary to this, the ZOI by ethanolic crude extract of honey against Pseudomonas aeruginosa was not decreased rather it shows a slight increment in ZOI up on combination with garlic (Table 2, Table 8). This is probably due to different mechanisms of action of the bioactive compounds in the ethanolic crude extracts of honey and garlic. Combined ethanolic crude extracts of honey-garlic, honey-ginger and honey-garlic-ginger also had antimicrobial activities against *Pseudomonas aeruginosa* with ZOI 8.33±0.58, 8±0.00 and 7.67±0.58 mm, respectively (Table 8) and they were significantly different ($p=0.000$) in which the aqueous and methanolic extracts did not showed inhibitory effect.

In contrast to the findings of the present study many researchers reported that combination of honey and ginger (Yalemwork et al., 2014 and Yahaya et al., 2012), combination of honey with ginger and lemon (Isiaka et al., 2015) and combination of honey and garlic (Berhanu, 2013 and Saad and Mona, 2013) had better inhibitory effect. This variation could be due to the concentration, the solvent used for extraction.

As indicated in Table 8, some combined crude extracts did not exhibit inhibitory activity at a concentration of 10 mg/disc. Accordingly, further evaluation of their antimicrobial activity was performed using broth dilution in the same manner performed for single extracts by mixing the individual crude extracts in the ratio of 1:1 from stock solution at increased concentration.

Table 9. Minimum inhibitory concentration of combined crude extracts against the test pathogens

Crude extracts	Solvents	Minimum inhibitory concentration (mg/ml) of the crude extract				
		Shigella boydii	*Staphylococcus aureus*	*Salmpnella* Typhimurium	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Honey-garlic	Water				125	
	Methanol				125	
Honey-ginger	Water	250	250	250	250	250
	Methanol	125	125	125	125	125
	Ethanol	62.5	62.5	62.5		62.5
Garlic-ginger	Water				250	
	Methanol				125	

Honey-garlic-ginger	Water				125	
	Methanol	62.5	125		125	125

As shown in Table 9, methanol extracts of combined crude extracts of honey-garlic, garlic- ginger and honey-garlic-ginger had inhibitory effect against *Pseudomonas aeruginosa* at a concentration of 125 mg/ml and above. Likewise aqueous crude extract of honey-garlic combination also exhibits inhibitory activity at 125 mg/ml and above while aqueous crude extracts of honey-ginger and garlic-ginger were inhibitory at 250 mg/ml and above. In addition, aqueous and methanolic extracts of honey-ginger showed antimicrobial activities against Shigella *boydii*, *Staphylococcus aureus*, *Salmonella* Typhimurium and *Klebsiella pneumoniae* at a concentration of 250 and 125 mg/ml respectively. Plus to this the ethanolic extract of honey-ginger was also inhibitory at 62.5 mg/ml.

4.3. Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Extracts with the Highest Zone of Inhibition

Minimum inhibitory concentration was determined for the crude extracts that have showed highest zone of inhibition among the treatments against each test pathogen. The results showed that the MICs ranged from 3.90 mg/ml- 15.625 mg/ml. The lowest MIC value was obtained from ethanolic extracts of garlic against *Salmonella* Typhimurium followed by ethanolic extract of garlic against *Staphylococcus aureus* and aqueous extract of garlic against *Shigella boydii* (Table 10). Iwalokun *et al*. (2004) and Tagoe and Gbadago (2009) reported the MIC of aqueous extracts of garlic against *Shigella* species was of 15.6 mg/ml; 50 mg/ml which is higher than the MIC obtained in the present study against *Shigella boydii*. Similarly, the MICs of ethanolic extract of garlic against *Staphylococcus aureus* (Abubakar (2009), *Salmonella* Typhimurium and *Klebsiella pneumoniae* (Nanasombat and Lohasupthawee (2005) reported were higher in concentration than the MIC in the present study. On the other hand, Gull *et al*. (2012) reported higher concentrations than the present study. The MIC of ethanolic extract of honey-garlic against

Pseudomonas aeuruginosa was almost similar with MIC of honey-garlic reported by Berhanu (2013).

Table 10. MIC and MBC values of crude extracts with highest zone of inhibitions

Test pathogens	MIC and MBC values(mg/ml)		
	MIC	MBC	Remark
Shigella boydii	7.81	15.625	Aqueous extract of garlic
Staphylococcus aureus	7.81	31.25	Ethanol extract of garlic
Salmonella Typhimurium	3.90	31.25	Ethanol extract of garlic
Pseudomonas aeruginosa	15.625	62.5	Ethanol extract of honey-garlic
Klebsiella pneumoniae	15.625	31.25	Ethanol extract of garlic

The MBC was also investigated by sub-culturing from the broth dilution with MIC value and above. According to the present study the MBC values were ranged from15.625 mg/ml to 62.5 mg/ml. In the present study the lowest MBC was obtained by aqueous extract of garlic on *Shigella boydii*. In addition, the MBC of ethanolic extracts of garlic against *Staphylococcus aureus, Salmonella* Typhimurium and *Klebsiella pneumonia* was 31.25 mg/ml. The MBC values of aqueous garlic extract in this study was lower than Garba *et al*. (2013) who reported MBC in the range of 100- 300 mg/ml but Berhanu (2013) reported MBC of garlic and honey-garlic in the range of 6.25-12.5% which is lower than the MBCs reported in this study.

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary

In the present study, honey was purchased from Mertule Maryam (East Gojam, North West Ethiopia) while garlic and ginger were purchased from Deker local market (Harer, Eastern Ethiopia) and their antimicrobial activities was investigated on five human pathogens. For their antimicrobial activities, extraction of the bioactive component in the form of crude extract was carried out using distilled water, methanol (98%) and ethanol (97%). The crude extracts of honey, garlic and ginger using the three solvents was tested for their antimicrobial activities against the test pathogens at 10 mg/disc using disc diffusion method. Here, despite for *P. aeruginosa*, all crude extracts of garlic had antimicrobial activities against 4 pathogens used in this study. Crude extracts of honey and ginger did not exhibit antimicrobial activities against the test pathogens at 10mg/disc except *Salmonella* Typhimurium that was susceptible for ethanolic extract of ginger while *pseudomonas aeruginosa* for both ethanolic extracts of honey and ginger. *Salmonella* Typhimurium was resistant for ceftriaxone and cefotaxime which are used as positive control in this study while it was significantly inhibited by ethanolic extract of garlic with ZOI of 14 mm.

However, in other pathogens the positive controls used had better inhibitory effect than the crude extracts. The antimicrobial activities of combined crude extracts were also evaluated. It was found that ethanolic crude extracts honey-garlic showed better inhibitory effect against *Pseudomonas aeruginosa* than honey alone. Ethanolic crude extracts of honey-ginger also exhibits antimicrobial activities against *Pseudomonas aeruginosa*.

In addition to this for those crude extracts which had no inhibitory effect at 10 mg/ml further investigation was carried out using broth dilution method. It was revealed that all extracts except aqueous extract of ginger exhibits antimicrobial activities at increased concentrations. In the case of combined crude extracts their activities were enhanced due to combinations at increased concentrations. MIC and MBC test of the crude extracts with the highest ZOI was also evaluated in which the MICs of the crude extracts were in the range of 3.90- 15.625 mg/ml while the MBCs were in the range of 15.625-62.5 mg/ml.

5.2. Conclusions

The percentage yields of crude extracts obtained using water, methanol and ethanol as extraction solvent varies.The finding of the present study indicated that crude extracts of honey, garlic and ginger as well as their combinations exhibits antimicrobial effect against the five human pathogens *(Shigella boydii, Staphylococcus aureus, Salmonella* Typhimurium*, Pseudomonas aeruginosa* and *Klebsiella pneumoniae)* though aqueous extract of ginger had not inhibitory effect at a concentration less than and equal to 500 mg/ml. This is an indication for the presence of bioactive compounds in the crude extracts and the antimicrobial activities of the extracts vary depending on the solvents used, test pathogen and the concentration. In the present study crude extracts obtained using ethanol as extraction solvent had better antimicrobial activities against the test pathogens except for *Shigella boydii* in which aqueous crude extract of garlic had better inhibitory activity.

Crude extracts of garlic were more effective than crude extracts of honey and ginger at a concentration of 10 mg/disc against *Shigella boydii, Staphylococcus aureus, Salmonella* Typhimurium*,* and *Klebsiella pneumoniae* while ethanolic crude extracts of honey and ginger

were effective than garlic against *Pseudomonas aeruginosa*. In the case of antimicrobial activities of combined crude extracts ethanolic extract of honey-garlic showed increased ZOI than individual extracts against *Pseudomonas aeruginosa*. On the other hand *Salmonella* Typhimurium was resistant to standard antibiotic discs used in the present study while it was susceptible to all crude extracts of garlic and ethanolic extracts of ginger. This resistance of *Salmonella* Typhimurium needs due attention in health care providers.

The MIC of the crude extracts with the highest ZOI were in the range of 3.90-15.625 mg/ml. This indicates that these crude extracts are effective at lower concentrations. In addition to this for crude extracts which had no inhibitory effect at 10 mg/ml further investigation was carried out using broth dilution method and their MIC were differently ranged against the test pathogens. Their activities were enhanced due to combinations at increased concentrations. Therefore concentration optimization of crude extracts is necessary to get better activities against human bacterial pathogens.

5.3. Recommendations

Based on the findings of this study the following recommendations are forwarded;

- Being the antimicrobial activities of extracts is dependent on the chemical constituents of the crude extract; phytochemical screening and characterization of components of the crude extract using different organic, inorganic, polar and non-polar solvent should be carried out for the development of new chemotherapy in the future.
- In this study, garlic was more effective than the other crude extracts. Therefore, it is recommended garlic to be used as home remedy for the treatments of different ailments.
- It was believed that medicinal materials of animal and plant origin enhance the effectiveness of chemotherapies. Therefore, it is necessary to conduct the combined effect of medicinal materials with chemotherapies since in this century drug resistance is a major concern.

- *Salmonella* Typhimurium was resistant for ceftriaxone and cefotaxime which are third generation drugs. Therefore antimicrobial susceptibility test of other serotypes of *Salmonllae* using these antibiotics and other chemotherapies should be carried out.
- For further implementations of these medicinal remedies *in-vivo* trial using laboratory animal is crucial for the future chemotherapy development.

7. REFERENCES

Abdulzahra, M.D. and Mohammed, H.F. 2014. The antibacterial effect of ginger and garlic extracts on some pathogenic bacteria isolated from patients with otitis media. *International Research Journal of Medical Sciences*, 2(5):1-5 www.isca.in,www.isca.me accessed on March, 2016

Abraha, T., Balcha, A. and Mirutse, G. 2013. An ethnobotanical study of medicinal plants used in Kilte Awulaelo District, Tigray Region of Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, 9: 65-98

Abubakar, M.M., 2009. Efficacy of crude extracts of garlic (*Allium sativum Linn*.) against nosocomial *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. *Journal of Medicinal Plants Research*, 3(4): 179-185,

Adeleke, OE, Olaitan, JO and Okepekpe, EI. 2006. Comparative antibacterial activity of honey and gentamicin against *Escherichia coli* and *Pseudomonas aeruginosa*. *Annals Burn Fire Disasters*, 19(4)

Adeshina, G.O., Mshelia, B.M. and Onaolapo, J.A. 2014. Antibacterial susceptibility of *Klebsiella pneumoniae* isolates from respiratory tract infections to honey and lemon. *Annual Research and Review in Biology*, 4(4):625-637

Ahmed El Sayed Hameda El Sayed and Aly Magda, M. 2014. Antibacterial activities of six medicinal plants used traditionally by Saudi people to treat common diseases. *British Biotechnology Journal* 4(4): 499-510

Ahmed, S. A., Jabbar, I. I. and Abdulwahed, H. E. 2012. Study the antibacterial activity of zingiber officinale roots against some of pathogenic bacteria. *Al- Mustansiriya Journal of Science,* 23(3): 63-70

Ahmed, T., Shittu, L.A.J., Bankole, M.A., Shittu, R.K., Adesanya, O.A., Bankole M.N.,and Ashiru, O.A. 2007. Comparative studies of the crude extracts of Sesame against some common pathogenic microorganisms. *Scientific Research and Essay*, 4(6): 584-589

Ajibola, A., Chamunorwa, JP. and Erlwanger, KH. 2012. Nutraceutical values of natural honey and its contribution to human health and wealth. *Nutrition Metabolism*, 9: 61.

Akinnibosun, F.I. and Itedjere, E. 2013. Evaluation of the antibacterial properties and synergistic effect of *Garcinia kola Heckel* (Family: *Guttiferae*) seed extract and honey on some bacteria. *African Journal of Microbiology Research*, 7(3): 174-180

Akintobi, OA, Nwanze, JC, Ogele JO, Idowu, AA, Onianwa, O. and Okonko IO, 2013. Antimicrobial Activity of *Allium sativum* (Garlic) Extract against Some Selected Pathogenic Bacteria. *Nature and Science*, 11(1):1-6.

Al-Jabri, A. A., Al Hosni, S. A., Nzeako, B. and Nsanze, H. 2005a. The anti-staphylococcal activity of Omani honey in combination with bovine milk. *British Journal of Biomedical Science,* 62 (2): 92-93.

Al-Jabri, A. A., Al Hosni, S. A., Nzeako, B.C, Al- Mahrooqi, Z.H. and Nsanze, H. 2005. Antibacterial activity of Omani honey alone and in combination with gentamicin. *Saudi Medical Journal*, 26 (5): 767-771.

Alli JA, Boboye BE., Okonko IO, Kolade AF and Nwanze JC. 2011. In-Vitro assessments of the effects of garlic (*Allium sativum*) extract on clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Advances in Applied Science Research*, 2(4):25-36.

Alvarez-Saurez, J., Tulipani, S., Romandini. S., Bertoli, F. and Battino, M. 2009.Contribution of honey in nutrition and human health review. *Mediterrean Journal of Nutrition Metabolism*, 6:1–19.

Andargachew Mulu, Belay Tessema, Fetene Derbie. 2004. *In vitro* assessment of the antimicrobial potential of honey on common human pathogens. *European Health Development*, 18:107–111.

Andrews, J.M., 2005. BSAC standardized disc susceptibility testing method (version4). *Journal of Antimicrobial Chemotherapy*, 56: 60-76.

Araya, Abera, B. and Giday, M. 2015. Study of plants traditionally used in public and animal health management in Seharti Samre District, Southern Tigray. *Ethiopia Journal of Ethnobiology and Ethnomedicine*, 11:22

Arriaga, E., Navarro-Calvo AL, Díaz-Carbajal, E. 2011. Botanical characterization of Mexican honeys from a subtropical region (Oaxaca) based on pollen analysis. *Grana*, 50: 40-45

Astal, Z.E., 2004. The inhibitory action of aqueous garlic extract on the growth of certain pathogenic bacteria. *European Food Research Technology*, 218: 460-464.

Badawy OFH, Shafii SSA, Tharwat EE, Kamal AM. 2004. Antibacterial activity of bee honey and its therapeutic usefulness against *Escherichia coli* O157:H7 and *Salmonella* Typhimurium infection. *Review Science Technology International Epiz*, 23: 1011-1122.

Baek, S.J., Kim, K.S., Nixon J.B., Wilson L.C. and Eling T.E. 2001. Cyclooxygenase inhibitors regulate the expression of the TGF-b superfamily member that has proapoptotic and anti-tumorigenic activities. *Molecular Pharmacology*, 59: 901-908.

Basualdo, C., Sgroy, V., Finola MS, Juam, M. 2007. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Veterinary Microbiology*, 124: 375-381

Beretta, G., Gelmini, F., Lodi, V., Piazzalunga, A. and MaffeiFacino, R. 2010. Profile of nitric oxide (NO) metabolites (nitrate, nitrite and N-nitroso groups) in honeys of different botanical origin: nitrate accumulation as index of origin, quality and of therapeutic opportunities. *Journal of Pharmacy and Biomedicine Annals*, 53: 343-349.

Berhanu, A. 2013. Synergistic antimicrobial effect of tenegn honey (trigona iridipennis) and garlic against standard and clinical pathogenic bacterial isolates. *International Journal of Microbiological Research*, 4(1):16–22.

Bibel, D. J., Miller, S. J., Brown, B. E., Pandey, B. B., Elias, P.M., Shinefield H.M. and Aly R. 1989. Antimicrobial activity of stratum corneum lipids from normal and essential fatty acid-deficient mice. *Dermatology*, 92: 632–638.

Borek, C. 2001. Antioxidant health effects of aged garlic extract. *Journal of Nutrition Science*, 131: 1010-1015.

Burt, S. A.; Reinders, R. D. 2003. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Letters in Applied Microbiology*, 36: 162–167.

Carrasco, FR, Schmidt, G, Romero, AL, Sartoretto, JL, Caparroz-Assef, SM, Bersani-Amado, CA and Cuman, RK. 2009. Immunomodulatory activity of *Zingiber officinale Roscoe*, *Salvia officinalis L*. and *Syzygium aromaticum* L. essential oils: evidence for humor- and cell-mediated responses, *Journal of Pharmacy and Pharmacology*, 61(7): 961–967.

Chorianopoulos, N. G., Giaouris, E. D., Skandamis, P. N., Haroutounian, S. A. and Nychas, G. J. E. 2008. Disinfectant test against monoculture and mixed-culture biofilms composed of technological, spoilage and pathogenic bacteria: bactericidal effect of essential oil and hydrosol of Satureja thymbra and comparison with standard acid base sanitizers. *Journal of Applied Micrbiology*, 104: 1586–1869.

Cowan, MM. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Review*, 12: 564-582.

Daeschel, M. A. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technology*, 43 (1), 164–167.

Daka, D. 2011. Antibacterial effect of garlic (*Allium sativum*) on *Staphyloccus aureus*: An *in vitro* study. *African Journal of Biotechnology*, 10 (4):666- 669.

Demissew, S. and Dagne, E. 2001. Basic and applied research on medicinal plants of Ethiopia. Proceedings of the national workshop on biodiversity conservation and use of medicinal plants in Ethiopia, 29-31.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. 2005. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4: 685-688.

Eja, M.E., Arikpo, G.E., Enyi-Idoh, K.H. and Ikpeme, E.M. 2011. An evaluation of the antimicrobial synergy of Garlic and Utazi (*Gongronema latifolium*) on *Escherichia coli* and *Staphylococcus aureus*. *Malaysian Journal of Microbiology*, 7: 45-49.

Ekweney, U.N. and Elegalan, N.N. 2005. Antibacterial activity of ginger and garlic extracts on *Escherichia coli* and *Salmonella* Typhi. *Journal of Molecular Medicine Advanced Science*, 1(4): 411-416.

El-Hamidi, M. and El-Shami, S.M. 2015. Scavenging activity of different garlic extracts and garlic powder and their antioxidant effect on heated sunflower oil. *American Journal of Food Technology*, 10 (4): 135-146

Ernst, E. and Pittler, M. H. 2008. Efficacy of ginger for nausea and vomiting: a systematic review of randomized clinical trials. *British Journal of Anesthesia*, 367-371.

Fabiola, B.H., Tania, U.B., Diogenes Aparicio, G.C., Jose, A.M. and Celso, V.N. 2003. Effect of essential oil of *Ocimum gratissimum* on the Trypanosomatid.Herpetomonas samuelpessoai. *Protozoological*, 42:269-276.

Fauci, AS. 2001. Infectious diseases: considerations for the 21st century. *Journal of Clinical Infectious Diseases*, 32: 675–685.

Ficker, C., Smith M.L., Akpagana K., Gbeassor M. and Zhang J. 2003. Bioassay guided isolation and identification of antifungal compounds from ginger. *Phytotherapy Research*, 17: 897-902.

Garba, I., Umar, A.I., Abdulrahman, A.B., Tijjani, M.B., Aliyu, M.S., Zango, U.U. and Muhammad, A. 2013. Phytochemical and antibacterial properties of garlic extracts. *Bayero Journal of Pure and Applied Sciences*, 6(2): 45 – 48.

Gheldof, N., Wang, X. and Engeseth, N. 2002. Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agriculture and Food Chemistry*, 50: 5870–5877.

Giday and Ameni. 2003. An ethnobotanical survey on plants of veterinary importance in two woredas of southern Tigray, Northern Ethiopia, Ethiopia. *Journal of Science*, 26(2): 123–136.

Gootz, TD. 2010. The global problem of antibiotic resistance. *Critical Review of Immunology*, 30, 79–93.

Groppo, FC, Ramacciato, JC, Motta, RHL, Ferraresi, PM and Sartoratto, A. 2007. Antimicrobial activity of garlic against oral *streptococci*. *International Journal of Dental Hygiene*, 5:109–115.

Gull, I., Saeed, M., Shaukat, H., Aslam, S.M., Samra, Z.Q. and 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*, 11:8.

Gutierrez, J., Rodriguez, G., Barry-Ryan, C. and Bourke, P. 2008. Efficacy of plant essential oils against foodborne pathogens and spoilage bacteria associated with ready-to-eat

vegetables: Antimicrobial and sensory screening. *Journal of Food Protection*, 71 (9): 1846–1854.

Haniyeh, K., Seyyed, MS. and Hussein, M. 2010. Preliminary study on the antibacterial activity of some medicinal plants of Khuzestan (Iran). *Asian Pacific Journal of Tropical Medicine*, 3: 180-184.

Harris, J. C., Cottrell, L., Plummer, S. and Lloyd D. 2001. Antimicrobial properties of *Allium sativum* (garlic). *Applied Microbiology Biotechnology*, 57:282–286.

Hasan, H., Raauf A. R., Abdrazik, B. and Hassan, B. A. 2012. Chemical Composition and Antimicrobial Activity of the Crude Extracts Isolated from *Zingiber officinale* by Different Solvents. *Pharmaceutica Analytica Acta*, 3(9): 1-5.

Hovana, E.K, James, U.S., James, E., Egbobor, E.M., Egba, A.G.,.Nwachukwu, O.A and Akpama, O.U. 2011. Antibacterial and Phytochemical Studies of *Allium sativum*. *New York Science Journal* 4:123-128.

ICMR Bulletin. 2003. Ginger: It's Role in Xenobiotic Metabolism, 33(6): 57-63 (http://icmr.nic.in/BUJUNEO3nwe.pdf).

Iqbal, S. and Bhanger, M.I. 2007. Stabilization of sunflower oil by garlic extract during accelerated storage. *Food Chemistry*, 100: 246-254.

Isiaka, I.H., Mansur, A. and Bahago, T. 2013. Anti-bacterial potency of fortified and unfortified honey on some clinical bacterial isolates. *IOSR Journal of Pharmacy*, 5: 50-55.

Iwalokun, BA, Ogunledun, A, Ogbolu, DO, Bamiro, SB and Jimi-Omojola, J. 2004. *In vitro* antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and *candida species* from Nigeria. *Journal of Medicinal Food*, 7:327–333.

Kalia, P., Kumar, N. R and Harjai, K. 2012. Phytochemical screening and antibacterial activity of different extracts of propolis. *International Journal of Pharmaceutical and Biological Research*, 3(6): 219-22.

Karaman, I., Sahin, F., Güllüce, M., Ö˘gütçü, H., Sengül M., Adıgüzel A. 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus L*. *Journal of Ethnopharmacology*, 85: 231–235.

Karuppiah, P. and Rajaram, S. 2012. Antibacterial properties of *Allium sativum* (garlic) cloves and *Zingiber officinale* (ginger) rhizomes against multi-drug resistant clinical pathogens. *Asian Pacific Journal of Tropical Biomedicine*, 2(8): 597-601.

Khodavandi, A., Alizadeh, F., Aala, F., Sekawi, Z. and Chong, P. P. 2010. *In vitro* investigation of antifungal activity of allicin alone and in combination with Azoles against *Candida species*. *Biomedical and Life Sciences, Mycopathologia*, 169: 287-295.

Kingsley A. 2001.Supplements. The use of honey in treatment of infected wounds. Case studies. *British Journal of Nursing*, 10(22): Sup 13-Sup 20.

Kumar, G., Karthik, L., and Bhaskara, K.V. 2011. A review on pharmacological and phytochemical properties of Zingiber officinale Roscoe (Zingiberaceae). *Journal of Pharmacy Research*, 4(9):2963–2966.

Kwakman, P.H.S. and Zaat, S.A.J. 2012.Antibacterial Components of Honey. *IUBMB Life*, 64(1): 48–55.

Lusby, PE, Coombes, AL and Wilkinson, JM. 2005. Bactericidal activity of different honeys against pathogenic bacteria. *Archives of Medical Research*, 36: 464–467.

Madureira, A.M., Ramalhete, C., Mulhovo, S., Duarte, A. and Ferreira, M.U. 2012. Antibacterial activity of some African medicinal plants used traditionally against infectious diseases. *Pharmaceutical Biology*, 50(4): 481–489.

Malu, SP, Obochi, GO, Tawo, EN and Nyong, BE, 2008. Antibacterial activity and medicinal properties of ginger (*Zingiber officinale*). *Global Journal of Pure and Applied Science*, 15:365–368.

Manisha, D.M and Shyamapada, M. 2011. Honey: Its antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, 1(2):154-160.

Manyi-Loh, C.E., Clarke, A.M., Mkwetshana, N.F. and Ndip R.N., 2010. Treatment of *Helicobacter pylori* infections: mitigating factors and prospective natural remedies. *African Journal of Biotechnology*, 9(14): 2032–2042

Meriga, B., Mopuri, R. and Murali, K.T. 2012. Insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum*. *Asian Pacific Journal of Tropical Medicine*, 5:391-395.

Mogessie, A. 1994. The *in vitro* antibacterial activity of 'Tazma mar' honey produced by sting less bee. *Ethiopian Journal of Health Development*, 8(1):109–117.

Mohapatra, D. P, Thakur, V. and Brar, S. K. 2010. Antibacterial efficacy of raw and processed honey. *Biotechnology Research International*, 2011: 6pages doi:10.4061/2011/917505

Molan, P.C. and Cooper, R.A. 2000. Honey and sugar as a dressing for wounds and ulcers. *Tropical Doctor*, 30(4): 249-250.

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

Morens, DM, Folkers, GK, Fauci, AS. 2008. Emerging infections: A perpetual challenge. *The Lancet Infectious Disease*, 8(11): 710–719.

Mulvey, MR, Grant, JM, Plewes, K, Roscoe, D and Boyd, DA. 2011. New Delhi metallo-β-lactamase in *Klebsiella pneumonia and Escherichia coli*, Canada. *Emerging Infectious Diseases*, 17: 103-106.

Namias, N. 2003. Honey in the management of infections. *Surgical Infection*, 4(2): 219–226.

Nanasombat, S. and Lohasupthawee, P. 2005. Antibacterial activity of crude ethanolic Extracts and essential oils of spices against *Salmonellae* and other enterobacteria. *KMITL Science and Technology Journal*, 5(3): 527-538.

Ndip, R.N., Malange, A.E., Echakachi, C.M., Malongue, A., Akoachere, J.F., Ndip, L.M. and Luma, H.N. 2007. *In vitro* antimicrobial activity of selected honeys on clinical isolates of Helicobacter pylori. *African journal of Health Sciences*, 7(4): 228–232.

Newman, DJ, Cragg, GM. 2007. Natural products as sources of new drugs over the last 25 years. *Journal of Natural Product*, 70: 461–477.

Nikolić, M., Vasić, S., Đurđević, J., Stefanović, O. and Čomić, L. 2014. Antibacterial and anti-biofilm activity of ginger (*zingiber officinale roscoe*) ethanolic extract. *Kragujevac Journal Science*, 36: 129-136.

Niranjan, K., Sathiyaseelan, V. and Jeyaseelan, E.C. 2013. Screening for anti-microbial and phytochemical properties of different solvents extracts of leafs of *Pongamia Pinnata*. *International Journal of Scientific and Research Publications*, 3: 1-3.

Nzeako and Hamdi. 2000. Antimicrobial potential of honey. *Medical Sciences*, 2: 75-79.

Okigbo, R. N. and Mmeka, E. C. 2008. Antimicrobial effects of three tropical plant extracts on *Staphylococcus aureus, Escherichia coli and Candida albicans*. *African Journal of Traditional, Complementary and Alternative Medicines*, 5 (3): 226 – 229.

Omoya, F. and. Akharaiyi, F. 2011. Mixture of honey and ginger extract for antibacterial assessment on some clinical isolates. *International Journal of Pharmaceutical and Biomedical Research*, 2(1): 39–47.

Onyeagba, R.A., Ugbogu, O.C., Okeke, C.U. and Iroakasi, O. 2004. Studies on the antimicrobial effects of garlic (*Allium sativum Linn*), ginger (*Zingiber officinale Roscoe*) and lime (*Citrus aurantifolia Linn*). *African Journal of Biotechnology*, 3 (10): 552-554.

Otunola, G.A., Oloyede, O.B., Oladiji, A.T. and Afolayan, A.J. 2010. Comparative analysis of the chemical composition of three spices *Allium sativum L., Zingiber officinale Rosc. and Capsicum frutescens L.* commonly consumed in Nigeria. *African Journal of Biotechnology*, 9(41): 6927–6931.

Palaksha, M.N., Ahmed, M. and Das, S. 2010. Antibacterial activity of garlic extracts on streptomycin-resistant *Staphylococcus aureus* and *Escherichia coli* solely and in synergism with streptomycin. *Journal of Natural Science, Biology and Medicine*, 1(1):12-15.

Patel, R.V., Thaker, V.T., 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*, S58-S61.

Portoi, G., Ching, L., Karimi-Tabesh, K., Korem, G., Tan, M.P. 2003. Prospective comparative study of the safety and effectiveness of ginger for the treatment of Nausea and vomiting in pregnancy. *American Journal of Obstetrics Gynecology*, 189(5): 1374- 1377.

Premkishore, K., Umapathy, T., Kathariya, M.D., Agrawal, A., Kumar, P.S.P and Kallampilly. G. 2013. Effect of Honey and aqueous Ginger extract against *Streptococcus mutans* isolated from extracted Carious Deciduous Teeth, *Journal of Indian Academy of Oral Medicine and Radiology*, 25(4): 265-267.

Rahmani Arshad, H., Al shabrmi Fahad, M. and Aly Salah, M. 2014. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. *International Journal of Physiology, Pathophysiology and Pharmacology*, 6(2): 125-136.

Rashmi, C., Vinay, D., Kumar, S., Abhimanyu, K. J. 2011. Detection of antimicrobial activity of *Oscimum sanctum* (Tulsi) and *Trigonella foenum graecum* (Methi) against some selected bacterial and fungal strains. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2(4): 809-813.

Saad, B. and Mona O. 2012. Antimicrobial activity of onion juice *(Allium cepa*), honey and onion-honey mixture on some sensitive and multi-resistant microorganisms. *Life Science Journal*, 9(2): 775-780.

Saad, B. and Mona O. 2013. Antimicrobial activity of garlic juice (*Allium sativum*), honey, and garlic -honey mixture on some sensetive and multiresistant microorganisms. *Life Science Journal*, 10(4): 2429- 2435.

Sasidharan, I. and Menon, A. N. 2010. Comparative chemical composition and antimicrobial activity fresh and dry ginger oils (*Zingiber officinale Roscoe*). *International Journal of Current Pharmacology Research*, 2(4): 40–43.

Sebiomo, A., Awofodu, A. D., Awosanya, A. O., Awotona, F. E. and Ajayi, A. J. 2011. Comparative studies of antibacterial effect of some antibiotics and ginger (*Zingiber officinale*) on two pathogenic bacteria. *Journal of Microbiology and Antimicrobials*, 3(1): 18-22.

Selcuk, H. and Nevin, K. 2002. Investigation of antimicrobial effect of honey collected from various regions of Turkey. *Pakistan Journal of biological sciences*, 5(3): 325-328.

Shears, P. 2000. Antimicrobial resistance in the tropics. *Tropical Doctor*, 30(2): 114-116.

Shenkute, Z. 2008. Personal interview with HMC Pharmacy Services pharmacist on topic of Ethiopian traditional medicine use in immigrant patients. Harborview Medical Center, Seattle, WA.

Shobana, S., Vidhya, V.G. and Ramya, M. 2009. Antibacterial activity of garlic varieties (ophioscordon and sativum) on enteric pathogens. *Current Research Journal of Biological Sciences*, 1(3): 123-126.

Subrahmanyam and Jibril, O. 2001. Antibacterial activity of honey on gram positive and gram negative bacterial. *Classification of Bacteria*, 223: 45-47.

Tagoe, D. and Gbadago, F. 2009. A comparison of the antimicrobial effectiveness of aqueous extracts of garlic, ginger and lime and two conventional antibiotics on *Escherichia coli, Salmonella species*, *Shigella species* and *Bacillus cereus*. *The Internet Journal of Microbiology*, 8 (2): 8390–839.

Taura, D.W, Yusha'u, M., Bello, U.A, Hassan, A., Saidu, J. and Panda, T.W. 2014. Antibacterial activity of *Psidium guajava* in clinical isolates. *Academia Journal of Microbiology Research*, 2(2): 079-083.

Tefere, B. 1996. Evaluation of herbal remedies with modern pharmaceuticals. Proceedings of the workshop on development and utilization of herbal remedies in Ethiopia, Nazereth: 19-25.

Temaru, E., Shimura, S., Amano, K., Karasama, T. 2007. Antimicrobial activity of honey from stingless honeybees (*Hymenopetra Apidae*; *Meliponinae*). *Polish Journal of Microbiology*, 56(4): 281–285.

Vallianou, N.G., Gounari, P., Skourtis, A., Panagos, J., Kazazis, C. 2014. Honey and its anti-inflammatory, anti-bacterial and anti-oxidant properties. *Gen Med (Los Angel) icine*, 2: 2-7.

Victor, I. U., Igeleke, C. L. 2012. Antimicrobial properties of the extracts of locally sold garlic and neem leaf in Benin City, Nigeria. *International Journal of Biosciences*, 2: 21-27.

Wainwright, M. 1989. Moulds in ancient and more recent medicine. *Mycologist*, 3 (1): 21–23.

WHO. 1999. Drug information. *WHO, Geneva*, 3(4):230-233.

WHO. 2000. General Guidelines for methodologies on research and evaluation of traditional medicine. *WHO/EDM/TRM*, 1: 1-8.

WHO. 2002. Traditional and alternative medicine, Fact Sheet 271, *World Health Organization*, Geneva http://www.who.int/mediacentre/news/releases/release38/en/.

Willix , D.J., Molan, P.C. and Harfoot, C.G., 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.

Yabaya, A., Orukotan, A. and Jonathan, M. 2010. Determination of anti-salmonella Typhi activity of the crude extract of *Allium sativum* (garlic). *Journal of Biological Sciences and Bioconservation*, 2: 22-28.

Yahaya, O., Yabefa, J.A., Umar, I.O., Datshen, M.M, Egbunu, Z.K. and Ameh, J. 2012. Combine Antimicrobial Effect of Ginger and Honey on Some Human Pathogens. *British Journal of Pharmacology and Toxicology*, 3(5): 237-239.

Yalemwork Ewnetu, Wossenseged Lemma and Nega Birhane. 2014. Synergetic antimicrobial effects of mixtures of Ethiopian honeys and ginger powder extracts on standard and resistant clinical bacteria isolates. *Evidence Based Complementary and Alternative Medicine*. 2014: 8 pages http://dx.doi.org/10.1155/2014/562804

Yassen, D. and Ammal Esmaeel Ibrahim. 2016. Antibacterial activity of crude extracts of ginger (*Zingiber officinale Roscoe*) on *Escherichia coli* and *Staphylococcus aureus*: A Study *in vitro*. *Indo American Journal of Pharmaceutical Research*, 6(06): 5830-35

Yisehak, T. R, Eyosyas, K., Cruz, C., Getachew, G., Nesibu, A. and Brahan, M. 2014. Potential antibacterial activity of crude extracts from *Aloe vera*, *Zingiber officinale* and *Vinca major*medicinal plants. *International Journal of Microbiological Research*, 5 (3): 202-207.

Zakia Metwali, Nihal Abdalla and Reem El Barrani. 2014. A Natural alternative to conventional antibiotics. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 5(4): 588-598.

8. APPENDIX

Appendix Table 1. ANOVA values for the comparison of the antimicrobial activities of crude extracts of honey, garlic, ginger and their combinations using different solvents against the test pathogens

Test pathogen	Crude extracts	DF	SS	MS	F-value	Sig (P-value)
Shigella	Garlic	2	6.22	3.111	7	0.027
Boydii	Honey-garlic	2	2	1.00	1.00	0.422
	Garlic-ginger	2	20.222	10.111	13.00	0.007
	Honey-garlic-ginger	2	176.889	88.444	199.00	0.000
Staphylococcus	Garlic	2	14.889	7.444	13.4	0.006
Auerus	Honey-garlic	2	1.556	0.778	0.875	0.464
	Garlic-ginger	2	6.222	3.111	5.6	0.042
	Honey-garlic-ginger	2	128	64	96	0.000
Salmonella	Garlic	2	13.556	6.778	6.1	0.036
Typhimurium	Ginger	2	186.889	93.444	841	0.000
	Honey-garlic	2	8.222	4.111	5.286	0.047
	Garlic-ginger	2	8.667	4.333	4.875	0.055
	Honey-garlic-ginger	2	2.889	1.444	3.28	0.111
Pseudomonas	Honey	2	107.556	53.778	484	0.000
Aeruginosa	Ginger	2	138.889	69.444	625	0.000
	Honey-garlic	2	162	81	243	0.000
	Honey-ginger	2	138.889	69.444	625	0.000
	Garlic-ginger	2	128	64	625	-
	Honey-garlic-ginger	2	117.556	58.778	529	0.000
Klebsiella	Garlic	2	1.556	0.778	1.750	0.252
pneumoniae	Honey-garlic	2	2.667	1.333	2.4	0.171
	Garlic-ginger	2	0.667	0.333	0.6	0.579
	Honey-garlic-ginger	2	139.556	69.778	628	0.000

DF- degree of freedom, MS mean square, SS sum of square

Appendix Table 2. Broth dilution of honey extracts against test pathogens

Test pathogen	Different concentration of the crude extracts (mg/ml)														
	Aqueous extract					Methanolic extract					Ethanolic extracts				
	500	250	125	62.5	31.25	500	250	125	62.5	31.25	500	250	125	62.5	31.25
Shigella boydii	-	-	-	+	+	-	-	-	+	+	-	-	-	-	+
Staphylococcus aureus	-	-	+	+	+	-	-	-	+	+	-	-	-	+	+
S. Typhimurium	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
P. aeruginosa	-	-	-	+	+	-	-	-	+	+					
K. pneumoniae	-	-	+	+	+	-	-	-	+	+	-	-	-	+	+

(-) there is no growth (+) there is growth

Appendix Table 3. Broth dilution of crude extract of garlic against *Pseudomonas aeruginosa*

Concentration of the crude extract (mg/ml)	Growth of *Pseudomonas aeruginosa*		
	Aqueous extract	Methanolic extract	Ethanolic extracts
500	-	-	-
250	-	-	-
125	+	+	-

62.5	+	+	+
31.25	+	+	+

(-) there is no growth (+) there is growth

Appendix Table 4. Broth dilution of ginger extracts against the test pathogens

Test pathogen	Different concentration of the crude extracts (mg/ml)														
	Aqueous extract					Methanolic extract					Ethanolic extracts				
	500	250	125	62.5	31.25	500	250	125	62.5	31.25	500	250	125	62.5	31.25
S. boydii	+	+	+	+	+	-	-	-	+	+	-	-	-	+	+
S. aureus	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+
S. Typhimurium	+	+	+	+	+	-	-	+	+	+					
P. aeruginosa	+	+	+	+	+	-	-	+	+	+					
K.pneumoniae	+	+	+	+	+	-	-	+	+	+	-	-	-	+	+

(-) there is no growth (+) there is growth

Appendix Table 5. Broth dilutions of combined crude extract against the test pathogens

Test pathogens	Solvent	Different concentration of the crude extracts (mg/ml)																			
		Honey-garlic					Honey-ginger					Garlic-ginger					Honey-garlic-ginger				
		500	250	125	62.5	31.25	500	250	125	62.5	31.25	500	250	125	62.5	31.25	500	250	125	62.5	31.25

Shigella boydii	W	-	-	+	+	+									
	M	-	-	-	+	+				-	-	-	-	+	
	Et	-	-	-	-	+									
Staphylococcus aureus	W	-	-	+	+	+									
	M	-	-	-	+	+				-	-	-	+	+	
	Et	-	-	-	+	+									

Salmonella Typhimurium	W						-	-	+	+	+										
	M						-	-	-	+	+										
	Et						-	-	-	+	+										
Pseudomonas aeruginosa	W	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	-	-	-	+	+
	M	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
Klebsiella pneumoniae	W						-	-	+	+	+										
	M						-	-	-	+	+						-	-	-	+	+
	Et						-	-	-	+	+										

NB: (-) there is no growth, (+) there is growth, (W) water, (M) methanol, (Et) ethanol

Appendix Table 6. Growth of colony from broth dilution with MIC on MHA for MBC determination

Crude extract concentration (mg/ml)	Growth of colony on MHA from broth dilutions with MIC				
	Shigella boydii	*Staphylococcus Auerus*	*Salmonella* Typhimurium	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
500	-	-	-	-	-
125	-	-	-	-	-
62.5	-	-	-	-	-
31.25	-	-	-	+	-
15.625	-	+	+	+	+
7.81	+	+	+		
3.90			+		

NB: (-) there is no growth (+) there is growth