EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF TANZANIAN HONEY FROM TWO AGRO-ECOLOGICAL ZONES

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A DISSERTATION SUBMITTED IN PARTIAL FULLFILMENT OF THE REQUIREMENT OF THE DEGREE OF MASTER OF SCIENCE IN FOOD QUALITY AND SAFETY ASSURANCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2016

ABSTRACT

A study was conducted to assess the quality of Tanzanian honey based on antioxidant properties and antimicrobial activities on bacteria and fungi in relation to its physico-chemical properties. Two types of honey samples (stinging and stingless bees' honey) were collected from central zone (Singida and Dodoma) and western zone (Tabora and Shinyanga). Honey samples were analysed for anti-microbial activities, anti-oxidant properties (total phenols, vitamin C) and physico-chemical properties pH, colour, pH, reducing sugar and minerals. Fifty four percent of tested honey samples inhibited microbial growth while 46% did not. The microbial inhibition zone ranged from 8.5 - 14.16 mm (stinging bee honey) and 10.56-15.13 mm (stingless bee honey). The stingless honey from Shinyanga town, Bukombe and Nzega and that from Singida town and Issuna were more effective in microbial growth inhibition. *Candida albicans* was more sensitive (23.1%) to stingless bee honey, followed by *Staphylococcus saprophyticus* (33%), *Salmonella typhi* (32%), *Escherichia coli* (19.2%) and *Aspergillus flavus* (16.0%). Antioxidant, total phenols and vitamin C content were significantly ($P<0.05$) higher in stingless bee honey than that of stinging bee honey. Antioxidant (FRAP) ranged from 322.16 - 973.57 µMFe(II)/100g, total phenol 13.87 – 33.55 mg/100g and vitamin C 2.54 - 10.99 mg/100. There was no significant ($P>0.05$) difference in pH between honey types. Potassium was highest while Zn was lowest in stinging and stingless honey samples. Colour grading of stingless bee honey ranged from extra light amber - light amber while that of stinging bee honey ranged from water white - light amber. There was a positive and significant ($P<0.001$) correlation between honey colour, antioxidant,

total phenol and mineral content. Stingless honey from western zone is superior to stinging bee honey in antioxidant and antimicrobial properties and therefore is recommended to be used as a valuable food product, preventive and curative medicine.

DECLARATION

I Flora Thomas Luvanda do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently in any other institution.

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ACKNOWLEDGEMENTS

First and foremost thanks go to Almighty God whose power makes me to be whom I am. Sincere thanks are due to my supervisors Prof. Monica Lyimo and Dr. Lucy Mlipano Chove, for their guidance, constructive criticisms, suggestions and diligent efforts that made this study possible.

I wish to express my sincere thanks and appreciation to my sponsor Tanzania Bureau of Standards (TBS) who granted me financial and moral support and study leave without which this study could not be possible.

My heartfelt thanks should go to Dr. Victor Kakengi who devoted his time assisting in data analysis and interpretation which is a core part of this dissertation. My sincere thanks are due to Sokoine University of Agriculture (SUA) for offering me an opportunity to pursue the course.

Special thanks should go to Mr. Phelemon Mkuchu, Mr. Sterwart. Mwanyika and Ms. Rose Shao for their tireless technical support, advice and encouragement during laboratory research period. Also, I highly appreciate my classmates especially, Salama Shekilango, Jamila Omary, Reward Mrema, Amina Yasin, Mahamud Sasamalo and Mwanahamisi Msangi who were always there for me whenever things became tough, during all the time from the course work to the completion of my research work. To those I did not mention I am immensely indebted for their help in and outside of the study, thank you and May the Almighty God bless you abundantly.

DEDICATION

This work is dedicated to my God who granted me the guidance, his grace and blessing to accomplish this work. To my God be glory. My beloved parents Mr. Thomas Zabron Luvanda and Late Mariam Luvanda who laid the foundation of my education, brothers Erick Luvanda, Collinely Luvanda and Frankline Luvanda, and finally my beloved children Moses, Mary, Amani and Baraka whose support, prayers and encouragement gave me the enthusiasm to accomplish this study.

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LIST OF ABBREVIATIONS AND ACRONYMS

AOAC	Association of Official Analytical Chemists
CAC	Codex Standard for Honey
Ca	Calcium
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
GAE	Gallic Acid Equivalent
HDL	High Density Lipoproteins
HMF	Hydromethyl Furfural
K	Potassium
LDL	Low Density Lipoproteins
Mg	Magnesium
N	Overall sample size
n	Number of observations
SUA	Sokoine University of Agriculture
SW	Sample Weight
TP	Total Phenol
UTI	Urinary Tract Infections
URT	United Republic of Tanzania
WHO	World Health Organisation
Zn	Zinc
+Ve	Positive
-Ve	Negative

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Honey is an organic, natural sweet substance, produced from nectar and saccharine exudation of plants by honey bees known as *Apis mellifera* and *Trigona meliponini* (Codex Standard, 2001). Nectar of flowers is gathered, modified, stored in the comb and transformed by honey bees into honey by process of regurgitation and evaporation (Stefan, 2009).

Honey is a natural product with many qualities that are beneficial for human beings. It is considered to be important in human nutrition and health (Alis *et al*., 2012). For thousands of years (since 2100 BC), ancient Greeks used honey as traditional food and healing agent (Alisi *et al*., 2012). Its properties make it potential to serve as a natural food with antioxidants and antimicrobial, which are responsible for its medicinal properties. It has powerful immune system booster and carbohydrates that provide strength and energy to the body. Presence of enzymes in honey helps to improve digestive system, and it decreases muscle fatigue of the body (Rodriguez, 2004).

Different types of honey have different composition depending on the type of honey bees, geographical origin and maturity property of honey before harvest (Alvarez *et al*., 2010). Honey contain carbohydrates, enzymes, hydromethyl furfural (HMF), proteins, minerals, amino acids, vitamins and antioxidants including phenolics and flavonoids (Candiracci *et al*., 2012). Its health benefit is associated with presence of these valuable nutrients and phytochemicals.

The most honey producing and consuming countries in the world are China, Turkey, Argentina and Mexico (FAOSTAT, 2014). Tanzania has potential of producing 138,000 metric tons of honey annually (URT, 2003). Almost all regions are involved in beekeeping and honey production. The main beekeeping and honey production regions are Western zone (Tabora, Shinyanga, Rukwa and Kigoma), Southern Zone (Lindi, Mbeya, Iringa and Ruvuma), Central Zone (Singida and Dodoma), Eastern Zone (Morogoro, Dar es Salaam and Coast) and North Eastern Zone (Manyara, Arusha, Tanga and Kilimanjaro) (URT, 2003). In Tanzania, honey is produced from different sources, including plants (perennial plants, fruits, sunflower, palm oil, rice, maize, sorghum) soil, and different geographical origins such as arid, wet or coast (URT, 1998). Tanzania has a tropical climate with regional variation due to topography (landscape of the country). The temperature is high in the mainland, tropical in the coastal regions and in the islands. The great climate variation in Tanzania results to variation in flora and fauna of Tanzania.

Currently consumption of honey is increasing because of its beneficial biological and physical chemical properties, including antioxidant and antibacterial activities. More than half of the honey produced in the country is consumed locally as food and medicine, while the rest is sold to other countries particularly European Union member countries as UK, Netherlands and German. Other countries are Oman, United Arab Emirates, Iran, Uganda, Kenya, and Rwanda (Mwakatobe and Mlingwa, 2014).

1.2 Problem Statement and Justification

Honey has a potent activity against bacteria and fungi. This potency is attributed by its biological properties as antioxidant properties, antibacterial activities and physico-chemical characteristics. Various bacteria including *Escherichia Coli*, *Salmonella typhi*, *Staphylococcus saprophyticus*, and fungi such as *Aspergillus flavus*, and *Candida albicans* are detrimental to health and food as they can cause diseases, food spoilage and food poisoning (Agrios, 2005). *Escherichia coli* cause sickness, food poisoning and are potential indicator organisms to test environmental hygiene for contamination (Feng *et al*., 2002). Bacteria such as *Salmonella typhi* cause illnesses such as typhoid fever, and food poisoning (Ryan and Ray, 2004). *Staphylococcus saprophyticus* is a common cause of Urinary Tract Infections (UTI) and food poisoning (Ryan and Ray, 2004). In the same way fungi including *Aspergillus flavus* cause diseases in many crops such as cereal grains, legumes, and tree nuts. Many strains produce toxic compounds known as mycotoxins (aflatoxin) which when consumed, are toxic to mammals (Agrios, 2005). *Candida albicans* is a causal agent of oral and genital infections in humans. All these pathogens, cause food borne diseases have become a cause of major health concerns. Alternative use of natural food products such as honey with biological properties can help in suppression and prevention of these pathogenic microorganisms.

Since the composition of honey varies with the agroecological zones, types of honey and procedures used for harvesting, handling and storage, it is likely that Tanzanian honey could have unique characteristics, differing from all other countries. Although, honey has been documented to possess potential medicinal properties worldwide, available studies in Tanzania have reported on physico-chemical properties (Gidamis

et al., 2004; Masoud, 2014). The same authors recommended further studies on antimicrobial and antioxidant activities of Tanzanian honey. Therefore, the objective of this study was to investigate on antioxidant and antimicrobial properties of two types of raw honey (from stingless and stinging bees' honey) selected from two zones of Tanzania. Information obtained will assist in understanding the quality of Tanzanian honey as a valuable food product attributed by antioxidant and antimicrobial properties.

1.3 Objectives of the Study

1.3.1 General objective

Assessment of the quality of Tanzanian honey based on antioxidant and antimicrobial activities in relation to its physico-chemical properties.

1.3.2 Specific objectives

1. To determine antimicrobial activities of bacteria (*Staphylococcus saprophyticus*, *Escherichia coli* and *Salmonella typhi*) and fungi (*Aspergillus flavus* and *Candida albicans*) on raw honey from stinging and stingless bees honey.
2. To analyse the physico-chemical properties (pH, reducing sugar and minerals) of raw honey from stinging and stingless bees honey.
3. To examine the antioxidant activities (total phenol and vitamin C) of raw honey from stinging and stingless bees honey.
4. To correlate the physicochemical properties of honey to the antioxidant and antimicrobial properties of raw Tanzanian honey from stinging and stingless bees honey

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction to Honey

Honey has been used for as long as medicine. The ancient Sumerians, Babylonians, and Egyptians all recorded its use, e.g., in Sumerian clay tablets estimated to be 4,000 years old. The ancient Egyptians used honey in embalming and made salves with it for treating diseases of the eye and skin, honey was used as a drug more than a nutrient (Singh *et al*., 2012). There are over 300 types of floral honey available in the marketplaces of the world.

The color, flavor, mineral and vitamin content of honey depend on the flower from which bees gather the nectar. In modern medicine, honey has been used for the treatment of respiratory disease, urinary disease, gastrointestinal disease, skin ulcers, wounds, eczema, psoriasis, dandruff, diaper dermatitis, and radiation mucositis (Kumar *et al*., 2010). Laboratory studies and clinical trials have shown that honey has a broad-spectrum antimicrobial activity (Osho and Bello, 2010), and increases antibody production against thymus-dependent and thymus-independent antigens (Tonks *et al*., 2007). Consumption of honey decreases the concentrations of prostaglandins in the plasma of normal individuals (Molan, 2001), and increases antioxidant agents, serum iron, and blood indices (Gheldof *et al*., 2003). Honey increases insulin secretion and decreases blood glucose levels (Erejuwa *et al*., 2012).

2.1.1 Honey types, content and source of variation between honey

Stinging honey bees (Apis mellifera) and stingless honey bees (Trigona meliponini) produce honey which is considered to be important in both treatment and nutritional purposes. Since 2100 BC ancient cultures used it as medicine (Alvarez, *et al.*, 2010).

Honey is characterised by its complex composition, which varies with the origin of the raw material as nectar or honeydew, bee species, climatic conditions, the available floral source and the storage conditions (Gheldof and Engeseth, 2002). Honey mainly consists of glucose and fructose but also contains amino acids, phenolic compounds, organic acids, vitamins, minerals, lipids, enzymes and other phytochemicals (Singh *et al*., 2012).

Stingless bees, sometimes called stingless honey bees or simply meliponines, are a large group of bees (about 500 species), comprising the tribe "Meliponini". They belong to the family "*Apidae*", and are closely related to common honey bees, carpenter bees, orchid bees, and bumblebees (Michener, 2007). Meliponines have stingers, but they are highly reduced and cannot be used for defense. Stingless and stinging honey bees are found in most tropical or subtropical regions of the world, such as Australia, Africa, Southeast Asia, and tropical America (Roubik, 1989). They are also quite diverse in Africa, including Tanzania (Eardley *et al*., 2009). The meliponines varies in color and appearance and they are small about 5mm (Roubik, 2006). Stingless bees produce honey (stingless honey) about 300 grams to 1 kg in a year. However, this depends on how big the colony is and nectar available locally. Most part of tropical area and South Americans consider this honey as medicine (Roubik, 2006).

Stingless bee honey is not very sweet and has a pleasant taste and aroma, compared to large bee honey which is very sweet. Stingless bee honey is medicinal because of the flowers and trees it visits (Parmas *et al*., 2000). Stingless bees don't mix pollen with honey. Large bees mix honey and pollen together (bee bread). Pollen produces enzymes that bring about chemical changes under mild conditions. This might

account for the difference in the two honey types. Stingless bee honey has higher water content, from 25 to 35% as compared to the honey from the genus *Apis* (larger bees) whose honey consists of about 20% or less water (Cortopasssi *et al*., 2006). This contributes to its less cloying taste, but also causes it to spoil more easily. If care is not taken stingless bee honey will spoil at room temperature. Traditionally people added two or three white pepper seeds to preserve it. Stingless bee's honey colour is mostly in shades of amber color whereas large bees honey comes in many varieties of colors (White, 2007).

2.2 Properties of Honey

2.2.1 Antimicrobial properties of honey and honey products

Honey has several antimicrobial systems (Cooper, 2007). Manuel *et al.* (2011) working on honey and bee hives related substances reported the use of honey and its products as antiseptic agents (example propolis). Manning (2000) revealed distinctive fatty acids profiles in pollen which are characteristically dominant in one or more fatty acids. The same author reported that pollens with high lipid concentrations are dominated by linoic, linolenic, myristic and dodecanoic acids which probably play a significant role in inhibiting the growth of the spore forming bacterial such as *Paenibacillus larvae* (American foulbrood), *Melissococcus pluton* (European foulbrood) and other microbes that inhibit the brood combs of beehives. Presence of antibacterial and antioxidant properties component in honey is what makes honey to be important as medicine (Stefan, 2009).

Various properties have been proposed to explain the effect of honey against bacterial growth; i) Presence of hydrogen peroxide resulting from the action of the glucose

oxidase enzyme (produced from hypopharyngeal glands of worker bees) on glucose in presence of oxygen inhibits microbial and fungal growth (Garcia *et al*., 1986) ; Wahdan, 1998; Molan, 1999a and Khan *et al*.,2007) ii) Inherent physico-chemical properties such as its high sugar content (Approximately 80% w/w) that can produce a high osmotic effect that dehydrate micro-organisms and its acid (pH of 3 – 4.5) (Molan, 1992;Bogdanov *et al*., 1997). iii) The presence of diverse organic acids including gluconic acids which remarkably creates an acidic micro environment and whose concentration varies considerably from one type of honey to another (White, 1978; Aparna and Rajalakshmj, 1999). The acidic environment prevents the growth of many micro-organisms. iv) Non-peroxidic substances such as polyphenols which possess anti-microbial activity (Cabrera *et al*., 2006). These compounds vary depending on plant species from which the bee gather their nectar (Cooper, 2007).

The enzyme is virtually inactive in honey itself, but in honey diluted with water it breaks down glucose to produce hydrogen peroxide and gluconic acid continuously. The Hydrogen peroxide produced by the reaction is microbicidal (Eva, 1999). Research on antibacterial activities of natural unprocessed honey revealed that honey has broad spectrum antibacterial activity when tested against pathogenic and food spoilage bacterial which showed higher antimicrobial activity (Irish *et al*., 2008). Antimicrobial properties of honeys from Mauritius were evaluated against clinical bacteria (*Escherichia coli Staphylococcus aureus*) and fungal (*Aspergillus niger* and *Candida albicans*) showed inhibition zones to range from 6 to 20 mm for undiluted honey samples, however, the fungus used was found to be more resistant than the bacteria (Mohammad *et al.,* 2012). Honey from Manuka in Newzeland has been reported to show antimicrobial activities against pathogenic bacteria (Cursons, 2010).

2.2.1.1 Bacteria and Fungi

(i) *Escherichia coli*

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped bacterium commonly found in the environment, foods, and the lower intestine of warm-blooded organisms (endotherms) people and animals (Vogt and Dippold, 2005). *Escherichia coli* is a potential indicator organisms to test environmental hygiene for contamination (Feng *et al*., 2002), and a cause of food poisoning (Center for Disease control and Prevention CDC, 2012). According to Shyamapada *et al*. (2010) honey showed excellent antimicrobial activity against *E. coli* and *Salmonella typhi*, respectively related to the urinary tract infection and enteric fever among human patients and thus the honey must be considered against such common infection.

(ii) *Salmonella typhi*

Salmonella typhi are found in both cold-blooded and warm-blooded animals, and in the environment. They cause illnesses such as typhoid fever, and food poisoning (Ryan and Ray 2004). Typhoid fever is a major cause of morbidity and mortality in the developing world. Report from World Health Organization (WHO, 2005) showed that 21 million cases of typhoid occur globally every year and over 200,000 people die each year, most of them at a very young age.

(iii) *Staphylococcus saprophyticus*

Staphylococcus saprophyticus is a gram-positive bacteria, found in the normal flora of the female genital tract. Is a common cause of Urinary Tract Infections (UTI) and food poisoning (Vogt and Dippold, 2005). French *et al*. (2005) reported that the growth of staphylococcus isolates was inhibited by Manuka honey at concentrations of 2.7-5% (v/v).

(iv) ***Aspergillus flavus***

Fungi including *Aspergillus flavus* is a saprotrophic and pathogenic microorganism (Masayuki and Katsuyai, 2010). It is found globally as a saprophyte in soils and causes disease on many crops such as cereal grains, legumes, and tree nuts. Many strains produce significant quantities of toxic compounds known as mycotoxins (aflatoxin) which when consumed, are toxic to mammals (Agrios, 2005). The pathogen can cause liver cancer through consumption of contaminated food or aspergillosis through invasive growth (Amaike *et al*., 2011). *Aspergillus flavus* is also an opportunistic human and animal pathogen, causing aspergillosis in immuno-compromised individuals (Amaike and Nancy, 2011).

(v) ***Candida albicans***

Candida albicans is a diploid toadstool that grows both as yeast and filamentous cells and is a constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestinal tract (Ryan and Ray, 2004). It is a causal agent of opportunistic oral and genital infections in human, an infection of the nail plate. Overgrowth of the fungus results in candidiasis (candidosis), which is often observed in immuno-compromised individuals such as HIV-infected patients (Schramm *et al*., 2003). Systemic fungal infections including those caused by *C. albicans* have emerged as important causes of morbidity and mortality in immuno-compromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation) (Enfert and Hube, 2007).

Since all these pathogens, cause diseases they have become a cause of major health concerns. Alternative use of natural food products such as honey with biological properties can help in suppression and prevention of these pathogenic microorganisms.

2.2.2 Antioxidant properties of honey

Antioxidant is a molecule that inhibits the chemical reaction involving the loss of electrons or an increase in oxidation state (oxidation reaction) and produce free radicals which can initiate chain reactions (Saratupa and Ganguly, 2014). The occurrence of chain reaction in a cell, causes cell damage or death by altering the oxidative state of certain components such as DNA and proteins that result in altered conformation (Inoue *et al.*, 2005). Antioxidants remove free radical intermediates by terminating the chain reactions and thus inhibiting other oxidation reactions. Free radicals are reactive molecules that arise from the products of the oxidation/reduction of oxygen and hydrogen (Aljadi and Kamaruddin, 2004).

Antioxidant properties in honey is a combination of a wide range of active substances. This activity may be due to the presence of various types of polyphenols. Various fruits, vegetables and food products such as honey contain phenolic acids and flavonoids which are considered as antioxidants (Gheldof *et al*., 2002). The antioxidant property of honey is potential to serve as natural food antioxidant as it can prevent the deteriorative oxidation reaction in foods, also the presence of chemical compounds such as flavonoids and phenolics can prevent the interaction between bacteria and food matrix hence, decreasing the food spoilage. Examples include, enzymatic browning reaction of fruits and vegetables and lipid oxidation in meat (Molan, 1992). Also antioxidants can act on scavenging of free radicals, such as hydroxyl and superoxide (Al- Mamary *et al.,* 2002). Analysis of samples from seven countries of central and southern region of Amazon states in Brazil showed higher antioxidant activity in samples that contained higher quantities of phenolic compounds (Isnandia *et al*., 2013). Antioxidants that naturally occur in honey may

contribute to antioxidant capacity. Antioxidant capacity is the ability and potential of honey in reducing oxidative reactions within food systems and human health. These oxidative reactions can cause adverse health effects such as chronic diseases and cancers (Gheldof *et al*., 2002). Compounds responsible to block these oxidative reactions are flavonoids, phenolic acids, ascorbic acid and organic acids as well as some enzymes including glucose oxidase and catalase (Gheldof *et al*., 2002). According to Manuela *et al*., (2006) polyphenols such as flavonoids and phenolic acids may function as antioxidants in honey. Not only could honey's antioxidants help to eliminate free radicals in the body but also part of the nutrient supply for growth of new tissues (Sham, 2010). It has been demonstrated that honey is similar in antioxidant capacity to many fruits and vegetables on a fresh-weight basis, as measured by the assay of absorbance capacity of oxygen radicals (Schramn *et al.,* 2003).

The antioxidant properties in honey have a beneficial effect on human health (Krystyna and Magdalena, 2009). It has been reported that free radicals and reactive oxygen species (ROS) such as hydroperoxyl, hydroxyl and superoxide are associated with aging and disease mechanisms (Krystyna and Magdalena, 2009). Reactive oxygen species can produce oxidative damage in biomolecules such as carbohydrates, lipids and nucleic acids which can alter various cell processes and cause cell death (Viudal *et al*., 2008). Several studies have found that the consumption of honey can improve the defense against oxidative stress (Al-Mamary *et al.,* 2002; Beretta *et al*., 2007). This function has been attributed to the natural phenolic compounds in honey (Van der Berg *et al*., 2008). These phytochemicals appear to exert their anti-oxidant capacities mainly through the decrease and removal

of reactive oxygen species (ROS) thus, diminishing the risk of pathologies and damage produced by free radicals (Beretta *et al*., 2007).

2.2.3 Phenols

Honey is rich in phenolic acids and flavonoids, which exhibit a wide range of biological effects and act as natural antioxidants. Phenolic compounds or polyphenols are one of the most important groups of compounds occurring in plants, in which they are widely distributed (Giorgi *et al*., 2011). The presence of phenols was suggested to contribute to the antioxidant and antimicrobial properties of honey. Montenegro *et al.* (2009) revealed that honey from unifloral that contain several natural phenolic compounds and show a broad spectrum of antibacterial and ant-fungal activities. The phenolic compounds commonly found in honey include phenolic acids, flavonoids and polyphenols. Honey with dark color has a higher total phenolic content and consequently a higher antioxidant capacity. Several works (Pia, 2010; Giorgi *et al.,* 2011) have reported that the antioxidant activity of many compounds including honey is proportional to the phenolic content.

The content of polyphenolic compounds (e.g., flavonoids and phenolic acids) in honey is strongly affected by floral and geographical origin as well as climate characteristic of the site. Isnandia *et al.* (2013) reported a range of total phenolic content of extract stingless bee honey from Amazonas to be 17 to 66 mg GAE/g (Gallic acid equivalent –GAE). Alijad and Kumardin (2004) evaluated the anti-oxidant of Malaysian honey originating from gelam and coconuts and its relationship to total phenols using Ciocalteau protocol reported a mean content of phenols in gelam honey of 21.4mg/g and 15.6mg/g in coconuts. Aline *et al*. (2005) quoted by

Syaliza *et al*. (2009), analysed 27 honey samples from 18 multifloral, 2 honeydews and 7 unifloral honeys in Burkina Faso using Folin- Ciocalteu method reported total phenols varied from 32.59 to 114.5 mg GAE/100g. Also Lihu *et al.,* (2005) working on several honey types to determine the potential for floral authentication using extraction and high pressure liquid chromatography (HPLC) method found the total phenolic contents range from 2.13mg/100g to 12.11 mg/100g of honey. In Yemen, Al-Mammary *et al*. (2002) reported a total phenolic content of diluted Yemen honey to vary from 56.32 to 246.21 mg/100g of honey as Catechin equivalents using Folin-Ciocalteu method. There is a variation in total phenol content in honey due to difference in floral types and method used (Pia, 2010).

2.2.4 Physico-chemical property of honey

The best-characterized honeys quality around the world are physicochemical proprieties of honeys. Most honeys are thick, viscous, sweet and tasty with an aromatic odor (Manuel *et al.*, 2011). Honey's taste, color, flavor, and density can greatly differ. The color depends entirely on the flowers from which the nectar that makes honey was collected from. Honey color can be whitish, brown, red, amber and even black. The density (specific gravity) of honey also varies (Manuel *et al.*, 2011).

The examination of physicochemical property of honey from Mauritius with respect to geographical origin and the type of honey were observed to vary with botanical origin (Mohammad *et al*., 2012). In Sudan analysis of protein structure, physicochemical properties and mineral composition of honey of different origin showed to vary with respect to origins. Honey originating from *Ziziphus spina, Acacia nilotica, Acacia seyal*, *Helianthus annus* and *Azadirachta indica* showed various chemical parameters.

The scientists reported honey parameters range, pH (4.2 – 5.4), sugar (78.0 -81.3 - ºBrix) and colour degree of 21.3 – 27.2 (Seif and Elfadil, 2009). Other studies for sixty two samples of *Apis mellifera* honey from the province of Chubut in Argentina, reported physiochemical values of pH (3.2 -3.5), moisture (5.4 – 18.4%), ash (0 – 0.54%) and colour (5 – 114 mm). In Tanzania, Masoud (2014) working on the quality of Tanzanian honey collected from 26 honey potential producing areas reported six parameters; water (21%), sugar (64.16 to 84.84g/100g), ash content (0.6%), pH (2.6-4.4), Hydroxymethyl furfural (5.0 to 26.4mg/kg) and colour (Light to Dark). The same author further reported honey colour from Dodoma (white), Manyoni (light amber) (central zone), Tabora (white – extra light amber), Geita (amber), Biharamulo (amber), Kibondo (dark amber), and Uvinza (extra light amber). Gidamis *et al*. (2004) showed that honey from selected areas in Tanzania (Dodoma, Tanga, Morogoro, Same, Arusha and Tabora) is of high quality and has important physico-chemical properties of the following; moisture 21.6 – 22.8%, total acidity 29.1-41.6 meq/kg, pH 4.2-4.87, free fatty acid 10.6-26.6 meq/kg, specific gravity 1.40-1.52 g cm^{-3} and HMF were far below the maximum acceptable level of 40mg/kg as recommended by Codex Alimentarius Commission Standards.

Honey as a substance in its own is used especially as food, sweetener, medicine and preservative. Many uses of honey depend on its high sugar content, which is the source of its sweetness and of its high energy value, about 3kcal/g. Honey consists mainly of the simple sugars mainly fructose and glucose; these are absorbed directly from the alimentary tract into the blood, and thus provide a rapid energy source. Most honey contain about 30-35% of glucose, which is less soluble in water than fructose and honey containing more glucose than average tend to granulate

(crystallize) rapidly, some even in a few days (Eva, 1999). Honey has a higher fructose content (usually 35-40%) than other natural foods. Fructose is very soluble in water, so a high fructose honey tends to remain in a liquid form. It is an exceptionally sweet sugar, which is very hygroscopic. The proportions of different sugars in a sample of honey depend on the plant sources of the nectar, and so do the flavor and aroma of the honey which produce organoleptic reactions (Eva, 1999)

2.2.5 Medicinal properties

2.2.5.1 Wounds and Ulcers

The use of honey as medicine is mentioned in the most ancient written records. Honey has been used for centuries as a popular 'home remedy' for wounds and ulcers. Today clinical observations and experimental studies have established that honey has effective antibacterial properties, as well as anti-inflammatory and analgesic properties (Jones, 2001). However, honey has varying degrees of such properties. It painlessly removes pus, scabs and dead tissues from wounds and stimulates new tissue growth, when used on burns, honey reduces the amount of scarring (Molan, 1992). There are indications that the anti-inflammatory properties of honey could be effective in the treatment of chronic leg ulcers. Excellent results of the therapy from patients with fungating wounds, recalcitrant leg ulcers and pressure sores using the unique Manuka factor containing honey known as Manuka honey has been reported (Brady *et al*., 2004). FAO (1996) reported honey to be used in treatments of ulcers, bed sores and other skin infections resulting from burns and wounds.

Treatment with honey is called apitherapy which includes replenishing energy, enhancing physical stamina and improving immune systems (Bogdanov, *et al*.,

2008). A number of properties inherent to honey might contribute to its ability to fight infection and promote healing. Its high sugar content allows it to draw infection and fluid from wounds by a process called 'osmosis' (Irish, 2008). Honey prevents bacterial growth through its acidic pH and through the work of an enzyme that produces small amounts of hydrogen peroxide. Its ability to keep the area around a wound moist and protected promotes fast healing and prevents scarring (Molan, 1992). Honey's high sugar content kills many kinds of bacteria, including some antibiotic-resistant germs. Unlike other topical antiseptics, honey causes no tissue damage. Honey also is considered to have a calming effect on the mind and promotes sleep.

2.2.5.2 Digestion and absorption

Natural honey contain several enzymes which enhance the digestion of food substances especially carbohydrates such as sugars and starch (Somerfield, 1991). The additional benefit of eating honey as a source of energy over the commonly used artificial sugar is that, the major sugar constituents of honey are present as monosaccharides (simple sugars). Unlike the refined sugar (sucrose) which normally has to undergo the processes of digestion into simpler forms prior to their absorption. These sugar molecules in honey are in pre-digested forms, and can be directly absorbed into the human system. As a sweetener, honey has nutritional advantages over sugar, providing some amount of small nutrients, which act to aid digestive processes in the body (Bogdanov, 2008).

2.2.5.3 Respiratory diseases

Honey has been used to treat cardiovascular disease and respiratory complaints (Al-Waili, 2004). Respiratory problems have traditionally been treated effectively with

honey, and pharmacological research referred the role of honey in inhibiting the inflammatory process present in respiratory problems (Kaal, 1991). Honey has been reported to treat 30 patients suffering from bronchial asthma (Mihailescu 1975). The World Health Organization (WHO) and American Academy of Pediatrics recommend honey as a natural cough remedy.

2.2.5.4 Exercise and athletic performance

The consumption of energy giving substances before during and after any form of physical exercise improves the individual's performance and increases the rejuvenation of muscles. This is also associated with dietary supplementation with natural honey which provides up to 17 g of carbohydrates for every tablespoon consumed and gives the much needed energy (Ajibola *et al*., 2012). Furthermore, honey can be an effective carbohydrate source and a better substitute to glucose for exercise and athletic performance, due to its constituent of various classes of sugars. People favour slow-burning sugars for sustenance as energy source during physical exercise. Honey is beneficial in this regard as it releases fructose slowly into the blood stream to produce a sustained energy boost and maintain homeostasis. The data obtained from the Sports Nutrition and Exercise Laboratory of one University showed that honey can be used effectively instead of glucose for energy replenishment during physical exercise (Kreider *et al*., 2002).

2.3 Nutritional value of honey

Honey consist of mainly sugars (79.6%) and water (17.2%). The sugars include fructose (38.2%) and Glucose (31.3%) which are simple sugars that are readily absorbed by the body. Other sugars include maltose (7.3%) and sucrose (1.3%). It

also contain several vitamins, minerals (0.17%) and some proteins (0.26%) (Honey Guideline, 2007). The other constituents of honey are amino acids, antibiotic-rich inhibine, phenol antioxidants, and micronutrients (Bogdanov, 2008). The sugars in honey are sweeter and give more energy than artificial sweeteners. These substances are of nutritional and health importance. Some of the vitamins found in honey include ascorbic acid, pantothenic acid, niacin and riboflavin; along with minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc (Alvarez *et al*., 2010).

2.3.1 Vitamins

Raw honey contain vitamins, such as A1, B1, B6, B12, C, D, E, and folic acid (Murray *et al*., 2001). Raw honey is rich in vitamin C (Ascorbic acid). This vitamin is one of the important vitamin to humans with health benefits as antioxidant, anti-atherogenic, anti-carcinogenic, enhancer of the availability and dietary absorption of iron from non-heme iron sources (Al-Waili, 2004). It boosts the immune system, helping to protect the body from infections and disease. It is important in wound repair and healing/regeneration process as it stimulates collagen synthesis also as a preservatives in food industry (Saratupa and Ganguly, 2014). Vitamin B6 is important to the human body, it is involved in chemical reactions that take place each minute. Vitamin B6 is actually a group of three different vitamins; pyridoxamine, pridoxal and pyridoxine (Bogdanov *et al*., 2008). This combination works in tandem with enzymes within the body to ensure that your body's metabolism is functioning properly ((Bogdanov *et al*., 2008). Vitamin B3 or Niacin, in honey helps to reduce levels of bad cholesterol or Low-Density Lipoproteins (LDL), and increase levels of beneficial cholesterol or High Density Lipoproteins (HDL). Vitamin B2, also known

as riboflavin is a water-soluble vitamin that helps to fuel the body's energy by converting carbohydrates into sugar. It also helps the breakdown of fats and amino acids. Vitamin B1 or thiamin in raw honey, helps to break down carbohydrates to fuel the body. It also neutralizes free radicals which can compromise the immune system and speed up the aging process. Raw honey contains vitamin B5, also known as pantothenic Acid. Vitamin B5 is present in blood plasma where it maintains the balance of hormones and keeps the nervous system in top condition (Murray *et al*., 2001)

2.3.2 Minerals

Honey contain minerals which help the body to grow, develop, and stay healthy. The body uses minerals to perform many different functions as building strong bones and transmitting nerve impulses (Pia, 2010). Some minerals are used to make hormones or maintain normal heartbeat. Minerals such as calcium helps in building strong bones, healthy teeth, proper functioning of nerves and muscles, blood vessels and certain hormones. Iron too is an essential mineral element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, electron transport and prevention of fatigue (Nazanin *et al*., 2014).

Masoud (2005) working with 26 stinging honey samples collected from potential honey producing areas in Tanzania reported an average ash content of 0.6%. Seif and Elifadil (2009) reported specific mineral content of honey that varied with floral types as follows *Ziziphus spina* (42.37 mg/kg), *Acacia nilotica* (56.83 mg/kg), *Acacia seyal* (35.63 mg/kg), *Helianthus annus* (82.92 mg/kg) and *Azadrachta indica* (58.02 mg/kg) for Calcium. Potasium in the honey also varied across floral types

ranging from 17.60 mg/kg for *Helianthus annus* to 74.66 mg/kg for *Acacia seyal*. Magnesium also exhibited significant variation with a minimum value in *Azadrachta indica* that contained 23.67 mg/kg to a maximum value in *Acacia nilotica* that had 177.15 mg/kg.

Iron was relatively lower compared to the preceded mentioned minerals with a least value in *Azachta indica* that contained as low as 2.05 mg/kg and higher in *Acacia seyal* with content of 33.65 mg/kg. Zinc had lowest values of all, ranging from 4.86 to 9.61 mg/kg in *Helianthus annus* and *Acacia nilotica* as well. It has been reported that K is the abundant and predominant element in honey (Rodriguesotero *et al*., 1994). Thyme honey from Spain was found to contain elements as K (679 mg/kg) and Mg (77 mg/kg) (Terrab *et al*., 2004). Despite its trivial content, mineral constituents make honey useful in the diet. Minerals, such as potassium, Chlorine, Sulphur, Sodium and Calcium originate from soil and get into honey via the plants. Minerals are among many components that affect honey colour. A deeply pigmented (darkly colored) honey is superior in nutritive value to one of light color and that the darker the honey the higher the mineral content therefore, the greater the percentage of minerals the greater the nutritive value of the honey (Root, 1980).

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1. Materials

Two types of honey i.e. raw stinging and stingless bees' honey were collected from beekeepers in two selected agroecological zones of Tanzania; Western zone covering Tabora (Inyonga, Tabora town and Nzega) and Shinyanga- (Kahama, Bukombe and Shinyanga town), Central zone covering Singida (Issuna, Manyoni and Singida town) and Dodoma (Kibaigwa, Bahi and Dodoma town). The central zone of Tanzania is dominated by savanna, bushland and thickets whereas the western zone is covered mainly with miombo woodland trees.

The test organisms included bacteria (*Staphylococcus sapropyticus, Escherichia coli, Salmonella typhi*) and fungi (*Aspergillus flavus* and *Candida albicans*) were obtained from Veterinary Microbiology laboratory- Sokoine University of Agriculture (SUA), Morogoro.

3.2 Methods

3.2.1 Sampling procedure

A purposive sampling procedure was used to select regions followed by simple random sampling procedure to collect honey samples from beekeepers in two selected zones (Western and Central) of Tanzania.

Two types of honey were collected in triplicate from each sampling area (3 replications), Thus, 3 replicate per honey type x 2 honey bee types x 3 sampling areas x 2 regions in each zone = 36 honey samples from each zone making a total of

72 honey samples that were collected from the two zones. The honey samples were packaged in sterilized plastic bottles (330ml) transported at ambient temperature (25 $\pm 2^{o}C$) and stored at the laboratory of Food Technology, Nutrition and Consumer Sciences, SUA, Morogoro.

3.3 Research design

A Completely randomized block design in factorial experiment with 3 replications was employed in the study where the principle factors were location at two levels (two zones) and bee type at two levels (honeybees and stingless honey bees' honey). The effect of these factors on pH, reducing sugar, color, antioxidant activity, antimicrobial activity, vitamin C, minerals and total phenol were determined. The following model was assumed

$$Y_{ijkl} = \mu + Z_i + R_j + A_k + T_l + (Z*R*A*T)_{ijkl} + e_{ijkl}, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where Y_{ijkl} = Dependent variable, μ=General mean, Z_i= the effect of i^{th} agro-ecological zone, R_j= Effect of j^{th} sampling region A_k= Effect of k^{th} sampling area T_l=The effect of l^{th} honey type, $(Z*R*A*T)_{ijkl}$= Interaction effect between i^{th} zone j^{th} region k^{th} area and l^{th} honey type e_{ijkl}= Error term or Random term.

3.4. *Antimicrobial sensitivity test*

Antimicrobial sensitivity test of the honey samples was determined by Agar Wells Diffusion method as described by Clinical Laboratory Standard Institute (2009). Mueller – Hinton Agar medium was used for antimicrobial susceptibility testing. The medium was prepared following the manufacturer's instructions. After autoclaving

(121^{o}C for 15minutes) the medium was left to cool to 50^{o}C. Then 25 ml per plate (15x100mm) was measured on a level pouring surface to a uniform depth of 4 mm and incubated in an incubator (35 ±2^{o}C) for 24 hours.

The test organisms included bacteria (*Staphylococcus sapropyticus, Escherichia coli, Salmonella typhi*) and fungi (*Aspergillus flavus and Candida albicans*), were streaked on to a non-inhibitory agar medium (broth agar) to obtain isolated colonies. After incubation at 35^{o}C overnight, 4 to 5 colonies were picked and inoculated into broth (Mueller- Hinton broth) and incubated at 35^{o}C for 24 hours. A sterile cotton swab was dipped into the suspension, pressed firmly against the inside wall of the tube just above the fluid level, then streaked over the entire surface of the medium rotating the plate approximately 60 degree after each application to ensure an even distribution of the inoculum, finally swabbed all around the edge of the agar surface. Small holes of 5 mm were made on the petri dishes with agar by using glass pastor and sterile loops, then 100 µl of honey sample was placed in the agar holes using sterile micropipette. The plates were inverted and incubated at 37 ±1^{o}C for 24 hours for tested bacteria and 48 hours for yeast. After incubation period the diameter of the zones of complete inhibition (including the diameter of the disk) was measured and recorded in millimeters (Plate 1). The measurements were made with ruler on the undersurface of the plate without opening the lids.

Plate 1: **Complete microbial growth inhibition zone measured using a ruler**

3.5 Chemical Analyses

3.5.1 Antioxidant activity

Antioxidant activity in honey samples was determined by using the Ferric reducing ability of plasma (FRAP) assay following the method described by Benzie and Strain (1996). About 0.1 g of honey was diluted with distilled water and made up to 10 ml. Then 200 µL of diluted honey (0.1 g/ml) was mixed with 1.5 ml of FRAP reagent. The reaction mixture was then incubated at 37°C for 4 minutes and its absorbance was measured spectrophotometrically at 593 nm against a blank that was prepared with distilled water. Fresh FRAP reagent was prepared by mixing 10 volumes of 300 mM/l acetate buffer (pH 3.6) with 1 volume of 10 m M TPTZ (2, 4, 6 – tripyridyl – s – triazine) solution in 40 mM/l HCl, containing 1 volume of 20 mM ferric chloride ($FeCl_3.6H_2O$). Trolox (vitamin E analogue) was used for the calibration curve and was prepared with serial dilutions of 0, 25, 50, 100, 150, 200, 250 and 300 µM/ml.

The standard curve was plotted and the unknowns were calculated using linear regression equation of the standard plot (appendix 7). The ferric reducing ability of honey sample were expressed as FRAP value (µM of Fe^{II}) per 100g of honey as per following formula:

$$\text{FRAP value}\left(\frac{\mu M\ Fe(II)}{100g}\right) = \frac{\text{Change in absorbance of sample}}{\text{Change in absorbance of standard}} \times \text{FRAP value of standard}$$

3.5.2 Vitamin C determination

Vitamin C (Ascorbic acid) was determined based on the oxidation reduction reaction principle according to AOAC (2007) procedure using method number 967.21. About 5g of the homogenized honey sample was weighed and extracted using 10 mls of 10% Trichloroacetic acid (TCA) and the extract was collected into a volumetric flask. The diluted sample extract was then filtered using whatman filter paper No.1. Then 10 mls of the clear filtrate was pipetted into 250 ml erlenmeyer flask. A blank solution was prepared by using 10 mls of 10% TCA solution. The burette was filled with standard indophenol solution. Slowly the contents of the flask was titrated with standard solution of indophenol until faint pink colour was obtained which persisted for 10 seconds. The volume of indophenol solution used to oxidize the ascorbic acid present in the sample extract and in the blank solution was recorded. Then vitamin C content in the honey sample extract was calculated using the following formula:

$$\text{Vitamin C content of the sample (mg/100g)} = \frac{(A-B)\ x\ C\ x\ V\ x\ 100}{D\ x\ S}$$

Whereby; A = Volume in ml of the indophenol solution used for sample

B = Volume in ml of the indophenol solution used for blank

C = Mass in mg of ascorbic acid equivalent to 1.0ml indophenols solution

S = Mass of sample in (g) taken for analysis

V = Total volume of extract in milliliters

D = Volume of sample filtrate in milliliters taken for analysis

3.5.3 Total phenolic compound.

Total phenol of the honey samples was determined by using the Folin-Ciocalteu reagent (FCR) as described by Singleton *et al*. (1999). Each sample was analysed using the spectrophotometer Wagtech CECIL 2021 where 20 µl sample was added to 100 µl FCR (diluted 1:10 with distilled water), mixed and incubated at 37^{o}C for 60 seconds prior to addition of 80µl 7.5% (w/v) sodium bicarbonate solution. The samples were mixed again and incubated at 37^{o}C for 15 minutes prior to absorbance reading at 760 nm. Total phenol was calculated against a calibrated standard curve of gallic acid (Appendix 1), and the results were presented as mg gallic acid equivalents (GAE) per 100 sample weight (SW).

$$\text{Total phenol } \left(\frac{\text{mg}}{\text{100g}}\right) = \frac{\text{Reading value in ppm x Dilution factor x 100}}{\text{sample weight}}$$

3.5.4 pH determination

pH of honey samples was determined using a digital portable pH meter – JENWAY, UK 3305P in accordance with Harmonised International Honey Commission (2009). In between the readings of different samples, the electrode was washed with distilled water and dried with tissue paper, and inserted into prepared honey samples and recorded. The experiment was done in triplicates.

3.5.5 Colour determination

Classification of honey colour was named and assigned a rank according to USDA Honey Colour Grading Chart (USDA, 1985) where samples of honey were placed in clean and clear McCartney bottles and observed against the colour grading chart (Plate 2) by Panaromic Hill Honey Collective (2013).

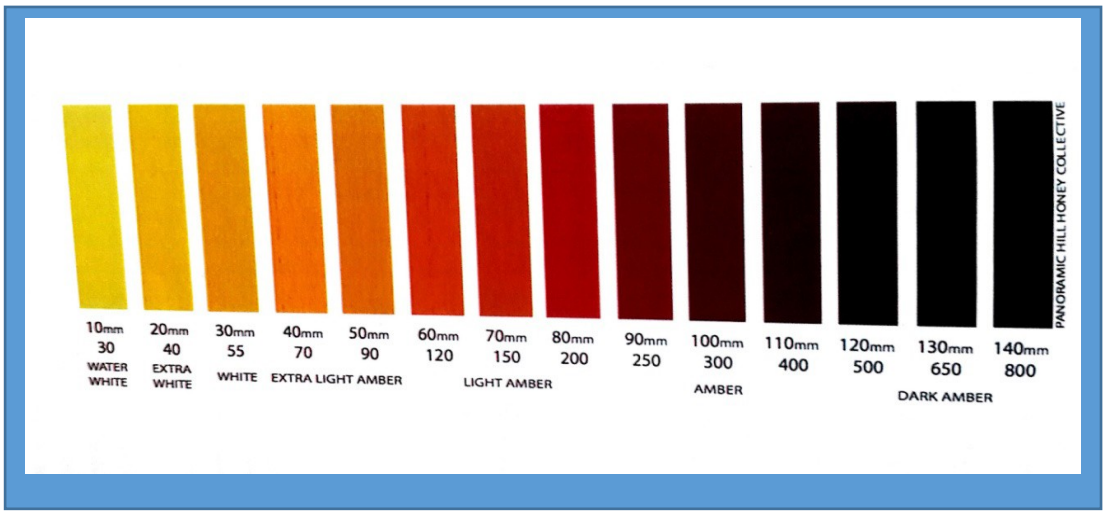

Plate 2: **Honey colour grade guide (Source: Panaromic Hill Honey Collective, 2013).**

3.5.6 Determination of total reducing sugar

Total reducing sugars of honey was determined following the AOAC (2000) procedures using method number 920.183. About 1g of honey sample in conical flask was dissolved using 100 ml hot water to dissolve all sugars. The solution was clarified by addition of 5 ml Carrez 1 solution followed by Carrez 2 solution then the content was filtered using whatman filter paper No. 1. Then 10 ml of the filtrate was placed in duplicate in conical flask mixed with 10 ml of copper reagent (sodium carbonate solution, copper sulphate solution and citric acid) then boiled for 30 minutes and left to cool, after then 1 ml of saturated solution KI was added followed

by 10 ml of 6N HCL. About three drops of 1%w/v starch was added as indicator, and the content was titrated against 0.1N (NaS_2O_3) up to the end point (blue black to cream). Luff Table (appendix 13) was used to convert titration volume to sugar content and the amount of reducing sugars per gram of honey samples was calculated as follows:

$$\text{Amount of total reducing sugar in} = \frac{\text{Reducing sugar (mg) from table}}{\text{Weight of sample (g)}} \times \frac{\text{Total volume of extract (ml)}}{\text{Volume of extract analysed}}$$

Original sample (mg/g)

3.5.7 Mineral analysis

Mineral content of honey samples (Fe, K, Zinc, Ca, Mg) were determined according to AOAC (2000) procedures using method number 999.11 by Atomic Absorption Spectrophotometer (AAS UNICAM 919) and Flame Analyzer (Model 2655-00). About 5 g of honey samples were incinerated in the muffle furnace at 550^{o}C for 4 hours and ash content of each sample was dissolved using 10 ml of 6N HCl. The obtained ash solution was filtered using whatman filter paper No.1. The serially diluted reference standard solutions for Atomic Absorption Spectrophotometer (Element wavelength flame-gases) were; zinc 213.9 nm, iron 248.3 nm, potassium 766.5 nm, calcium 422.7 nm and Magnesium 285.2 nm.

The Standard curve plot (Appendix 2, 3, 4, 5, 6) of absorbance against the known concentration of standard solutions (0.5, 1, 1.5, 2. 0, 2 .5 and 3.0 ppm) was used to determine the concentration of minerals in samples and expressed as in the following formula.

$$\text{Mineral Content } \left(\frac{\text{mg}}{\text{100g}}\right) = \frac{\text{Reading Value in ppm x Dilution factor x 100}}{\text{Sample weight(g)}}$$

3.6 Statistical Data Analysis

Data obtained from antimicrobial analysis, antioxidant analysis and physico-chemical analysis were analyzed using SAS software (version 9.1) for Analysis. Analysis of Variance (ANOVA) was used to determine the influence of main factor to the dependent variables, the factor influence was considered to be significant when $p<0.05$. Mean comparison was done using probability of difference (pdiff) and results were presented as Least Squares Mean ± SE. Statistical Package for Social Scientists (SPSS,Version 17) software was used to estimate the percentage of various measured parameters.

CHAPTER FOUR

4.0 Results and Discussion

4.1 Antimicrobial Properties of Raw Honey from Stinging and Stingless Bee Honey

The general ability of stinging and stingless bee honey to inhibit pathogenic microbial growth is demonstrated in Appendix 8. Results in Tables 1a, b and c show ability of honey to inhibit microbial growth from selected zones, regions and areas respectively. Table 1d exhibits two types of honey collected from various honey producing zones, regions and areas with respect to its ability to inhibit micro-organisms growth.

Honey collected from western zone had more ability of inhibiting microbial growth than that from central zone although, the inhibition zone (diameter) between western and central zone was not significantly (P>0.05) different (Table 1a).

Table 1a: Effect of honey collected from central and western zones of Tanzania on microbial growth (N =360)

Zone	Response %		Inhibition zone
	+ Ve	-Ve	(mm)
Central	22.2	27.8	11.87± 0.43[a]
Western	31.9	18.1	12.24±0.30[a]
Total/Significance level	**54.2**	**45.8**	**NS**

Note: NS = Not significant at P>0.05
+ve response = Ability of honey to inhibit microbial growth
-ve response = Inability of honey to inhibit microbial growth

Honey from Tabora and Shinyanga in the western zone and Singida in central zone had relatively higher ability to prevent microbial growth than honey from Dodoma. Shinyanga honey exhibited significantly larger zone diameter of inhibition followed by Singida, Dodoma and finally Tabora (Table 1b).

Table 1b: Effect of honey collected from various regions of Tanzania on response of microbial growth (N =360)

Region	Response		Inhibition zone
	+Ve	-Ve	(mm)
Dodoma	9.2	15.8	11.31 ± 0.57^{b}
Singida	13.1	11.9	12.13 ± 0.48^{ab}
Tabora	16.4	8.6	10.97 ± 0.42^{b}
Shinyanga	15.6	9.4	13.80 ± 0.43^{a}
Total/Significance level	**54.2**	**45.8**	*

Note: * = Significant at $P<0.05$.

+ve response = Ability of honey to inhibit microbial growth

-ve response = Inability of honey to inhibit microbial growth

Referring to the areas where samples of honey were collected, it is clearly shown in Table 1c that honey samples from Nzega and Inyonga had highest ability to resist microbial growth, followed by samples from Shinyanga town, Bukombe and Kahama. In contrast, honey from Kibaigwa in Dodoma region showed lowest ability to resist microbial growth and significantly ($P<0.05$) lowest inhibition zone. The difference in ability to resist microbial growth could probably be due to differences in vegetation types between these areas (Kumar *et al*., 2010). Different vegetation types contain different floral types that varies in pollen and nectar which influence the honey composition.

Table 1c: Effect of honey collected from various areas of Tanzania on microbial growth (N =360)

Areas	Response		Inhibition Zone
	+Ve	-Ve	(mm)
Kibaigwa	2.5	5.8	9.78 ± 1.04^{c}
Dodoma town	3.1	5.3	13.80 ± 0.99^{a}
Bahi	3.6	4.7	10.46 ± 0.87^{bc}
Issuna	4.4	3.9	13.63 ± 0.78^{a}
Manyoni	4.2	4.2	10.60 ± 0.81^{bc}
Singida town	4.4	3.9	12.06 ± 0.81^{b}
Nzega	5.8	2.5	10.90 ± 0.68^{bc}
Inyonga	5.8	2.5	10.62 ± 0.68^{bc}
Tabora town	4.7	3.6	11.47 ± 0.76^{b}
Kahama	5.0	3.3	13.78 ± 0.74^{a}
Bukombe	5.0	3.3	15.11 ± 0.74^{a}
Shinyanga town	5.6	2.8	12.65 ± 0.70^{b}
Total/Significance level	**54.2**	**45.8**	*

Note: * = Significant at P<0.05

+ve response = Ability of honey to inhibit microbial growth

-ve response = Inability of honey to inhibit microbial growth

The results of this study showed that about 54 % of all tested honey samples regardless of the type of honey or the area collected or the tested microorganism, showed positive response to inhibit microbial growth, whereas 46% showed negative response (Table 1c). Inability to inhibit microbial growth could be attributed to the fact that vegetation where this honey samples were collected contain nectar and

pollen that do not contain medicinal properties that can kill pathogenic microorganisms (Manning, 2000). Alternatively, the pathogenic microorganisms used to test honey inhibition could not be affected by the tested honey.

Results in Table 1d further support that stingless honey had more ability to inhibit microbial growth than stinging bee honey suggesting that stingless bee honey has higher medicinal properties than stinging bee honey. The superiority of stingless bee honey could be attributed to the flowers and trees visited. Also, stingless bees do not mix pollen with honey whereas larger bees mix honey and pollen together to make the so called bee bread (Cortopassi *et al.*, 2006). Another reason could be due to the smaller size (5 mm) of the stingless bee that enable the bees to penetrate more to the flower and extract medicinal ingredients compared to larger bees that can't penetrate down deep to the flower (Roubik, 2006).

The microbial inhibition zone ranged from 8.5 (stinging bee honey) to 15.13 mm (stingless honey). The observed inhibition zone in this study is more or less similar to that reported by Rahman *et al*. (2010) (13 - 15) who worked with honey and propolis samples that showed the ability to inhibit growth of *Escherichia coli* and *Staphylococcus aureus*, where propolis attained higher inhibition limit. Mohapatra *et al*. (2010) reported higher inhibition zone resulted from raw and processed honey from India on gram positive bacteria (*S. aureus*, *B. subtilis*, *B. cereus, E. faecalis* and *Micrococcus luteus*) and gram negative bacteria (*E. coli, P. aureginosa* and *S. typhi*) where inhibition zone ranged from 6.94 mm to 37.97 mm. The reported upper limit is higher than the observed upper limit in this study. The difference could be attributed to differences in vegetation type, processing of honey and type of tested micro-organisms.

Table 1d: Ability of stingless and stinging bee honey from central and western zones in Tanzania to inhibit microbial growth (N = 360)

Zone	Region	Area	Honey type	Response%		Inhibition zone (mm)
				+Ve	-Ve	
Central	Dodoma	Bahi	Stingless	3.24	0.8	10.79±0.88[b]
			Stinging	0.2	3.78	12.16±3.06[b]
		Town	Stingless	2.43	1.62	13.25±2.16[a]
			Stinging	0.54	3.5	10.16±1.08[bc]
		Kibaigwa	Stingless	2.43	1.62	10.56±1.02[b]
			Stinging	0	4.1	NA
	Singida	Issuna	Stingless	4.1	0	13.40±0.79[a]
			Stinging	0.27	3.78	14.16±1.53[a]
		Manyoni	Stingless	3.24	0.8	11.71±0.88[b]
			Stinging	3.5	3.24	9.51±1.76[c]
		Town	Stingless	4.1	0	11.82±0.82[b]
			Stinging	0.27	3.78	12.16±3.06[b]
	Tabora	Inyonga	Stingless	3.78	0.27	11.27±0.92[b]
			Stinging	1.89	2.16	10.21±1.25[bc]
		Nzega	Stingless	4.1	0	11.33±0.79[b]
			Stinging	1.62	2.43	8.67±1.25[d]
		Town	Stingless	2.97	1.08	12.13±0.92[b]
			Stinging	1.62	2.43	8.88±1.25[d]
Western	Shinyanga	Bukombe	Stingless	4.1	0	15.13±0.79[a]
			Stinging	0.8	3.24	12.16±1.37[b]
		Kahama	Stingless	3.78	0.27	14.63±0.82[a]
			Stinging	1.08	2.97	10.34±1.53[bc]
		Town	Stingless	4.1	0	13.53±0.79[a]
			Stinging	1.35	2.70	8.50±1.37[d]

Note: Means bearing the same superscript along the column are not statistically different according to pdiff at P>0.05

+ve response = Ability of honey to inhibit microbial growth

-ve response = Inability of honey to inhibit microbial growth

The effectiveness of stingless and stinging bee honey to inhibit growth of micro-organisms is shown in Table 2. The results showed that *Candida albicans* was more inhibited by stingless bee honey, followed by *Staphylococcus saprophyticus*, *Salmonella typhi*, *Escherichia coli* and *Aspergillus flavus*.

The growth of *Escherichia coli* was not affected by stinging honey at all, contrary *Aspergillus flavus* and *Candida albicans* were more affected. The reason for bacteria

being more resistant than fungi is that bacteria cells have a high spontaneous mutation rate (about 10 – 7 per cell division) this means that they can change their characteristics rapidly thus, providing a greater variation on which natural selection can act which helps them to survive in ever – changing environment (French *et al.,* 2005).These results are different from that reported by Mohamad (2012) who tested antimicrobial properties of stinging honey from Mauritius using bacteria (*Escherichia coli* and *Staphylococcus aureus)* and fungal (*Asperigilus niger* and *Candida albicans)* and observed that fungi were more resistant than the bacteria, this could probably be due to the concentration of honey used where Mohamad (2012) diluted the honey used while in this study honey was not diluted.

Table 2: The effect of honey types on the growth of pathogenic micro-organisms (N = 360)

Honey type	Micro-organism	Response %		Inhibition zone
		+Ve	-Ve	mm
Stingless	*Escherichia coli*	19.2	25.0	9.22 ± 0.54^{b}
	Salmonella typhi	20.5	16.7	10.47 ± 0.53^{ab}
	Staphylococcus saprophyticus	21.2	12.5	10.86 ± 1.22^{ab}
	Candida albicans	23.1	0.0	11.67 ± 0.50^{a}
	Aspergillus flavus	16.0	45.8	10.97 ± 0.60^{ab}
Sub-Total		**100.0**	**100.0**	
Stinging	*Escherichia coli*	0.0	25.5	0.0
	Salmonella typhi	7.7	23.4	0.83 ± 0.06^{f}
	Staphylococcus saprophyticus	15.4	21.3	1.78 ± 0.09^{e}
	Candida albicans	30.8	17.0	2.89 ± 0.10^{d}
	Aspergillus flavus	46.2	12.8	6.56 ± 0.48^{c}
Sub-Total		**100.0**	**100.0**	***

Note: Means bearing the same superscript along the column are not statistically different according to pdiff at P>0.05

+ve response = Ability of honey to inhibit microbial growth

-ve response = Inability of honey to inhibit microbial growth

The general characteristics of honey to prevent bacterial or fungal growth have been explained by various scientists (Garcial *et al.,* 1986; Wahdan, 1998; Molan, 1999a

and Khan *et al*., 2007) who reported inhibition of pathogenic microbial growth from presence of hydrogen peroxide resulting from the action of glucose oxidase enzyme produced from hypopharyngeal glands of workers bees on glucose in presence of oxygen that inhibits microbial and fungal growth. Presence of inherent physical-chemical properties such as high sugar content (about 80% w/w) that results into high osmotic effect that dehydrate the micro-organisms has been reported to contribute inhibition of microbial growth (Molan, 1992; Bogdanov *et al.,* 1997). White (1978); Aparna and Rajalashmj (1999) suggested inhibition of microbial growth to be due to presence of diverse organic acids such as gluconic acids that remarkably creates an acidic micro-environment (pH 3 – 4.5) that prevents growth of many micro-organisms. Apart from hydrogen peroxide as a factor that inhibits microbial growth Cabrera *et al.* (2006) elucidated inhibition of microbial growth to be due to presence of non-peroxidic substances such as polyphenols which possess anti-microbial activity.

4.2 Physico-chemical Properties of Stingless and Stinging bees honey

4.2.1 pH and total reducing sugars of honey

Results in Table 3a show that there were no significant ($P>0.05$) difference in pH of the honey. Total reducing sugars was significantly ($P<0.001$) higher in honey from western zone than that of central zone.

Table 3a: Effect of honey collected from western and central zones on pH and total reducing sugars content

Zone	pH	Total reducing sugars (mg/g)
Central	3.46[a]	211.97[b]
Western	3.45[a]	267.58[a]
Mean	**3.45**	**239.77**
SE	±0.02	±10.04
Significance	NS	***

Note: Means bearing same superscript within the same column are not statistically different (p≥ 0.05) according to pdiff.

NS =Not significant (P>0.05)

Looking at the effect of regions (Table 3b) it is clear that honey collected from Shinyanga had significantly (P<0.05) higher total reducing sugars than that of Tabora, Singida and Dodoma regions. The difference could be due to difference in vegetation growing in areas where the samples were collected

Table 3b: Effect of region on pH and total reducing sugars content of honey

Region	pH	Total reducing sugars (mg/g)
Dodoma	3.43[ab]	182.62[c]
Shinyanga	3.41[b]	304.88[a]
Singida	3.49[a]	241.31[b]
Tabora	3.48[a]	230.27[b]
Significance	*	*
Overall mean	**3.45**	**239.77**
SE	±0.02	±13.13

Note: Means bearing same superscript within the same column are not statistically different (p≥ 0.05) according to pdiff.

* =Significant at P<0.05

Results in Table 3c show that there were no significant (P>0.05) variations in pH in honey between areas where honey samples were collected. Honey from Shinyanga town had highest total reducing sugars content (322.03 mg/g) and that of Kibaigwa had the lowest total reducing sugars content (172.04 mg/g) (Table 3c). These observations suggest that honey form Shinyanga town could be a better source of energy to the body compared to one collected from Kibaigwa (Eva, 1999).

The results of the present study showed that area where honey samples were collected, honey type and interactions between the two factors significantly (P<0.01) affected the pH and total reducing sugars content of the tested honey samples (Table 3d). Comparisons between sampling areas and honey types showed that there were no significant (P>0.05) difference in pH between stinging and stingless bee honey (Table 3d). The observed pH values in this study is within the range reported by various authors within and outside Tanzania. Masoud (2014) working with 26 honey samples from Tanzania reported a pH range of 2.6 to 4.4. The results of the present study also comply with those reported by Aloisi (2010) who worked with Apis honey and reported a pH range of 3.2 – 3.5. The honey samples used in this study seemed to be of lower pH than those used by Gidamis *et al.* (2004) while working with Tanzanian honey from Dodoma, Tanga, Morogoro, Same, Arusha and Tabora and reported a pH range of 4.4 – 4.87. The difference could be due to difference in soil type and vegetation growing in areas where the samples were collected. The honey samples in the present study might have potential medicinal properties as its acidic nature could have resulted from organic acids which remarkably creates an acidic micro-environment that inhibits pathogenic microbial growth (Aparna and Rajalakshmj, 1999).

Simple sugar content, such as glucose in particular have been reported to play two important roles in antimicrobial growth; one is that high sugar content can produce high osmotic effect that can dehydrate microbial cells (Molan, 1992; Bogdanov *et al*., 1997). Second, gluconic acids which remarkably creates an acidic micro – environment prevents the growth of many micro-organisms (Cooper, 2007). Therefore, the results of this study suggest that stingless honey from Shinyanga town, Kahama, Bukombe and Nzega in western zone and that from Singida town and Issuna might contain more medicinal properties than stinging bee honey.

Table 3c: Effect of honey collected from various areas on pH and total reducing sugars content

Area	pH	Total reducing sugars (mg/g)
Bahi	3.39[a]	176.33[b]
Bukombe	3.36[a]	296.52[a]
Dodoma town	3.44[a]	199.49[b]
Inyonga	3.54[a]	223.07[b]
Issuna	3.51[a]	283.13[a]
Kahama	3.45[a]	296.09[a]
Kibaigwa	3.44[a]	172.04[b]
Manyoni	3.48[a]	245.01[b]
Nzega	3.44[a]	212.52[b]
Shinyanga town	3.44[a]	322.03[a]
Singida town	3.47[a]	195.78[b]
Tabora town	3.46[a]	255.23[b]
Overall mean	3.45	239.77
SE	±0.04	±22.49
Significance	NS	*

Note: Means bearing same superscript within the same column are not statistically different according to pdiff (P>0.05) **NS** =Not significant (P>0.05) * = Significant at P<0.05

Table 3d: The effect of zones, regions, areas and honey types on pH and total reducing sugars content

Zone	Region	Area	Honey Type	pH	Total reducing sugars (mg/g)
Central	Dodoma	Bahi	Stingless	3.45[a]	259.54[cd]
			Stinging	3.34[a]	93.13[e]
		Town	Stingless	3.59[a]	294.78[bc]
			Stinging	3.29[a]	104.22[e]
		Kibaigwa	Stingless	3.50[a]	240.26[d]
			Stinging	3.39[a]	103.82[e]
	Singida	Issuna	Stingless	3.42[a]	288.94[bc]
			Stinging	3.59[a]	277.32[bc]
		Manyoni	Stingless	3.42[a]	226.95[cd]
			Stinging	3.55[a]	263.07b[cd]
		Town	Stingless	3.41[a]	252.92[cd]
			Stinging	3.53[a]	138.66[de]
Western	Tabora	Inyonga	Stingless	3.41[a]	204.89[d]
			Stinging	3.68[a]	241.25[d]
		Nzega	Stingless	3.42[a]	310.73[bc]
			Stinging	3.45[a]	114.31[e]
		Town	Stingless	3.41[a]	303.94[bc]
			Stinging	3.51[a]	206.53[d]
	Shinyanga	Bukombe	Stingless	3.30[a]	318.77[bc]
			Stinging	3.42[a]	274.27[bc]
		Kahama	Stingless	3.42[a]	413.18[a]
			Stinging	3.47[a]	179.01[d]
		Town	Stingless	3.41[a]	320.53[b]
			Stinging	3.46[a]	323.53[b]
			SE	±0.01	±0.12
			Significance	**NS**	***

Note: Means bearing same superscript within the same column are not statistically different (p≥ 0.05) according to pdiff.
NS =Not significant (P>0.05)

*** = Significant at P<0.001

4.2.2 Honey colour

Results of the present study show that honey samples from western zone had colour representation ranging from light amber to amber than honey samples from central zone which had colour representation ranging from water white to amber (Table 4a). On the other hand honey samples from Dodoma region had relatively light colour that ranged from water white to light amber (Table 4b).

Table 4a: Effect of zones on colour grading of honey (N = 72)

Zone	Colour	Percent
Central	Water white	1.4
	Extra white	5.6
	White	2.8
	Extra light amber	19.4
	Light amber	13.9
	Amber	6.9
Western	Extra white	8.3
	White	12.5
	Extra light amber	1.4
	Light amber	13.9
	Amber	13.9
Total		**100**

Note: Grading was done according to USDA Honey Colour Grading Chart (USDA, 1985)

Table 4b: Effect of regions on colour grading of honey (N = 72)

Region	Colour	Percent
Dodoma	Water white	1.4
	Extra white	5.6
	White	1.4
	Extra light amber	8.3
	Light amber	8.3
Singida	White	1.4
	Extra light amber	11.1
	Light amber	5.6
	Amber	6.9
Tabora	Extra white	5.6
	White	6.9
	Light amber	4.2
	Amber	8.3
Shinyanga	Extra white	2.8
	White	5.6
	Extra light amber	1.4
	Light amber	9.7
	Amber	5.6
Total		**100**

Note: Grading was done according to USDA Honey Colour Grading Chart (USDA, 1985)
Values in parantheses presents number of observations (n)

Considering honey bee type stingless bee honey showed colour grading of extra light amber (4.2%), amber (20.8%) and light amber (25.0%). Stinging honey bee was represented in most colour grading ranging from water white (1.4%) to light amber (2.8%) (Appendix 9). The honey colour distribution by honey types and area where the honey samples were collected is presented in Table 4c.

Results showed that stingless bee honey had more of light amber to amber colour (Appendix 10). Honey color have been related with nutritive value of the honey where deeply pigmented honey (darkly coloured) honey is superior in nutritive value

to one of light colour and that the darker the honey the higher the mineral content (Root, 1980). Observations in this study suggest that stingless bee honey had relatively higher nutritive and medicinal value than stinging bee honey. It has been also reported that honey with dark colour have a higher total phenolic content and consequently higher anti-oxidant capacity (Montenegro *et al*., 2009). These observations further support earlier findings (Table 2) that stingless bee honey had more medicinal properties than stinging bee honey.

Table 4c: Colour grading of stinging and stingless honey bees sampled from various areas in central and western zones in Tanzania (N=72)

Area	Colour	Honey type	
		Stingless (%)	Stinging (%)
Bahi	Extra light amber	0	4.2
	Light amber	4.2	0
Bukombe	Extra light amber	0	1.4
	Light amber	0	2.8
	Amber	4.2	0
Dodoma town	Extra white	0	2.8
	Light amber	4.2	0
	White	0	1.4
Inyonga	Light amber	1.4	0
	White	0	4.2
	Amber	2.8	0
Issuna	Extra light amber	0	2.8
	Light amber	1.4	0
	White	0	1.4
	Amber	2.8	0
Kahama	Extra white	0	2.8
	Light amber	4.2	0
	White	0	1.4
Kibaigwa	Extra light amber	4.2	0
	Extra white	0	2.8
	Water white	0	1.4
Manyoni	Extra light amber	0	4.2
	Light amber	1.4	0
	Amber	2.8	0
Nzega	Extra white	0	4.2
	Amber	4.2	0
Shinyanga town	Light amber	2.8	0
	White	0	4.2
	Amber	1.4	0
Singida town	Extra light amber	0	4.2
	Light amber	2.8	0
	Amber	1.4	0
Tabora town	Extra white	0	1.4
	Light amber	2.8	0
	White	0	2.8
	Amber	1.4	0

Note: Grading was done according to USDA Honey Colour Grading Chart (USDA, 1985)

4.2.3 Mineral profile of stinging and stingless honey bee

The results showed that the mineral content of honey samples did not vary significantly (P>0.05) between central and western zones for Zn, Fe and Mg. However, Ca was significantly (P<0.001) higher in honey collected from central zone while K was higher in honey from western zone (Table 5a). The differences in mineral content of honey could probably be due to difference in soil types that have an influence on floral mineral content which bees extract pollen and nectar (Seif and Alfadil, 2009).

Table 5a: The Effect of honey collected from western and eastern zones on mineral content

Zone	Mineral content (mg/kg)				
	Zn	**Fe**	**Ca**	**Mg**	**K**
Central	2.59[a]	7.17[a]	237.56[a]	154.17[a]	379.0[b]
Western	2.02[a]	8.55[a]	162.0[b]	143.91[a]	531.56[a]
SE	±0.23	±0.82	±19.55	±18.96	±154.15
Significance	NS	NS	**	NS	*

Means bearing different superscript along the same column are significantly different according to pdiff P<0.05

Results in Table 5b show the variability of mineral content of honey between regions. It was observed that there was no significant (P>0.05) difference on Mg content of honey collected from the four regions, however, Ca content was significantly (P<0.05) higher in honey samples collected from Singida than that obtained from Shinyanga, Tabora and Dodoma. A different trend was observed for K where honey collected from Tabora, Shinyanga and Singida regions had significantly (P<0.05) higher content compared to that of Dodoma region.

Table 5b: Effect of honey collected from various regions of Tanzania on mineral content

Region	(mg/kg)				
	Zn	**Fe**	**Ca**	**Mg**	**K**
Dodoma	2.24[a]	3.68[b]	169.55[b]	134.41[a]	281.79[b]
Shinyanga	1.78[b]	6.88[ab]	131.36[b]	130.43[a]	504.82[a]
Singida	2.93[a]	10.67[a]	305.57[a]	173.94[a]	476.22[a]
Tabora	2.27[a]	10.23[a]	192.65[b]	157.38[a]	558.31[a]
Significance	*	*	***	NS	*
Overall mean	2.31	7.86	199.78	149.04	455.29
SE	±0.33	±0.98	±24.97	±26.91	±76.34

Means bearing similar superscript along the same column are not significantly different according to pdiff (P>0.05)

Table 5c showed that honey collected from Issuna had higher Zn content followed by Nzega, Manyoni, Bahi, Dodoma town and Singida town. Iron was higher in honey from Inyonga followed by Shinyanga town, Singida town and Issuna. Calcium was significantly (P<0.05) highest in honey collected from Singida town and lowest in honey collected from Kahama (P<0.05). Nzega honey yielded highest Mg whereas Kibaigwa honey had the lowest Mg content. Potassium was significantly higher in Inyonga honey and lowest in Dodoma town honey (P<0.05).

Table 5c: Effect of honey collected from various areas on mineral content

Area	(mg/kg)				
	Zn	**Fe**	**Ca**	**Mg**	**K**
Bahi	2.63[a]	7.12[c]	215.09[b]	155.34[a]	447.89[a]
Bukombe	1.12[b]	4.65[d]	119.82[c]	127.79[a]	436.35[a]
Dodoma town	2.62[a]	1.61[e]	159.84[c]	155.53[a]	111.85[c]
Inyonga	1.57[b]	15.34[a]	216.45[b]	114.95[a]	602.47[a]
Issuna	3.29[a]	10.80[ab]	238.17[b]	175.48[a]	343.55[a]
Kahama	1.67[b]	4.22[d]	91.72[d]	141.23[a]	496.56[a]
Kibaigwa	1.47[b]	2.32[e]	133.73[c]	92.38[b]	285.61[b]
Manyoni	2.89[a]	10.38[ab]	274.27[b]	166.02[a]	551.59[a]
Nzega	2.95[a]	8.44[b]	190.71[b]	189.29[a]	531.22[a]
Shinyanga town	2.55[a]	11.76[ab]	182.54[b]	122.26[a]	581.55[a]
Singida town	2.62[a]	10.81[ab]	404.26[a]	180.32[a]	533.52[a]
Tabora town	2.29[a]	6.92[c]	170.80[b]	167.90[a]	541.25[a]
Overall mean	2.31	7.86	199.78	149.04	455.29
SE	±0.57	±1.31	±41.46	±48.62	±134.91

Means bearing similar superscript along the same column are not significantly different according to pdiff (P>0.05)

The results in Table 5d showed that Potassium had the highest concentration which was significantly (P<0.05) different from the rest in both types of honey. Honey sampled from Dodoma town had the lowest concentration. Kibaigwa honey had the least Ca content (P<0.05). Zinc was the lowest in both honey with minimum value in Kibaigwa honey and high value in Issuna honey (Table 5d). The dominance of K

content in honey is similar to the observations made by Rodriguesotero *et al*. (1994) who reported that K was the abundant and predominant element in honey. Also thyme honey from Spain was found to contain relatively higher K concentration of 679 mg/kg (Terrab *et al.,* 1994). Seif and Elifadili (2009) reported variation in K content to be due to floral types whereby a range of 17.60 mg/kg for *Helianthus annus* to 74.66 mg/kg for *Acacia seyal* was reported. The overall mean concentration of K observed in this study (Table 5d) is within the range reported by other workers (Terrab *et al.,* 1994). However, in the present study the variation of K concentration between areas and honey types (stinging bee honey from Dodoma town and for stingless bee honey from Singida town) (Table 5d) could be attributed to bee types and variation in floral types.

Table 5d: Effect of zones, regions, areas and honey type on mineral content

Zone	Region	Area	Honey Type	(mg/kg)				
				Zn	Fe	Ca	Mg	K
Central	Dodoma	Bahi	Stingless	4.12^{c}	7.84^{dc}	282.50^{cd}	259.57^{bc}	758.41^{d}
			Stinging	1.13^{g}	6.39^{de}	147.67^{d}	51.10^{d}	137.37^{f}
		Town	Stingless	3.90^{c}	1.34^{g}	228.42^{d}	292.41^{bc}	143.28^{c}
			Stinging	1.34^{g}	1.88^{g}	91.25^{f}	18.65^{f}	80.42^{f}
		Kibaigwa	Stingless	2.56^{e}	2.23^{g}	237.50^{d}	177.08^{c}	470.76^{e}
			Stinging	0.38^{h}	2.40^{g}	29.95^{g}	7.67^{f}	101.29^{f}
	Singida	Issuna	Stingless	5.29^{a}	11.67^{bc}	287.62^{c}	273.92^{bc}	423.37^{c}
			Stinging	1.28^{g}	9.93^{c}	188.73^{d}	77.04^{d}	260.73^{f}
		Manyoni	Stingless	4.55^{b}	12.36^{b}	442.0^{b}	277.46^{bc}	931.39^{c}
			Stinging	1.22^{g}	8.41^{c}	106.53^{ef}	54.57^{d}	171.81^{f}
		Town	Stingless	4.43^{bc}	12.10^{b}	508.70^{a}	281.98^{bc}	951.10^{c}
			Stinging	0.82^{h}	9.52^{c}	299.82^{c}	78.65^{d}	115.94^{f}
Western	Tabora	Inyonga	Stingless	1.56^{f}	12.53^{b}	221.53^{cd}	164.82^{c}	751.85^{d}
			Stinging	1.57^{f}	18.15^{a}	211.36^{cd}	65.07^{d}	453.09^{e}
		Nzega	Stingless	4.56^{b}	10.80^{bc}	319.46^{c}	350.52^{a}	894.72^{c}
			Stinging	1.34^{g}	6.08d^{e}	61.96^{f}	28.07^{e}	167.72^{f}
		Town	Stingless	3.22^{d}	8.32^{c}	275.81^{cd}	310.76^{b}	961.38^{b}
			Stinging	1.36^{g}	5.52^{e}	65.79^{f}	25.04^{e}	121.12^{f}
	Shinyanga	Bukombe	Stingless	1.35^{g}	7.05^{d}	133.50^{e}	185.23^{c}	719.64^{a}
			Stinging	0.88^{h}	2.26^{g}	106.14^{ef}	70.36^{d}	153.05^{f}
		Kahama	Stingless	1.42^{g}	3.93^{fe}	94.76^{f}	244.01^{bc}	792.59^{b}
			Stinging	1.92^{f}	4.50^{e}	88.68^{f}	38.45^{e}	200.54^{f}
		Town	Stingless	3.31^{d}	19.87^{a}	274.02^{cd}	205.87^{c}	911.48^{a}
			Stinging	1.80^{f}	3.64^{fe}	91.06^{f}	38.66^{e}	251.63^{f}
			SE	±0.039	±0.182	±4.346	±3.138	±23.822
			Sign. level	***	***	***	***	***

- **Note:** Means bearing same superscript within the same column are not statistically different (p≥ 0.05) according to pdiff.

Stingless honey showed relatively higher Zn concentration where honey from Issuna was highest and significantly ($P<0.05$) different from all other honey samples (Table 5d). Other stingless honeys from Nzega, Manyoni, Singida town had higher Zn content that did not differ significantly ($P>0.05$) from each other (Table 5d). Stinging bees honey had significantly lower values for Zn for all samples as compared to stingless bees' honey. It was further observed that stinging bees honey from Kibaigwa had the lowest Zn concentration (Table 5d). Generally Zn was present in lowest concentrations in relation to other minerals. These findings comply with that reported by Rodriguesotero *et al*. (1994) who found Zn to be relatively lower while working with stinging bee honey originating from various floral types. The concentration of Zn observed in this study was relatively lower to that observed by Rodriguesotero *et al*. (1994) who reported a minimum of Zn concentration of 4.86 mg/kg to 9.61 mg/kg. The difference could probably be due to difference in vegetation and soil types. Soil and vegetation have an influence on the mineral content of honey. Root (1980) reported that minerals such as K, Cl, S, Na, Zn and Ca originate from soil and get into honey via the plants, furthermore, the plant mineral uptake varies with plant species.

Total iron (Fe) content was slightly higher (Table 5d) in stingless bee honey than that contained in stinging bee honey. Stingless bee honey from Shinyanga town had significantly ($P<0.05$) highest Fe content that did not differ from the stinging bee honey from Inyonga. There were no significant differences ($P>0.05$) in Fe content of stingless bee honey from Inyonga, Issuna, Manyoni, Singida town and Nzega. The stingless bee honey from Dodoma town had the lowest Fe content (Table 5d). According to this study, the observed iron concentration range of honey from

Dodoma and Shinyanga town had a lower minimum value of 2.05mg/kg reported by Seif and Alfadil, (2009) who worked on the honey originating from *Azadirachta indica* and *Acasia seyal.* The difference in iron content could be attributed to differences in vegetation types where honey was extracted.

Generally, the total levels of Ca, Mg and K were significantly ($P<0.05$) higher in stingless bee honeys than that of stinging bee honeys collected from various areas (Table 5d). The reasons contributing to the difference in the mineral profile between the two bee types could probably be due to smaller size of stingless honey bees that enable them to penetrate deeper to the flower and extract more nutrients, also the behaviour of stingless bee of not mixing pollen with honey unlike stinging bee that mix the two could contribute to the mineral superiority of stingless bee honey (Cortopassi *et al*., 2006). The concentration of calcium observed in the present study was different from the results reported by Seif and Elifadil (2009) who tested honey and found the concentration of Ca (mg/kg) to range from 42.37 to 82.92. The difference could probably be due to difference in soil that influences floral types and floral content (Berreta, 1999).

The values of magnesium recorded in current study (Table 5d) are similar to the results reported by Seif and Elifadil (2009) who tested honeys from different vegetation (*Ziziphusspina, Acacia nilotica, Acacia seyal, Helianthus annus and Azadracta indica*) and found magnesium concentration to range between 23.67 to 177.15 mg/kg. Also Parmas *et al*. (2000) determined mineral composition of honey from western Spain by flame photometric method and reported mg concentration of 23.9 mg/kg.

4.3 Antioxidant, Total Phenolic Compounds and Vitamin C Content of Stinging and Stingless Bee Honey

Tables 6a, 6b and 6c present the effect of zone, regions and areas where samples were collected on antioxidant, total phenols and vitamin C content of honey. Table 6d shows the interaction effect of zone, regions and areas on the honey antioxidant, total phenols and vitamin C content.

Results in Table 6a show that there were no significant (P>0.05) differences in antioxidant properties (FRAP values) and total phenols of honey between two zones. However, honey from central zone had significantly (P<0.05) higher vitamin C than that from the western zone.

Table 6a: Effect of honey collected from western and central zones on antioxidant, total phenol and vitamin c content of honey

Zone	FRAP values (µM Fe(II)/100g)	Total phenol (mg/100g)	Vitamin c (mg/100g)
Central	539.90[a]	18.41[a]	4.77[a]
Western	611.58[a]	15.24[a]	3.51[b]
Mean	575.74	16.82	4.14
SE	±44.14	±1.30	±0.36
Significance	NS	NS	*

Note: Means bearing same superscript within the same column are not statistically different (p≥ 0.05) according to pdiff.
NS =Not significant (P>0.05)

Looking at the effect of regions (Table 6b) it is clear that there were variations in FRAP values, total phenols and vitamin C between regions. The variation could be

attributed to difference in soil types that influences the vegetation of the area (Terrab *et al*., 2004). Antioxidant properties (FRAP values) was significantly (P<0.05) higher in Shinyanga honey but did not differ with that of Singida and Tabora (P>0.05). The higher FRAP values of Shinyanga honey may be due to its stronger antioxidant properties compared with all the other honey from other regions, indicating a greater reduction of Fe^{3+} to Fe^{2+} ions. Honey from Singida had significantly (P<0.05) higher vitamin C content than that collected from Tabora, Shinyanga and Dodoma. These observations suggest that honey from Singida could be a rich source of this vitamin than the ones collected from Tabora, Shinyanga and Dodoma.

Table 6b: Effect of region on antioxidant, total phenol and vitamin C content of honey

Region	FRAP values (µM Fe(II)/100g)	Total phenol (mg/100g)	Vitamin C (mg/100g)
Dodoma	440.47^{b}	15.71^{b}	3.88^{b}
Shinyanga	711.11^{a}	13.36^{b}	3.94^{b}
Singida	639.33^{a}	21.10^{a}	5.66^{a}
Tabora	512.04^{ab}	17.12^{ab}	3.07^{b}
Significance	*	*	*
Overall mean	575.74	16.82	4.14
SE	±58.56	±1.78	±0.50

Note: Means bearing same superscript within the same column are not statistically different (p≥ 0.05) according to pdiff.
***** =Significant at P<0.05

Results in Table 6c show that there were no significant (P>0.05) variations in total phenols in honey between areas where honey samples were collected. Honey from

Singida town yielded significantly (P<0.05) highest vitamin C that did not differ from honey collected from Dodoma town, Shinyanga town, Manyoni, Issuna and Bukombe. The results of the present study showed that honey from Inyonga had significantly (P<0.05) higher FRAP value that did not differ with that from Bukombe, Issuna and Shinyanga town, suggesting that honey from these areas could be used as a potential source of these compounds that are important in removing the free radicals from the body that play role in body immunity (Organic Facts, 2016). The honey collected from Kibaigwa had the lowest FRAP value content.

Table 6c: Effect of honey collected from various areas on antioxidant, total phenol and vitamin C content of honey

Area	FRAP values (µM Fe(II)/100g)	Total phenol (mg/100g)	Vitamin C (mg/100g)
Bahi	625.42^{b}	17.91^{a}	3.83^{b}
Bukombe	815.57^{a}	12.88^{a}	4.25^{ab}
Dodoma town	431.92^{c}	17.21^{a}	6.29^{a}
Inyonga	836.37^{a}	22.39^{a}	3.92^{b}
Issuna	795.66^{a}	22.10^{a}	4.66^{a}
Kahama	613.15^{b}	14.11^{a}	2.07^{c}
Kibaigwa	264.06^{d}	12.0^{a}	1.51^{c}
Manyoni	541.49^{bc}	20.20^{a}	5.44^{a}
Nzega	415.95^{c}	15.36^{a}	3.06^{b}
Shinyanga town	704.61^{a}	13.08^{a}	5.52^{a}
Singida town	580.86^{bc}	21.0^{a}	6.86^{a}
Tabora town	283.81^{d}	13.61^{a}	2.24^{c}
Overall mean	575.74	16.82	4.14
SE	±56.59	±3.11	±0.80
Significance	*	NS	*

Note: Means bearing same superscript within the same column are not statistically different according to pdiff (P>0.05) **NS** =Not significant (P>0.05) * = Significant at P<0.05

The results of the present study showed that area where honey samples were collected, honey type and interactions between the two factors significantly (P<0.01) affected the FRAP value, total phenols and vitamin C content of the tested honey samples (Table 6d).

Table 6d: The effect of zones, regions, areas and honey types on antioxidant, total phenols and vitamin C content

Zone	Region	Area	Honey Type	FRAP values (µMFe(II)/100g)	Tphenol (mg/100g)	Vitamin C (mg/100g)
Central	Dodoma	Bahi	Stingless	816.07^{a}	24.69^{bc}	5.45^{d}
			Stinging	434.76^{c}	11.14^{h}	2.22^{ef}
		Town	Stingless	640.71^{b}	26.39^{b}	10.99^{a}
			Stinging	223.12^{d}	8.03^{i}	1.60^{f}
		Kibaigwa	Stingless	455.99^{c}	17.07^{ef}	2.54^{ef}
			Stinging	72.14^{e}	6.93^{i}	0.47^{g}
	Singida	Issuna	Stingless	970.48^{a}	25.23^{b}	7.69^{c}
			Stinging	620.83^{b}	18.98^{de}	1.63^{f}
		Manyoni	Stingless	612.03^{b}	27.23^{b}	8.67^{bc}
			Stinging	470.95^{c}	13.18^{gh}	2.21^{ef}
		Town	Stingless	645.90^{b}	25.67^{b}	9.80^{b}
			Stinging	515.82^{c}	16.35^{ef}	3.92^{e}
Western	Tabora	Inyonga	Stingless	822.50^{a}	33.55^{a}	5.48^{d}
			Stinging	850.24^{a}	11.24^{h}	2.35^{ef}
		Nzega	Stingless	557.26b^{c}	24.07^{bc}	3.95^{e}
			Stinging	274.64^{d}	6.64^{i}	2.17^{ef}
		Town	Stingless	322.16^{d}	22.09^{c}	2.74^{ef}
			Stinging	245.45^{d}	5.13^{i}	1.73^{f}
	Shinyanga	Bukombe	Stingless	848.90^{a}	15.13^{f}	5.51^{d}
			Stinging	782.23^{a}	10.65^{h}	2.97^{e}
		Kahama	Stingless	871.07^{a}	13.87^{gh}	2.53^{ef}
			Stinging	355.24^{d}	14.36^{fg}	1.61^{f}
		Town	Stingless	973.57^{a}	19.96^{d}	9.25^{b}
			Stinging	435.64^{c}	6.19^{i}	1.78^{f}
			SE	±61.79	±0.26	±4.70
			Significance	*	***	***

Note: Means bearing same superscript within the same column are not statistically different (p≥ 0.05) according to pdiff.
NS =Not significant (P>0.05)

*** = Significant at P<0.001

The results of the present study have shown that FRAP values, total phenols and Vitamin C content were significantly (P<0.001) higher in stingless bee honey than that of stinging bee honey in all sampled areas from all the regions in respective zones. The FRAP values range of 72.14 – 973.57 µM Fe(II)/100 g observed in present study are higher than the range of Bengal honey reported by Angira *et al*. (2013) (101.6 – 622.1 µM Fe(II)/100 g) and that reported by Moniruzzaman *et al.* (2014) working with monofloral Bangladeshi honey who observed a FRAP range of 116 – 786.3 µM Fe(II)/100 g. The difference could be attributed to difference in vegetation types. However, the lower FRAP value (of Kibaigwa honey) suggests that

the honey have relatively lower antioxidant properties compared with all the other collected honey samples which might be associated by a less reduction of Fe^{3+} to Fe^{2+} ions (Moniruzzaman *et al.,* 2013).

The observed total phenol (5.13-33.55 mg/100 g) in this study were lower than the range reported by Aline *et al*. (2005) who analysed 27 honey samples from 18 multiflora, 2 honey dews and 7 unifloral honeys in Burkina Faso and reported total phenols of 32.59 to 114.5 mg/100 g. However, the total phenols in the present study were greater than those reported by Lihu *et al*. (2005) working on several honeys using HPLC and obtained total phenols ranging from 2.13 to 12.11 mg/100 g. The variation in phenol content between these tested honey samples could be attributed to difference in floral types, method of analysis, geographic origin as well as climatic characteristics of the sampled areas. Honey with high non-peroxidic substances such as polyphenolic contents is anticipated to possess higher antimicrobial characteristics (Aline *et al*., 2005).

4.4 Correlation between honey colour, total phenols and minerals

Table 7 presents the correlation coefficient between honey colours, total phenols and mineral content in the honey samples. There was a positive and significant ($P<0.001$) correlation between honey colour and total phenolic compounds contained in the honey samples. These observations suggest that the deeper the honey colour the higher the phenolic compounds contained in the honey. Likewise, a significant ($P<0.01$) and positive correlation existed between honey colour and mineral content and total phenols contained in honey. Mineral content, total phenols and anti-oxidant

have been reported to be positively related (Montenegro *et al*., 2009). Therefore, honey with deep colour is expected to have high anti-microbial activities, thus, the deeper the color the honey has the broader the spectrum of anti-microbial, fungal and yeast (Kumar *et al*., 2010).

Table 7: Correlation coefficient between honey bee colour, total phenols and mineral content

	Colour	**Total phenols**	**Zn**	**Fe**	**Ca**	**Mg**	**K**
Colour	1						
Total phenols	0.7729***	1					
Zn	0.6739***	0.6928***	1				
Fe	0.4225***	0.4295***	0.3462**	1			
Ca	0.6505***	0.7549***	0.7554***	0.5456***	1		
Mg	0.8587***	0.7821***	0.8279***	0.2916*	0.7238***	1	
K	0.7598***	0.4045***	0.4133***	0.3644**	0.3644***	0.6969***	1

Note: *** = P< 0.001 ** = P< 0.01 * = P<0.05

CHAPTER FIVE

5.0 Conclusion and Recommendations

5.1 Conclusion

Honey samples collected from the western zone had more ability to inhibit microbial growth than those collected from the central zone. In addition, honey from Shinyanga in the western zone had highest ability to prevent microbial growth followed by Singida, Dodoma and Tabora. On the other hand, honey samples from Nzega and Inyonga had highest ability to resist microbial growth followed by honey samples from Shinyanga town, Bukombe and Kahama. *Candida albicans* was more sensitive to inhibition by stingless bee honey followed by *Staphylococcus saprophyticcus, Salmonella typhi,* whereas *Escherichia coli* was not affected by stinging bee honey. Stinging bee honey was more effective to inhibit growth of *Aspergillus flavus* and *Candida albicans.* It was generally observed that stingless bee honey had higher values for total phenols, antioxidant and vitamin C contents than stinging bee honey. Honey from Inyonga, Manyoni, Dodoma town, Issuna, Singida town, Shinyanga town and Nzega exhibited relatively higher total phenol, antioxidant and vitamin C content. There was no significant ($P>0.05$) difference in pH between stinging and stingless honey samples. However, total reducing sugars and mineral content were higher in stingless than stinging bee honey samples. Total reducing sugar was higher in honey from Kahama, Shinyanga town, Bukombe, Tabora town, Nzega, Issuna, Dodoma town and Bahi. High reducing sugars was also observed in stinging bee honey from Shinyanga town. Similar trend was observed for mineral content, where stingless bee honey contained more minerals thus, had darker

colour (light amber to amber) than the stinging bees' honey. There was a positive and significant ($P<0.05$) correlation between total phenols, honey color, and mineral content of honey, suggesting that the deeper the colour of the honey, the higher the mineral and total phenol content. Therefore, honey with deep colour is expected to have high anti-microbial activities thus, the deeper the color the honey has the broader the spectrum of anti-microbial growth. Generally, stingless honey is superior to stinging bee honey in antimicrobial and antioxidant properties, total reducing sugars, total phenol, vitamin C and mineral content.

5.2 Recommendations for further studies

Stingless honey from the Western and Central zones of Tanzania expressed more medicinal and anti-oxidant properties that are of medicinal importance than that of stinging bees honey therefore, research institutions such as National Medical Research Institute (NIMRI), SUA and other institutions are recommended to carry out further research on;

i. Clinical studies investigating and comparing both the short -term and long-term effects of Tanzania honey supplementation in patients, such as diabetic, in wounds, respiratory diseases, typhoid and UTI patients.

ii. More variables on anti-microbial and physical-chemical properties of Tanzania honey.

iii. Identification and quantification of individual phenolic acids which attribute more to antioxidant and medicinal properties of Tanzania honey.

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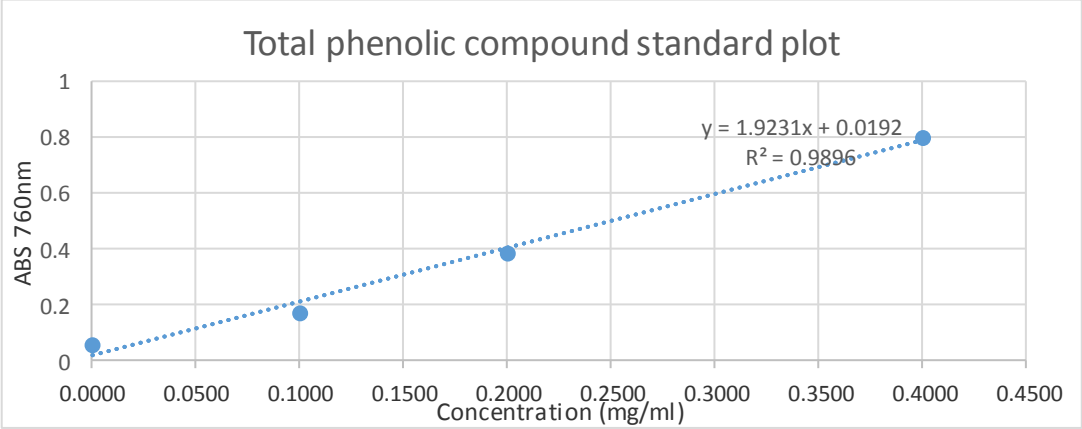
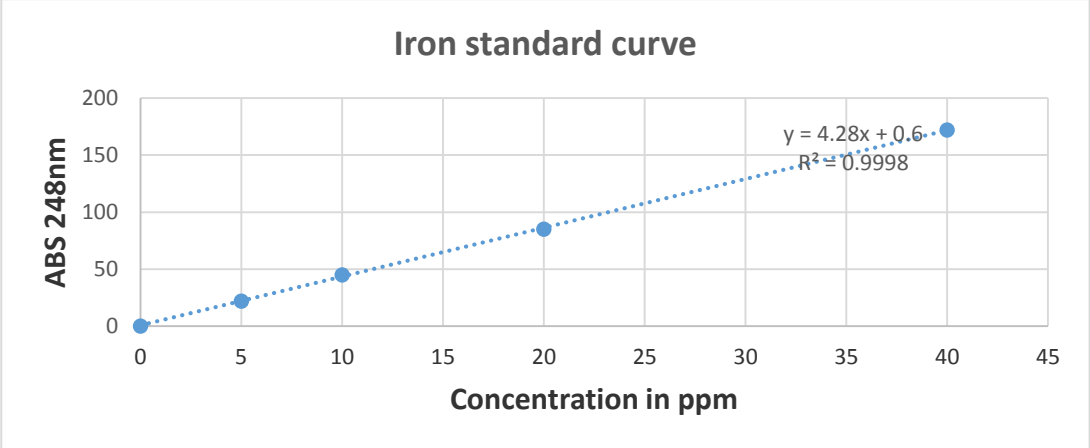
APPENDICES

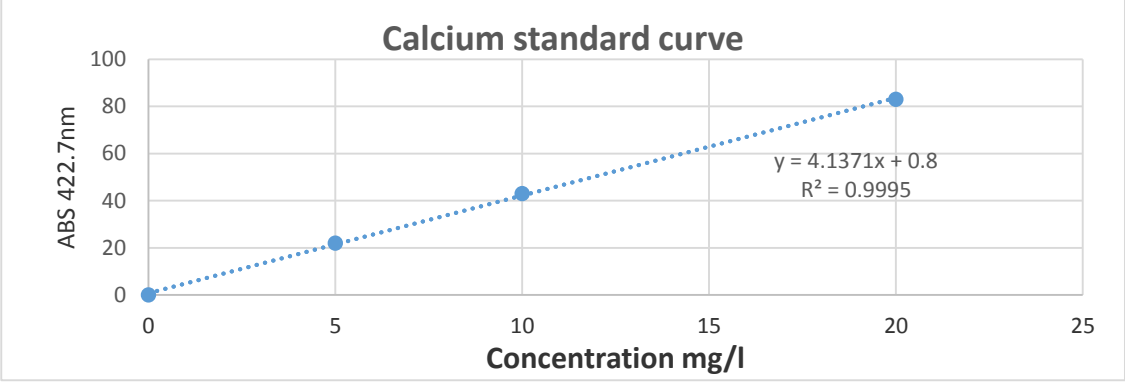
Appendix 1: Total phenolic compound standard curve



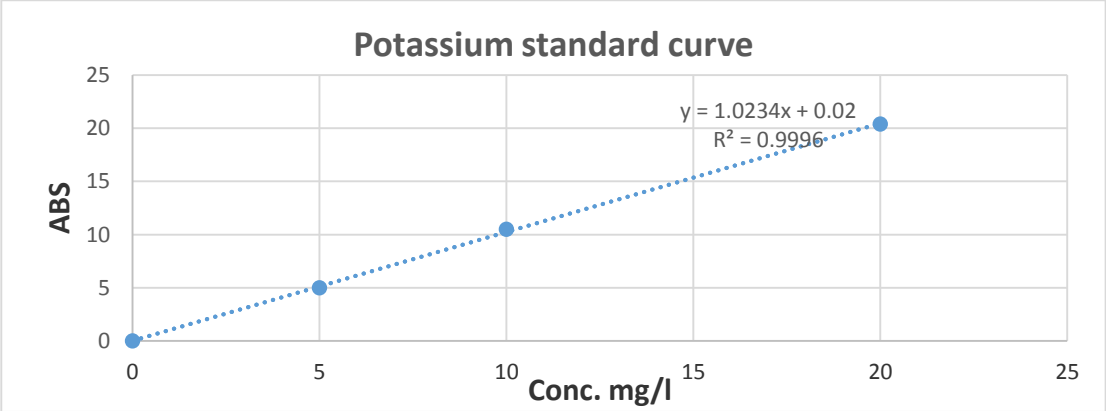
Appendix 2: Iron standard curve



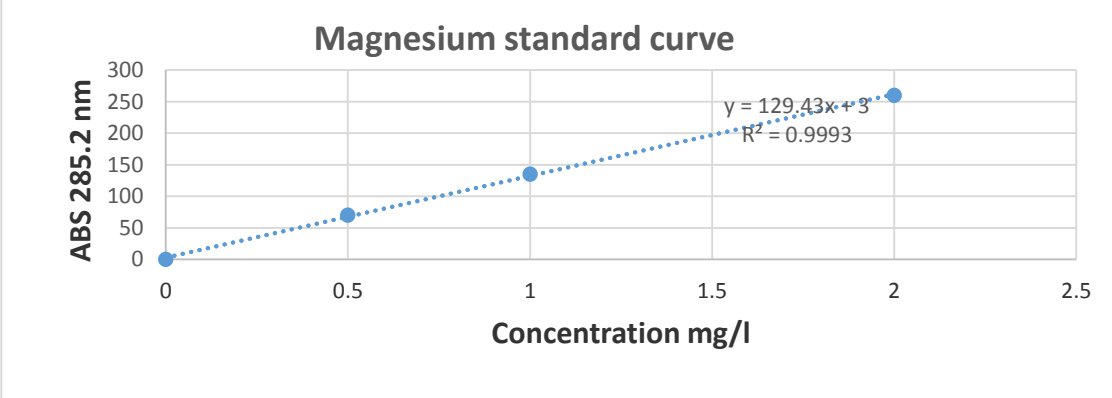
Appendix 3: Calcium standard curve



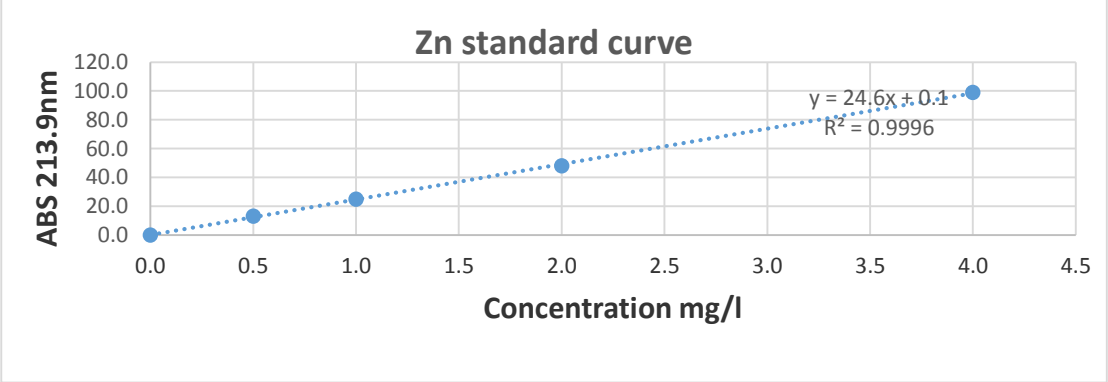
Appendix 4: Potassium standard curve



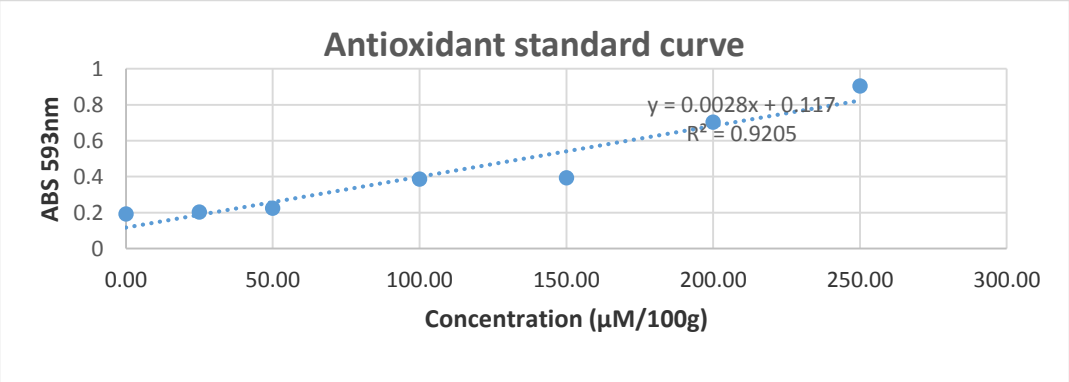
Appendix 5: Magnesium standard curve



Appendix 6: Zinc standard curve



Appendix 7: Antioxidant standard curve



Appendix 8: Ability (%) of honey to inhibit microbial growth

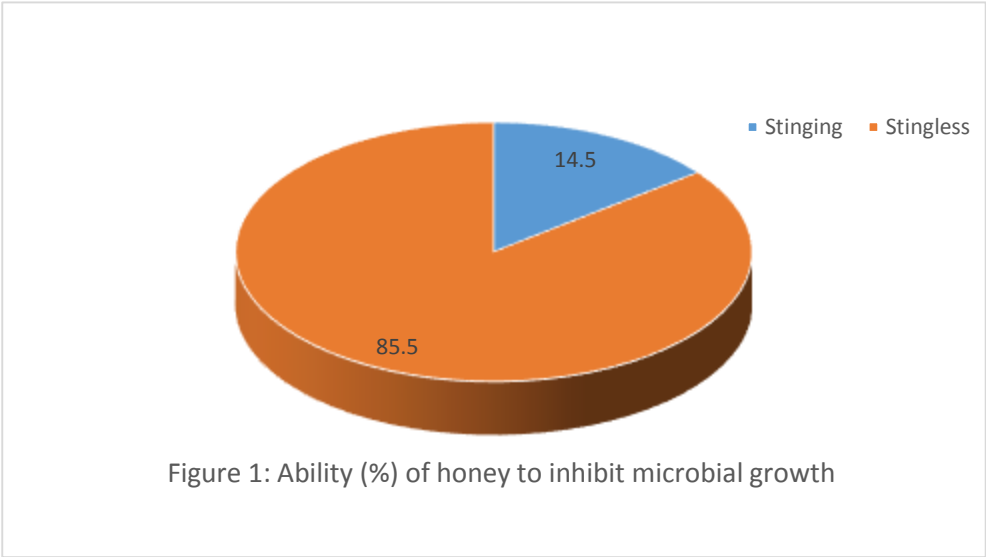


Figure 1: Ability (%) of honey to inhibit microbial growth

Appendix 9: Honey colour grading (%)

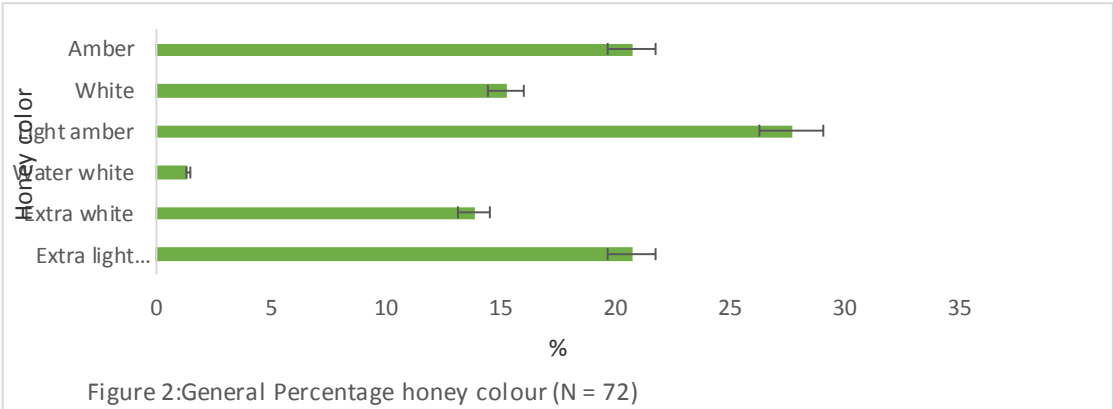


Figure 2:General Percentage honey colour (N = 72)

Appendix 10: Colour grading (%) of stinging and stingless honey bees collected from two zones in Tanzania

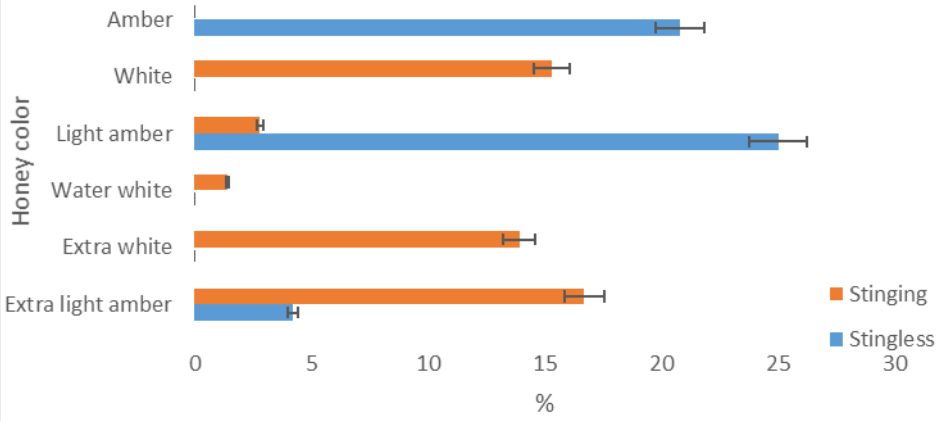


Figure 3:Percentage color grading of stinging and stingless honey bees collected from two zones in Tanzania (N = 72)

Appendix 11a: ANOVA for Dependent Variable: Zn

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	11	30.84536111	2.80412374	50.76	<.0001
HONEYTYPE	1	79.54808889	79.54808889	1440.04	<.0001
AREA*HONEYTYPE	11	36.07781111	3.27980101	59.37	<.0001
Error	48	2.6515333	0.0552403		
Corrected Total	71	149.1227944			

R-Square	Coeff Var	Root MSE	Zn Mean
0.982219	10.19541	0.235033	2.305278

Appendix 11b: ANOVA for Dependent Variable: Fe

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	11	1140.090544	103.644595	87.15	<.0001
HONEYTYPE	1	122.931200	122.931200	103.37	<.0001
AREA*HONEYTYPE	11	440.828100	40.075282	33.70	<.0001
Error	48	57.085667	1.189285		
Corrected Total	71	1760.935511			

R-Square	Coeff Var	Root MSE	Fe Mean
0.967582	13.86774	1.090543	7.863889

Appendix 11c: ANOVA for Dependent Variable: Ca

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	11	447573.3907	40688.4901	59.83	<.0001
HONEYTYPE	1	412632.0710	412632.0710	606.75	<.0001
AREA*HONEYTYPE	11	173574.4634	15779.4967	23.20	<.0001
Error	48	32643.351	680.070		
Corrected Total	71	1066423.277			

R-Square	Coeff Var	Root MSE	Ca Mean
0.969390	13.05329	26.07815	199.7822

Appendix 11d: ANOVA for Dependent Variable: Mg

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	11	57754.4240	5250.4022	14.81	<.0001
HONEYTYPE	1	762808.0542	762808.0542	2151.23	<.0001
AREA*HONEYTYPE	11	70994.2116	6454.0192	18.20	<.0001
Error	48	17020.4062	354.5918		
Corrected Total	71	908577.0961			

R-Square	Coeff Var	Root MSE	Mg Mean
0.981267	12.63456	18.83061	149.0404

Appendix 11e: ANOVA for Dependent Variable: K

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	11	13378938.75	1216267.16	44.69	<.0001
HONEYTYPE	1	37969425.10	37969425.10	1395.03	<.0001
AREA*HONEYTYPE	11	13551887.93	1231989.81	45.26	<.0001
Error	48	1306443.83	27217.58		
Corrected Total	71	66206695.61			

R-Square	Coeff Var	Root MSE	K Mean
0.980267	18.14726	164.9775	909.1044

Appendix 12a: ANOVA for Dependent Variable: Total Phenols

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	11	970.763444	88.251222	35.35	<.0001
HONEYTYPE	1	2669.368889	2669.368889	1069.17	<.0001
AREA*HONEYTYPE	11	699.751244	63.613749	25.48	<.0001
Error	48	119.840800	2.496683		
Corrected Total	71	4459.724378			

R-Square	Coeff Var	Root MSE	TPHENOLS Mean
0.973128	9.391631	1.580090	16.82444

Appendix 12b: ANOVA for Dependent Variable: VITAMINC

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	11	384.6860688	34.9714608	32.62	<.0001
HONTYP	1	622.8768063	622.8768063	580.91	<.0001
AREA*HONTYP	11	255.7082688	23.2462063	21.68	<.0001
Error	120	128.668550	1.072238		
Corrected Total	143	1391.939694			

R-Square	Coeff Var	Root MSE	VITAMINC Mean
0.907562	25.03071	1.035489	4.136875

Appendix 12c: ANOVA for Dependent Variable: Total reducing sugars

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	11	341935.2908	31085.0264	19.54	<.0001
HONTYP	1	311544.4461	311544.4461	195.86	<.0001
AREA*HONTYP	11	298471.6575	27133.7870	17.06	<.0001
Error	120	190880.719	1590.673		
Corrected Total	143	1142832.113			

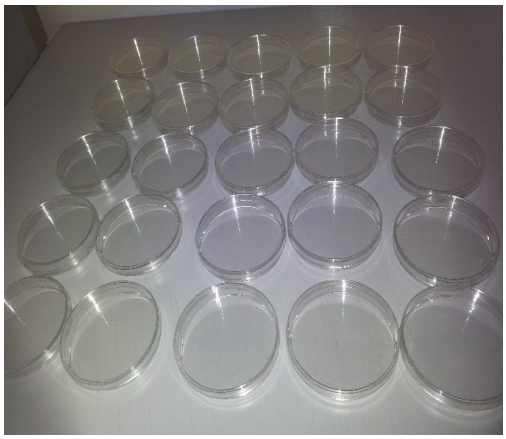
R-Square	Coeff Var	Root MSE	Total reducing sugars Mean
0.832976	16.63386	39.88324	239.7714

Appendix 13: Luff table

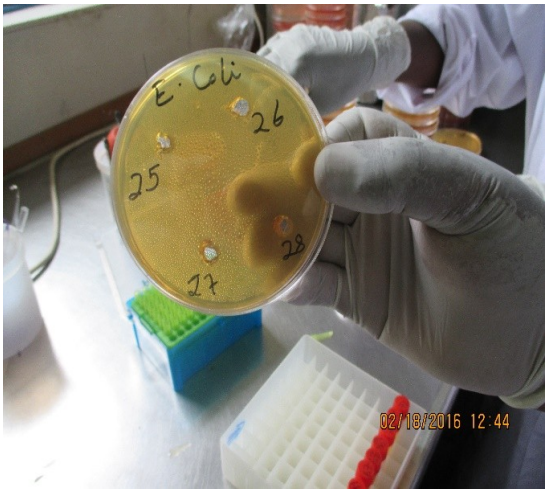
Titration ml of 0.1N thiosulphate (T)	mg glucose or fructose	Ratio
1	2.4	0.417
2	4.8	0.417
3	7.2	0.417
4	9.7	0.412
5	12.2	0.410
6	14.7	0.408
7	17.2	0.407
8	19.8	0.404
9	22.4	0.402
10	25.0	0.400
11	27.6	0.399
12	30.3	0.396
13	33.0	0.394
14	35.7	0.392
15	38.5	0.390
16	41.3	0.387
17	44.2	0.385
18	47.1	0.382
19	50.0	0.380
20	53.0	0.377
21	56.0	0.375
22	59.1	0.372
23	62.2	0.370

Appendix 14: Laboratory work

Sampled honey labelled and packed in 330 mls container and stored at ambient temperature



Empty Petri dishes and Petri dishes with Mueller Hinton Agar medium used for antimicrobial susceptibility test



5mm holes made on the susceptibility test Agar and honey sample (100µl) dropped on 5mm holes using micro-pipette

Color reading using USDA Honey color grading