EVALUATION OF BIOACTIVE COMPONENTS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF HONEY PRODUCED FROM *APIS MELLIFERA* AND *MELIPONULA BECCARII* ALONG VALUE CHAIN FROM GERA DISTRICT TO JIMMA TOWN SOUTH WESTERN ETHIOPIA

M.Sc. THESIS

ZEBENAY NIGUSE

MAY 2024

JIMMA, ETHIOPIA

EVALUATION OF BIOACTIVE COMPONENTS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF HONEY PRODUCED FROM *APIS MELLIFERA* AND *MELIPONULA BECCARII* HONEY ALONG VALUE CHAIN FROM GERA DISTRICT TO JIMMA TOWN SOUTH WESTERN ETHIOPIA

By

Zebenay Niguse

MSc. Thesis

Submitted to Department of Animal Sciences, College of Agriculture and Veterinary Medicine, Jimma University in Partial Fulfillment for the Degree of Master of Science in Animal Production

Major Advisor: Zemene Worku (Ass. Prof, PhD candidate)

Co-Advisor: Metekia Tamiru (PhD candidate)

May 2024

Jimma, Ethiopia

APPROVAL SHEET OF THESIS

JIMMA UNIVERSITY COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

Thesis Submission for External Defense Request Form (F-07)

Name of Student: Zebenay Niguse ID No: Rm0948/14

Program of Study: Animal Production

Title: Evaluation of Bioactive Components, Antioxidant and Antibacterial Activities of Honey Produced from *Apis mellifera* and *Meliponula beccarii* Honey along Value Chain from Gera District to Jimma Town South Western Ethiopia

I have incorporated the suggestions and modifications given during the internal thesis defense and got the approval of my advisors. I hereby kindly request the department to allow me submit my thesis for external thesis defense.

Name of student Zebenay Niguse Signature__________ Date___________

We the Thesis advisors have verified that the student has incorporated the suggestion and modifications during the internal thesis defense and the thesis is ready to submitted, hence we recommend the thesis to be submitted for external defense.

Major Advisor: Mr. Zemene Worku (Ass. Prof) Signature __________Date__________

Co-Advisor: Metekia Tamiru (PhD candidate) Signature ___________Date__________

Decision/suggestion of Department Graduate council (GDC)

____

____

____

DGC _________________________ Signature ______________Date_______________

Chairperson, CGS_________________Signature ____________ Date ______________

DEDICATION

I dedicate this thesis to the Almighty God. Thank you for the guidance, power of mind, protection and skills and for giving me a healthy life. Finally, I dedicate this thesis to my parents, who have supported me throughout my work: my father Niguse Ayele and my mother Aselefech Birhanu.

STATEMENT OF AUTHOR

With my signature below, I declare and confirm that this work is my own work. I have followed all ethical and technical principles of science in the preparation, data collection, data analysis, and completion of this work. All scientific content contained in the thesis has been acknowledged by citation. This thesis is submitted in partial fulfillment of the requirements for the MSc. Degree in Animal Production from Jimma University. The thesis will be deposited in the library of Jimma University College of Agriculture and Veterinary Medicine and is available to borrowers in accordance with the library's rules and regulations. I declare that this thesis has not been submitted to any other institution for the award of an academic degree.

Name: **Zebenay Niguse**

Place: **Jimma, Ethiopia**

Date of submission: **May, 2024**

Signature: ________________

BIOGRAPHICAL SKETCH

The author, Zebenay Niguse was born on October 06, 1998 G.C in Aleltu wereda, North Shoa zone, and Oromia region. She completed her primary education at Fitche Gelila elementary school, secondary and preparatory education at Aleltu Secondary and Preparatory School. Then, she joined Jimma University in 2017 G.C. and graduated with a B.Sc in Animal Sciences in February 2021 G.C. with very great distinction. After graduating, she was employed as Graduate Assistant I at Jimma University in June 2021 G.C. After three months, she got an opportunity to start her MSc studies in Animal Production at Jimma University College of Agriculture and Veterinary Medicine.

ACKNOWLEDGEMENT

This work was made possible with the help of Almighty God. Next, I would like express my great honor and thanks to my advisors, Mr. **Zemene Worku** and Mr. **Metekia Tamiru** for their encouragement, supervision, patient guidance, and constructive criticism they have put on my Thesis for its current shape.

Also, I would like to thanks laboratory technicians of post-harvest management and veterinary medicine for their continuous guidance, encouragement, and support during the experimental work. I am grateful to the Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) for allowing and sponsoring me to pursue my MSc. Finally, I would like to thank my families, friends and colleagues who have supported me in various directions to enable me to successfully complete this work.

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BHT	Butylated Hydroxytoluene
CE	Catechin Equivalent
DPPH	2, 2-Diphenyl -1- Picryhdrazyl
EPHI	Ethiopian Public Health Institute
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic acid equivalent
Kg	Kilogram
LSD	List Significance Difference
MBC	Minimum Bactericidal Concentration
MGO	Methylglyoxal
MIC	Minimum Inhibition Concentration
pH	Power of Hydrogen
ROS	Reactive Oxygen Species
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
UV	Ultraviolet
WHO	World Health Organization

TABLE OF CONTENTS

Contents **Pages**

CONTINUED……..

LIST OF TABLES

LIST OF FIGURE

EVALUATION OF BIOACTIVE COMPONENTS, ANTIOXIDANT ACTIVITIES, AND IN VITRO ANTIBACTERIAL ACTIVITIES OF HONEY PRODUCED FROM *APIS MELLIFERA* AND *MELIPONULA BECCARII* ALONG THE VALUE CHAIN FROM GERA DISTRICT TO JIMMA TOWN SOUTH WESTERN ETHIOPIA

ABSTRACT

Honey is a natural sweetener, rich in bioactive constituents, with complex chemical properties and health benefits. However, these contents can vary with the species of bee and at different value chains. Thus, this study aimed to investigate the bioactive components, antioxidant, and antibacterial activities of honey produced from Apis mellifera and Meliponula beccarii honey along the value chain. The honey samples of Apis mellifera and Meliponula beccarii were collected from four different value chains, namely Gera farmers, Gera Chira local collectors, Agaro town retailers, and Jimma town retailers. The bioactive components, antioxidant, and antibacterial activities of the samples were determined using standard procedures. A two-way analysis of variance was performed using Minitab software version 19 and SAS software version 9.3 to evaluate the bioactive components, antioxidant, and antibacterial activities of honey from Apis mellifera and Meliponula beccarii across the value chain. The bioactive components, antioxidant activities, and antibacterial activities of the honey samples were significantly ($p < 0.05$) affected by bee species and value chain. The result showed that higher total phenolic content (102.81 ± 0.52 mg GAE/100g), total flavonoid content (117.94 ± 0.54 mg CE/100 g), vitamin C content (42.09 ± 0.25 mg/100 g), and beta-carotene content (5.59 ± 0.03 mg/100g) were found in Meliponula beccarii honey, and it showed stronger 2, 2-diphenyl-1- picrylhydrazyl scavenging activity (35.28 ± 0.53%) and stronger ferric reducing antioxidant power (387 ± 0.09 µmol $Fe_2SO_4.7H_2O$/100 g) than Apis mellifera bee honey. But higher alkaloid content (12 ± 0.80%) was found in Apis mellifera bee honey. Meliponula beccarii honey showed better inhibition against Escherichia coli (27.67 ± 1.53 mm) and Staphylococcus aureus (26.00 ± 2.65 mm) at 100% honey concentration. Based on the findings of this study, it could be concluded that bee species and value chain affected the bioactive components, antioxidant, and antibacterial activities of honey. Meliponula beccarii honey is preferred due to its antioxidant properties and antibacterial activity, and quality control measures should be implemented throughout the honey value chain.

Keywords: *Antibacterial, Antioxidant, Apis mellifera, Honey, Meliponula beccari*

1. INTRODUCTION

1.1. Background and Justification

Honey, a natural sweetener with complex chemical properties, is produced by bees collecting nectar from plants and/or insect excretions (Zulkhairi *et al.,* 2018). Commercial production of honey is carried out by two kinds of bees: *Apis* sp. (honeybee) and *Melipona* sp. (stingless bee) (Rosli *et al.,* 2020). It is believed that stingless bees yield less honey than honey bees, which makes them less desirable to humans economically (Chidi *et al.,* 2017). More than 200 distinct chemicals can be found in honey produced by *Meliponiniae* bees (Izzati *et al.,* 2021). Honey contains a range of substances in trace amounts, such as vitamins, enzymes, proteins, minerals, flavonoids, phenolic acids, and other organic acids (Ashagrie, 2021). Darker honey has a higher overall phenolic content, it has been observed to have improved antioxidant action (De Araújo *et al.*, 2023). The phenolic and flavonoid chemicals that makeup honey are thought to be responsible for most of its biological activity (Al-Kafaween *et al.,* 2023). The presence of polyphenols in honey is believed to be from the plant's nectar while polyphenols quality and quantity depend on the geographical region, floral source, climatic conditions, and bee type (Rosli *et al.,* 2020; Boudiar *et al.,* 2022).

Honey has long been valued for its nutritional and medicinal properties (Al-Kafaween *et al.,* 2023). Honey from a stingless bee was reported to have antimicrobial and antioxidant properties (Biluca *et al.,* 2020; Mahmood *et al.,* 2021) and is suggested as an alternative medicine to help in wound healing (Al-Mamary *et al.*, 2002; Mahmood *et al.,* 2021). Due to its excellent therapeutic qualities and safety for human use, honey produced by *Meliponiniae* bees has recently acquired favor (Ngaini *et al.,* 2023). In both human and animal health, honey has been rediscovered as a treatment for burns, gastrointestinal problems, asthma, infected wounds, and skin ulcers (Wasihun & Kasa, 2016). For many years, honey has been used as a more potent functional food in alternative and traditional medicine to treat conditions caused by microbial infections (Nordin *et al.,* 2018). Honey has antioxidant (Blasa *et al.,* 2007; Lachman *et al.,* 2010), antibacterial (Lusby *et al.,* 2005; Deng *et al.,* 2018; Bucekova *et al.,* 2019; Ofijan *et al.,* 2022), antifungal (Irish *et al.,* 2006; Aurongzeb *et al.,* 2019), anti-viral, (Ikeda *et al.,* 2011; Wan Yusuf *et al.,* 2019) and anti- inflammatory properties (Biluca *et al.*, 2020).

Previous studies have shown that the antibacterial properties are associated with intrinsic characteristics like high osmolarity and acidity, alike compounds such as hydrogen peroxide (H_2O_2), methyl syringate and methylglyoxal, defensin-1, nitric oxide metabolites and phenolic acids and flavonoids strongly linked to antibacterial activity (Combarros-Fuertes *et al.,* 2019; Grecka *et al.,* 2019; Hau-Yama *et al.,* 2020). Compared to most common honey bees, stingless honey has less sugar and greater antibacterial activity (Rosli *et al.,* 2020). The ability of honey to fight different types of microorganisms is determined by various variables, including the kind and natural structure of the nectar and the environmental circumstances in which the bees were raised (Al-Kafaween *et al.,* 2023).

Ethiopia's diverse climate, abundant bee flora (over 800 honey plant species identified (Kebede *et al.,* 2012)), and established reputation for high-quality honey production make its southwest region a prime area for honey production (Adgaba, 2007; Abdi *et al.,* 2023). High-quality honey is produced in the hive, but low-quality honey is produced during inadequate handling from harvest to sale. The accessibility of floral resources and the treatment of beekeepers and retailers have an impact on the quality of honey as well (Clearwater *et al.,* 2018; Khansaritoreh *et al.,* 2021). Honey sources and floral types, bee species, geographical origin, climate, harvesting time, processing, and storage conditions, are some of the factors that are linked to and affect the honey's bioactive components, antioxidant activities, and antibacterial activity (Moniruzzaman *et al.,* 2013; Adadi & Obeng, 2017; Bucekova *et al.,* 2019; Ofijan *et al.,* 2022). The sophisticated issue of adulterating honey nowadays has a significant effect on the product's safety and quality (Ávila *et al.,* 2019). Moreover because of post-harvest processing, yeasts and spore-forming bacteria are frequently discovered among the microorganisms in honey (Nordin *et al.,* 2018).

Fewer studies have been conducted on the bioactive components, antioxidant activities, and antibacterial activities of *Apis mellifera* and *Meliponula beccarii* honey. A study conducted by Ofijan (2023) showed the harvesting period and antioxidant properties of unifloral (*Apis mellifera L.*) honey based on floral origin. In addition, Ofijan *et al.* (2022) investigated western Ethiopian A*pis mellifera L.* and *Meliponulla beccarii L.* honey based on their in vitro antimicrobial properties. In a study conducted by Hawine (2020), Ethiopian honey was analyzed, focusing on its botanical origin, physicochemical properties, antimicrobial effects, phytochemical content, antioxidant activity, sensorial characteristics, and glycemic index derived from *Coffea arabica* and *Vernonia amygdalina.* Similarly, Hailu & Belay (2020) also investigated Melissopalynology and antioxidant properties used to differentiate Ethiopian *Schefflera abyssinica* and *polyfloral* honey. Sime *et al.* (2015) examined Ethiopian honey based on total phenols and antioxidant activities of natural honey and propolis collected from different geographical regions. While existing research has investigated aspects of honey quality, including antioxidant properties, phytochemical content, and antimicrobial activity, these studies often suffer from limitations. They tend to focus on isolated characteristics, such as geographical origin or a single property like antioxidant, phytochemical content, and antimicrobial activity. Additionally, the scope is frequently limited by investigating only specific honey types (unifloral *Apis mellifera*) or honey from particular regions. Most importantly, a critical gap exists in understanding how these properties change along the value chain, particularly from producer to retailer. Understanding this transformation is essential, as the value chain can significantly impact honey's properties and potentially influence its overall effectiveness. Therefore, the current study aimed to investigate the bioactive components, antioxidant activities, and in vitro antibacterial activities of honey produced from *Apis mellifera* and *Meliponula beccarii* honey along the value chain.

1.2. Objectives of the study

1.2.1. General objective

- To evaluate bioactive components, antioxidant and antibacterial activities of honey produced from *Apis mellifera* and *Meliponula beccarii* honey along the value chain from Gera district to Jimma town southwestern Ethiopia.

1.2.2. Specific objectives

- To evaluate the bioactive components of honey produced from *Apis mellifera* and *Meliponula beccarii*
- To evaluate the antioxidant properties of honey produced from *Apis mellifera* and *Meliponula beccarii*
- To examine antibacterial activities of honey produced from *Apis mellifera* and *Meliponula beccarii*

2. LITERATURE REVIEW

2.1. Honey and its Importance

Currently, stingless bee honey and conventional *Apis mellifera* honey are the two varieties of honey produced worldwide (Ávila *et al.,* 2018). The Latin name for honeybees is *Apis*, and they use the nectar that they collect from plants to make honey after regurgitating and digesting it (Bicudo de Almeida-Muradian *et al.,* 2020). To prevent honey from fermenting, their wings fan the honey, evaporating the water content in the nectar (Ranneh *et al.,* 2021). Stingless bees get their name because, in contrast to *Apis mellifera* bees, which are known around the world for producing traditional honey, they do not sting (Roubik *et al.,* 2013; Braghini *et al.,* 2022). The stingless bee is characterized by its ability to gather nectar from creeping plants, travel short distances in search of food, and build hives that are horizontally rather than vertically oriented to store pollen and nectar(Nur Eszaty *et al.,* 2022). The stingless bee's mandibles produce mandibular secretions during construction, which are added to cerumen, a combination similar to propolis, where the bees store their honey (Vit *et al.,* 2004; Nordin *et al.,* 2018). According to Ávila *et al*. (2018), stingless bee honey is renowned for its unique flavor and perfume, as well as its fluid texture and gradual crystallization (Biluca *et al.,* 2016).

In Ethiopia, beekeeping and the collection of honey from wild stingless bees are important for socioeconomic development and can account for as much as 50% of household income. Widely utilized in both traditional and contemporary medicine, stingless bee honey (referred to as *tazima* (*tazma*) *mar* locally), pollen, and wax have long been known to possess potent antibacterial, immune-stimulatory, anti-inflammatory, antioxidant, and wound-healing qualities (Jemberie *et al.,* 2020).

The potent antioxidant capacity of honey is a result of the forest's biodiversity (Nur Eszaty *et al.,* 2022). This is because there are a lot of flavonoid and phenolic chemicals present, which increase antioxidant activity (Ibrahimi & Hajdari, 2020). The most prevalent polyphenols in this beehive product, flavonoids, and phenolic acids, are the main antioxidants (Pontis *et al.,* 2014; Keng *et al.,* 2017). Compared to honey made by *Apis mellifera*, stingless bee honey has a higher concentration of flavonoids and phenolic acids (Ávila *et al.,* 2018). By releasing hydrogen from one of the hydroxyl groups in free radicals, this phenolic molecule stabilizes those (Cianciosi *et al.,* 2018).

In comparison to *Apis mellifera* honey, stingless bee honey from Cuba was also demonstrated to have higher levels of flavonoids, carotenoids, ascorbic acid, phenolic compounds, free amino acids, and protein, as well as stronger antioxidant activity (Alvarez-Suarez *et al.*, 2018). A study by Kek *et al.* (2018) found that the antioxidant activity of stingless bee honey generated by *Heterotrigona itama* bees was double that of *Apis mellifera* honey. Ascorbic acid, tocopherol, and phenolic compounds, on the other hand, are non-enzymatic substances that neutralize reactive oxygen species by preventing and interrupting the harmful chain events that free radicals generate (Chettri & Kumari, 2020). Stingless bee honey's phenolic and flavonoid content scavenges free radicals and produces more stable and less reactive radical species as a result, making it a potent antioxidant (Nur Eszaty *et al.*, 2022).

Honey's antibacterial properties work well against a wide range of dangerous microbes ((Minden-Birkenmaier & Bowlin, 2018; Cebrero *et al.*, 2020). A low pH, a high osmolarity, and hydrogen peroxide are some of the factors that contribute to its antibacterial qualities (Stagos *et al.*, 2018). According to Cianciosi *et al.* (2018) and Purbafrani *et al.* (2014), it can help promote the growth of new tissue to mend lesions as well as heal burns, wounds, skin ulcers, and inflammations. The variables include the honey's geographical distribution during collection, the time of year, the source of the flowers, processing, storage conditions, and the bacteria's susceptibility to the antibiotic substances in honey (Omar *et al.*, 2019).

There are two types of antibacterial qualities in stingless bee honey: peroxide and non-peroxide (Abd Jalil *et al.*, 2017). Non-peroxide activity may also be the cause of stingless bee honey's antibacterial activity, which is more stable and consistent than that of *Apis mellifera* honey (Nweze *et al.*, 2017; Ávila *et al.*, 2018). Manuka honey was discovered to include antimicrobial peptides Nishio *et al.* (2016). However, Ng *et al.* (2020) observed that the honey from stingless bees was devoid of methylglyoxal (MGO), dihydroxyacetone, and phenolic, which are typical of *manuka* plant nectars. Furthermore, stingless bee honey's very acidic environment could contribute to its remarkable antibacterial properties and potential lethality against a range of microorganisms (Minden-Birkenmaier & Bowlin, 2018; Rosli *et al.*, 2020; Mark *et al.*, 2021).

Furthermore, stingless bee honey can prevent human gastrointestinal infections and reduce the duration of illness for eye disorders brought on by *S. aureus* and *P. aeruginosa*

(Yaacob *et al.,* 2018). The phenolic compounds in stingless bee honey may act in concert to cause this effect (Borsato *et al.,* 2014). According to Patricia *et al.* (2015), the synergistic impact of the hydrogen peroxide and sugar in stingless bee honey works in tandem to promote wound healing. Phenolic acid from an extract of stingless bee honey, such as p-coumaric acid, caffeic acid, and salicylic acid, also reduced the generation of ROS at the site of injury (Yaacob *et al.,* 2018). Several pro-inflammatory cytokines/chemokines were shown to be downregulated by flavonoid chemicals, including kaempferol, quercetin, luteolin, genistein, apigenin, galanin, and naringenin (Biluca *et al.,* 2020). They protected cells against the cytotoxicity brought on by pro-inflammatory mediators by acting as free radical scavengers (Abd Jalil *et al.,* 2017).

2.2. Bioactive components of Honey

Honey's antioxidant activity is derived from phytochemicals (Molan, 2006), which also can prevent other molecules from oxidizing (Meo *et al.,* 2017). The majority of phytochemicals found in all plants are called phenolic phytochemicals, and honey is made from the nectar or pollen of plants that honeybees visit (Alvarez-Suarez *et al.,* 2018). Polyphenols, primarily flavonoids, phenolic acids, and their derivatives, are found in honey. According to Istasse *et al.* (2016), these substances come from a variety of sources, including nectar, pollen, honeydew, and propolis. Phenolic molecules and flavonoids are mostly responsible for antioxidant activity (Becerril-sánchez *et al.,* 2021). Therefore, according to Afroz *et al*. (2016), the functional and therapeutic qualities of honey are enhanced by the presence of phenolic acids.

Numerous vital biologically active substances, such as flavonoids (Qamer *et al.,* 2016), phenolic (Muhammad *et al.,* 2015), vitamins A (retinol), and C (ascorbic acid), are found in honey. According to Afrin *et al.* (2020), honey contains two main categories of phenolic compounds: phenolic acids and flavonoids. Honey's primary functional ingredients are flavonoids. Their nuclear structure is C6–C3–C6, connecting two benzene rings through a pyran ring (Da Silva *et al.,* 2016). The three main groups of flavonoids that arise from ring replacement are flavonols, flavones, and flavanones (Prithviraj Karak, 2019). Additionally, according to Meo *et al.* (2017), proline, glutamic acid, aspartic acid, arginine, and cysteine are some amino acids with physiological significance. Depending on the weather and geographic conditions, honey has different amounts of flavonoids, phenolic, amino acids, proteins, ascorbic acid, and carotenoid contents as well as antibacterial and antioxidant qualities (Alvarez-Suarez *et al.*, 2010).

2.2.1. Total Phenolic Content

According to Gül & Pehlivan, (2018), honey is a naturally occurring dietary antioxidant that contains components that are likely responsible for its redox characteristics. Phenols with a single carboxylic acid functional group are generally referred to as phenolic acids (Robbins, 2003). Two unique carbon frameworks are seen in naturally occurring phenolic acids: the hydroxyl benzoic and hydroxyl cinnamic structures (Koche *et al.*, 2016). Foods' color, texture, and antioxidant and nutritional value have all been linked to phenolic acids (Becerril-sánchez *et al.,* 2021).

According to Biluca *et al.* (2016), the total phenolic component concentration of the stingless bee honey samples ranged from 10.3 to 98.0 mg GAE 100 g^{-1}. The honey samples had significant total phenolic contents, ranging from 233.3 ± 24.0 mg GAE/kg to 693.3 ± 26.8 mg GAE/kg (Adgaba *et al.,* 2020). The TPC in honey varied from 23.1 to 158 mg/100 g (Combarros-Fuertes *et al.*, 2019). The phenolic content of Brazilian stingless bee honey is higher than that of *Apis* spp., weighing 106.01 ± 9.85 mg GAE/100 g versus 92.34 ± 13.55 mg GAE/100 g for *Apis* spp. (Duarte *et al.,* 2012). Similar to Tualang honey from *Apis* spp., which has 183 mg GAE/100 mg, stingless bee honey in Malaysia has about 235 mg GAE/100 mg of total phenolic content (Ranneh *et al.,* 2018; Ranneh *et al.,* 2021).

2.2.2. Total Flavonoid Contents

The majority of low molecular weight phenolic chemicals found in human diets that are found abundantly throughout the plant kingdom are called flavonoids (Poquet *et al.,* 2009). They belong to a family of 19 secondary plant metabolites, and it is believed that their chelating and antioxidant qualities, which together account for the majority of their antioxidant capacity, have positive health effects (Williams *et al.*, 2004). Flavonoids work by scavenging or chelating substances to exert their effects. Honey's anti-inflammatory and antioxidant properties are mostly attributed to flavonoids (Afroz *et al.,* 2016).

Honey is rich in flavonoids. Popular natural antioxidants and flavonoids have a variety of biological impacts (Małgorzata Brodowska, 2017). Honey's components, flavonol, flavone, anthocyanidin, and organic acid, are primarily responsible for its nutritional importance (Bian *et al.,* 2022). Total flavonoid content ranged between 1.65 and 5.93 mg CE/100 g of honey Combarros-Fuertes *et al.* (2019), 7.97 - 44.99 mg CE/100 $g^{-1}$of honey (Galhardo *et al.*, 2021), 10.70–25.71 mg CE/100 g (Shamsudin *et al.*, 2022). The flavonoid content of Malaysian stingless bee honey is 100 mg CE equivalent to 100 mg (Ranneh *et al.,* 2018; Ranneh *et al.*, 2021).

2.2.3. Vitamin C (L-ascorbic acid)

Because it is recognized to be a rich source of antioxidants, such as ascorbic acid, glucose oxidase, catalase, phenolic compounds, carotenoids, organic acids, amino acids, and proteins, honey continues to have a significant position in terms of nutrition (Özcan & Al Juhaimi, 2015). One of the primary characteristics of honey is its botanical origin (Adgaba *et al.,* 2017). It has been noted that processing, seasonal and environmental conditions, the type of flower used to gather nectar, and the honey's composition and antioxidant capability all affect these qualities (Gül & Pehlivan, 2018). Ascorbic acid, also known as vitamin C, is a water-soluble vitamin that occurs naturally in some foods (Aysun, 2009).

It is an antioxidant that dissolves in water and reacts quickly with peroxyl and superoxide radicals (Gupta, 2015). According to Bahar *et al.* (2022), vitamin C equivalents ranged from 412.22 to 1117.78 µmol/mg or from 725.99 to 1968.59 µg/mg or from 7.26 to 19.69 mg/100g of honey. Samples of honey were found to contain vitamin C. Nonetheless, there were significant differences in their levels, which varied from 0.34 to 75.9 mg/100 g of honey (Combarros-Fuertes *et al.,* 2019).

2.2.4. Beta Carotene

Carotenoids are the type of organic nutritional substances that are pigments present in both plants and animals (Pasarin & Rovinaru, 2018). The carotenoid content of Malaysian Kelulut Honey and peat land Kelulut Honey of Banjarbaru were determined to be 4.61 mg/100g (Keng *et al.,* 2017) and 0.617 ± 0.001 mg/100g (Satriadi *et al.,* 2023), respectively. Variations in bee feed type and season may impact the amount of carotenoids (Ahmed, 2015). A high amount of carotenoids is a result of a dry season and a diverse bee diet (Mahmood *et al.,* 2021). Carotenoids' primary advantage is that they function as antioxidants. Additionally, it can be converted into vital vitamins (Keng *et al.,* 2017).

2.2.5. Alkaloids

Alkaloids are naturally occurring compounds found in fungi, plants, and animals that have nitrogen in their structure (Wink, 2013). Alkaloids are important for plant survival because they defend plants from insects, herbivores, and microorganisms (exhibiting antibacterial and antifungal properties) (Koche *et al.*, 2016). Alkaloids are nitrogen-containing molecules with low molecular weight that are usually alkaline because they have a nitrogen atom in a heterocyclic ring (Matsuura & Fett-Neto, 2015). These metabolites can be categorized into over 20 different classes (including *pyrrolidine* alkaloids, tropane alkaloids, *piperidine* alkaloids, pyridine alkaloids, *quinolizidine* alkaloids, and *indole* alkaloids, among others) based on their precursor (e.g., indole alkaloids are alkaloids derived from tryptophan) (Matsuura & Fett-Neto, 2015). Alkaloids play a significant role in human health as chemicals, medications, vitamins, and other medicinal products (Saranra*j et al.*, 2016).

Table 1: Bioactive components and Antioxidant Activities of *Meliponula beccarii* and *Apis mellifera* honey

Bioactive contents	**Percentages/ Contents**		**References**
	Apis Mellifera	*Meliponula beccarii*	
TPC (mg GAE/100 g^{-1})	11.39 - 61.27 23.1 - 158	52.64 -74.72 10.3 - 98.0 23.33 - 69.33	Galhardo *et al.* (2021); Combarros-Fuertes *et al.* (2019); Shamsudin *et al.* (2022); Biluca *et al.* (2016) Adgaba *et al.* (2020)
TFC (mg CE/100 g^{-1})	7.97 - 44.99 1.65 - 5.93	10.70–25.71	Galhardo *et al.* (2021); Combarros-Fuertes *et al.* (2019) Shamsudin *et al.* (2022)
Alkaloid (mg/g)	2.58	119.71	Igbang *et al.* (2018) Mahani *et al.* (2022)
Vitamin C (mg/100g)	7.26 - 19.69	0.34 - 75.9	Bahar *et al.* (2022) Combarros-Fuertes *et al.* (2019)
Beta carotene (mg/kg)	0.617	1.55-3.09 8.64-9.49 0.05 - 0.25	Satriadi *et al.* (2023) Keng *et al.* (2017) Mahmood *et al.* (2021)
DPPH (µmol ET/ g^{-1})	0.04 - 0.16	11.27–24.09	Galhardo *et al.* (2021) Shamsudin *et al.* (2022)
FRAP (µmol FeSO4/ g^{-1})	0.03 -11.12	77.88–164.88	Galhardo *et al.* (2021) Shamsudin *et al.* (2022)

Alkaloids are a type of chemical compounds that are alkaline and have poisonous properties. They can be employed as detoxifiers to help the body eliminate pollutants(Thawabteh *et al.,* 2021). Alkaloids are among the substances that give honey its bitter flavor (Mahani *et al.,* 2022). Furthermore, alkaloids in particular function as antimicrobials, which can avert a range of illnesses brought on by microbial infections (Yap & Abu Bakar, 2014). Alkaloids are beneficial to health; to create honey with the best alkaloid levels, it is necessary to analyze the alkaloid content of probable honey sources (Wang *et al.,* 2019). The total alkaloid content of honey was 119.71 ±1.66 mg/100g; 2,58 mg/100g (Igbang *et al.,* 2018; Mahani *et al.,* 2022) respectively.

2.3. Antioxidant Activities

Antioxidants can be categorized into enzymatic and non-enzymatic types based on their mode of action(Mirończuk-Chodakowska *et al.,* 2018). Enzyme-based antioxidants eliminate and break down free radicals, while non-enzymatic antioxidants work by preventing the spread of free radicals(Alkadi, 2022). Vitamin C, vitamin E, plant polyphenols, carotenoids, and glutathione are a few examples of non-enzymatic antioxidants (Shahidi & Zhong, 2010). The integrity and functionality of cell membranes are preserved by antioxidant enzymes, which reduce the levels of lipid hydroperoxide and H_2O_2, thus preventing lipid peroxidation (Elsayed Azab *et al.,* 2019). According to Nimse & Pal (2015), there are two categories of non-enzymatic antioxidants: natural antioxidants and synthetic antioxidants. Various naturally occurring antioxidants include phenolic compounds, phenolic acids, flavonoids, coumarins, sesquiterpene lactones, terpenoids, and their derivatives(Seca & Moujir, 2020).

Honey is a rich source of natural antioxidants, and plays a major role in health maintenance and disease prevention (Habib *et al.,* 2014). It provides a variety of nutrients that are necessary for human health(Bogdanov *et al.,* 2008). Its elevated phenolic content contributes to its antioxidant properties (Kishore *et al.,* 2011). According to Alvarez-Suarez *et al.* (2014), honey is a raw material used to make pharmaceutical products for wound treatment, anti-cancer therapy, antibacterial agents, and supplements for pregnant women.

Honey contains different chemicals depending on the climate and geography(Tomczyk *et al.,* 2019). For instance, rosemary honey contains flavanol kaempferol, while sunflower honey has quercetin (Inhan-Garip *et al.,* 2011). Depending on the source of the flowers, honey has varying levels of antioxidant activity(Rababah *et al.,* 2014). Season, environment, and farming practices are a few significant outside variables that impact the makeup and therapeutic value of honey (Bogdanov *et al.,* 2008; Kıvrak & Kıvrak, 2017). Good nutritional values found in stingless bee honey can help treat disorders linked to oxidative stress (Satriadi *et al.,* 2023).

2.3.1. 2, 2-diphenyl-2-picrylhydrazyl

DPPH has been widely utilized to assess a sample's capacity to scavenge free radicals(Chua *et al.,* 2013). Because the antioxidant activity of honey has been demonstrated to be directly correlated with its phenolic acid and flavonoid content, the DPPH assay is widely employed to assess the radical-scavenging potential of honey (Beretta *et al.,* 2005). The antioxidant activity of *Apis mellifera* honey is 0.12 mg/mL of DPPH (Galhardo *et al.,* 2021). The free radical scavenging activity (IC50) of stingless bee honey ranges from 11.27 to 24.09 mg/mL (Shamsudin *et al.,* 2022).

2.3.2. Ferric Reducing Antioxidant Power

Honey's antioxidant and reducing power have been evaluated using ferric reducing antioxidant power (FRAP), a commonly used technique for determining antioxidant levels(Alzahrani *et al.,* 2012). The FRAP assay uses the ability of a sample to reduce the Fe3+/Fe2+ pair to directly assess the amount of reductants or antioxidants present in the sample (Bakchiche *et al.,* 2017). The greatest ferric ion reduction ability is exhibited by darker honey (Ferreira *et al.,* 2009). The lowering capacity of honey may affect the FRAP values (Kishore *et al.,* 2011). Greater conversion of ferric ions to ferrous ions is indicated by high FRAP levels(Moniruzzaman *et al.,* 2012). Ferric-reducing antioxidant power and the total phenol content of honey are highly associated (Chua *et al.,* 2013).

The reducing power of the stingless bee honey produced by the different bee species (Irshad *et al.,* 2012). The average total antioxidant capacities of the honey samples as Ferric Reducing Antioxidant Power (FRAP) were varying 225.4 – 465.7 FRAP (lM Fe(II)/100 g with a mean of 320.3 lM Fe(II)/100 g (Adgaba *et al.,* 2020). The antioxidant content of *A. mellifera* honey ranged from 19.00 - 73.27 eq. mg GA / 100 g (average 48.31 ± 14.80 mg GA equivalent / 100 g) (Duarte *et al.,* 2012). Stingless bee honey contains

77.88–164.88 µmol FeSO4.7H2O/100 g honey ferric reducing antioxidant power (FRAP)(Shamsudin *et al., 2*022). *Apis mellifera* honey contains antioxidant activity of 2.68 µmol FE (II)/g^{-1} of FRAP (Galhardo *et al.*, 2021).

2.4. Antibacterial Activity of Honey

Honey's powerful osmotic action, naturally low pH, and capacity to create hydrogen peroxide which is essential to honey's antimicrobial activity and phytochemical components have all been linked to its antibacterial efficacy (Nur Eszaty *et al.,* 2022). Flavonoids and phenolic compounds contribute to the antibacterial properties of both peroxide and non-peroxide honey (Hossain *et al.,* 2022). Honey's low water content, high sugar content, acidity, and hydrogen peroxide levels all contribute to its antibacterial effect (Mavric *et al.,* 2008). Honey's antibacterial activity differs from that of antibiotics, which either breaks down the bacterial cell wall or obstructs intracellular metabolic processes. Four characteristics of honey are connected to its antibacterial activity(Massoura, 2019). Honey primarily dehydrates bacteria by taking moisture out of the air. Honey has antibacterial properties due to a variety of factors, not just the high sugar content that stops microbes from growing (Machado De-Melo *et al.,* 2018). Next, honey has an acidity level that is low enough to prevent the majority of microbes from growing, ranging from 3.2 to 4.5 (Almasaudi, 2021). The third and most important antibacterial component is hydrogen peroxide, which is produced by the glucose oxidase; however, other authors say that the non-peroxide activity is more significant(Nolan *et al.*, 2019). Lastly, a number of phytochemical components for honey's antibacterial activity have been found (Eteraf-Oskouei & Najafi, 2013).

Natural honey contains enzymes, mostly glucose oxidase, which reacts to make hydrogen peroxide (Brudzynski, 2020). When bees produce honey, the hypo pharyngeal gland helps release glucose oxidase into the nectar of flowers through an enzymatic process (Ajibola, 2015). These enzymes play a critical role in assessing the peroxide activity level in honey, which is responsible for many biological processes, including its antibacterial activity (Bacayo *et al.,* 2019). Hydrolyzing glucose to produce hydrogen peroxide (H_2O_2) in the presence of a high concentration of active glucose oxidase induces oxidative stress, which helps prevent bacterial colonization (Samarghandian *et al.,* 2017). According to Brown *et al.* (2020), the antimicrobial activity of honey is therefore a result of many chemicals and compounds with various chemical properties, depending on the honey's botanical origin.

2.4.1. Agar Diffusion Assay

Agar well diffusion assays are widely used to evaluate natural compounds' antibacterial properties. The foundation of this assay is the measurement of the area surrounding the sample, which can be put into an agar well or onto a paper disc (Chowdhury *et al.,* 2015). A detectable growth inhibition zone is the outcome of this (Hossain *et al.*, 2022). The antimicrobial activity of honey from the good diffusion method ranged from 9.7 ± 2.0 mm by the A. mellifera honey against clinical E.coli to 18.3 ± 3.2 mm by *M. beccari* honey against clinical S. aureus(Ofijan *et al.,* 2022). The antibacterial activity of honey obtained by the good diffusion method varied, with *A. mellifera* honey showing 9.7 ± 2.0 mm against clinical *E. coli* and *M. beccari* honey showing 18.3 ± 3.2 mm against clinical *S. aureus*(Cui *et al.,* 2021).

2.4.2. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Hindering the growth of tested human pathogens at comparatively lower concentrations, the majority of the analyzed honey samples had excellent antibacterial capabilities, with an average range of 10.5 ± 0.5–28.6 ± 0.9 of mean inhibition concentration (MIC) (% v/v)(Zainol *et al.,* 2013). According to Adgaba *et al.* (2020), *Staphylococcus aureus* and *Staphylococcus epidermis*, two gram-positive bacteria, exhibit increased susceptibility to *C. macrostachys* and *V. amygdalina* honeys, with MIC values of 10.5 ± 0.9 and 10.5 ± 0.5 % v/v, respectively. Regarding gram-negative bacteria *(E. coli)*, the MIC (% v/v) of the honey samples under investigation ranged from 14.0 ± 0.7 – 25.5 ± 1.1% v/v on average. When compared to fresh *A. mellifera* honey samples and stingless bee *(M. beccarii)* honey samples demonstrated a comparatively lower minimum inhibitory concentration against all test pathogens. According to Nwankwo, *et al*. (2014), the MBC of E. coli was found to be 30% v/v of the sample, whereas that of S. aureus was 20%.

3. MATERIALS AND METHODS

3.1. Description of Study Site

This study investigated the honey value chain in southwestern Ethiopia. It began with farmers (producers) and collectors in the Gera district (Figure 1). Located approximately 440 km from Addis Ababa and 93 km from Jimma Town, Gera district is a mountainous forest area within the Jimma zone of Oromia National Regional State (7°15'N - 8°45'N latitude and 35°30'E - 37°30'E longitude). The study was also cover the retailers in Agaro and Jimma towns following the honey value chain.

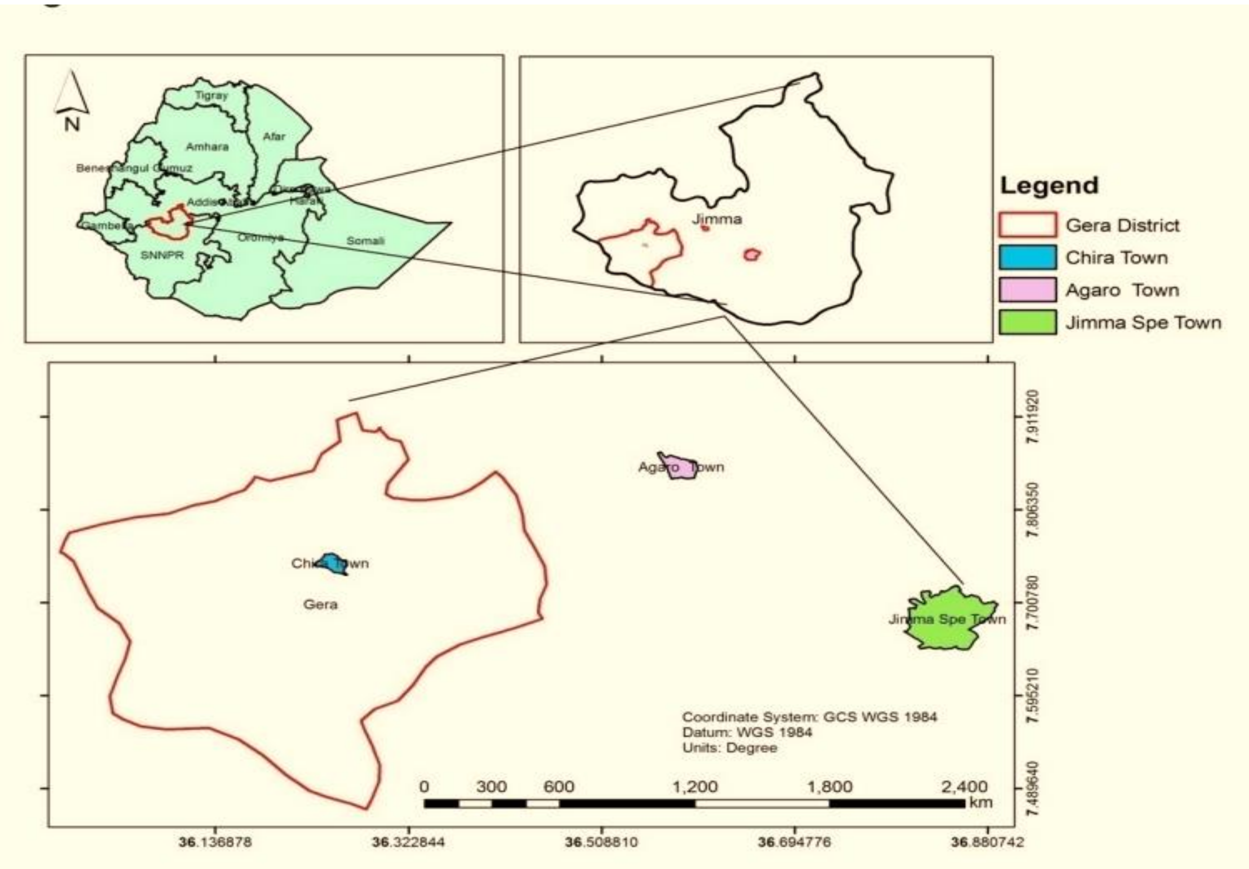


Figure 1. Map of the study Area

3.2. Experimental site

The analyses were conducted at the post-harvest laboratory and microbiology laboratory of Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Jimma, Oromia, Ethiopia. JUCAVM is found 352 km southwest of Addis Ababa and geographically, located at 7^{o}33" N latitude and 36.57^{o}E longitude with an elevation of 1710 m.a.s.l.

3.3. Honey Sample Collection

3.3.1. Sample Source

Honey samples of *Apis mellifera* and *Meliponula beccarii* were collected from four locations in southwestern Ethiopia, including Gera district (farmers), Gera Chira (local collectors), Agaro town (retailers), and Jimma town (retailers). A purposive sampling strategy was employed to target specific locations within the value chain (beekeepers, local collectors, and retailers) (Source). The sample chosen in this study were *Apis mellifera* and *Meliponula beccarii* honey. The honey samples were stored at 4ºC (source).

3.3.2. Sample Pooling

A total of 24 honey samples were initially collected (12 for each bee species, 3 from each value chain stage), composite samples were further formed in order to ensure representative honey samples at each stage of the value chain, address the limited sample volumes of *Meliponula beccarii* from farmers, and achieve cost-effective analysis. In order to establish a single composite sample for that value chain stage and bee species (*Apis mellifera* and *Meliponula beccarii*), equal volumes of honey from each actor within a value chain stage were pooled in sterile containers. This resulted in a total of 8 composite samples (4 for each honey type).

3.4. Experimental Design

Treatments were arranged in a factorial design with three replications. The intended factors were bee species with two levels (*Apis mellifera* and *Meliponula beccarii* honey) and a value chain with four levels (farmers Gera, Gera Chira local collectors, retailers Agaro, and retailers Jimma). There were 2*4 (8) treatment combinations and a total of 24 experimental units.

3.5. Data Collected and Analysis Methods

Bioactive components such as total flavonoid, total phenolic, beta carotene, vitamin C and alkaloid content and antioxidant activities like DPPH (*2, 2-Diphenyl -1- Picrylhdrazyl*) scavenging activity and (IC50) and Ferric reducing antioxidant power (FRAP) and finally in vitro antibacterial activities such as agar well diffusion assay, minimum inhibition concentration and minimum bactericidal concentration of both types of honey samples were determined using standard methods as described below.

3.5.1. Determination of Bioactive Components

3.5.1.1. Determination of Total Flavonoid Contents

The total flavonoid content of honey was determined using a method by Alvarez-Suarez *et al.* (2010). Briefly, 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ solution were added to 1 mL of the honey sample extracted with methanol in the test tube, and after shaking for 6 minutes, 150 µL 10% $AlCl_3$ solution was added and then after 5 minutes, 2 mL of 1M NaOH solution was added to complete the reaction, followed by the addition of 2.4 mL of distilled water. Next to that, a standard solution was prepared by dissolving 0.05 g of the catechin standard in 40 mL of methanol. A series of catechin standards were prepared at different concentrations (0, 5, 10, 15, 20, 25, 40, 50,100 µg/mL) to the final volume of 1 mL with methanol, and all solutions were added similarly to the sample. The absorbance of the sample and the catechin standard was measured at a wavelength of 510 nm by UV-Vis spectrophotometer (T80, Ltd, UK). The total flavonoid content of the honey sample was calculated from the calibration curve from the standard curve ($y = 0.0028x + 0.00831$, $R^2 = 0.9906$, $p < 0.001$). The concentration was expressed as milligrams of catechin equivalents per 100 grams of honey (mg CE/100 g) using the following equation.

$$TFC\left(\frac{mgCE}{100g}\right) = \frac{C * V}{m} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.1$$

Where TFC = total flavonoid content (mg CE/100 g honey); c = concentration established from the catechin calibration curve (µg/mL); V = volume of honey solution in milliliter; m = weight of honey in gram

3.5.1.2. Determination of Total Phenolic Content

The total phenolic content was estimated by the *Folin-Ciocalteu* method with slight modifications by (Alvarez-Suarez *et al.*, 2010). Firstly, 1 mL of each honey sample extract (in triplicate) was mixed with 5 mL of *Folin-Ciocalteu* reagent. Then, mixtures were shaken and kept at room temperature for 6 minutes and then, 4 mL of sodium carbonate (7.5%, v/v) was added to the mixture, mixed thoroughly, and incubated for two hours in the dark place at room temperature. Then, absorbance was read at 760 nm using a UV-Vis spectrophotometer (T80, Ltd, UK) using methanol as a blank. The total phenol content was calculated from the calibration curve ($y = 0.0069x + 0.0056$, R^2 is 0.9981, $p < 0.001$), from the Gallic acid standard at various concentrations of (0, 25, 50,100,150,250,500 μg/mL) and the results were expressed as milligram of Gallic acid equivalent per 100 gram of honey (mg GAE/100 g) using the following equation obtained from the calibration curve.

$$TPC\left(\frac{mgGAE}{100g}\right) = \frac{GAEC * V_t}{W * v} * 100 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots .2$$

Where: TPC = total phenol content, GAEC = Gallic acid equivalent concentration, W = weight of the extracted honey sample, Vt = original volume of extracted honey sample, v = volume of eluate analyzed.

3.5.1.3. Determination of Vitamin C

The vitamin C content of the honey sample was determined by the 2,6-dichloroindophenol titration method according to (Alvarez-Suarez *et al.*, 2010). Precisely, 2 grams of honey sample was extracted with 50 mL solution made from 3% metaphosphoric acid (HPO_3) and 40 mL acetic acid in 500 mL distilled water. Then, the honey sample was filtered using Whatman filter paper No.1. Next, titration was performed using an indophenol standard solution prepared by dissolving 50 mg of 2, 6-dichloroindophenol sodium salt and 42 mg of standard $NaHCO_3$ in 200 mL with distilled water. The titration was performed until the pink endpoint was observed. A standard solution of ascorbic acid was prepared dissolving 50 mg L-ascorbic acid and 50 mL previously prepared extraction solvents of HPO_3-acetic acid. Finally, the vitamin C content of the honey sample was calculated using the following equation.

$$Vit\ C\left(\frac{mg}{100g}\right) = \frac{(A-B)*C*50}{10S} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots ..3$$

Where, Vit C = vitamin C, A = volume in mL of 2, 6-dichloro indophenol sodium salt solution used for the sample, B = volume in mL of 2, 6- dichloro indophenol sodium salt solution used for the blank, C = mass in mg of ascorbic acid corresponds to 1 mL standard indophenol solution S = sample weight taken, 50 = honey volume and 10 = honey volume used for ascorbic acid determination

3.5.1.4. Determination of Beta Carotene

The beta carotene content of honey was determined as described by (Barros *et al.*, 2007). Exactly, 2 grams of honey sample was mixed with 1 g of $CaCl_2$ $2H_2O$ and 50 mL of extraction solvents (25% acetone, 50% hexane, 25% ethanol containing 0.1% BHT) by shaking at room temperature for 30 minutes. Then, 15 mL of distilled water was added and shaken for 15 minutes. Next, the organic phase containing the beta carotene was separated from the water phase using a separator funnel and filtered using Whatman No. 1 filter paper. Then, stock beta carotene standard solution (Sigma Aldrich of USA) was prepared by dissolving an accurately weighed 0.01 g of carotene in the solvent (25% acetone, 25% ethanol, and 50% hexane) used to extract samples and made up to a volume of 100 mL using the same solvent. From the stock solution prepared a series of standard (0.1, 0.2, 0.4, 0.6, 0.8 and 1 g/mL, $y = 0.0969x + 0.0569$, $R^2 = 0.9963$, $p < 0.001$) were used to construct calibration curve line from which beta carotene was calculated and expressed in mg/100g. Then, the absorbance of the sample extract and the beta carotene standard solution was read at a wavelength of 450 nm using a UV-Vis spectrophotometer (T80, Ltd, UK).

3.5.1.5. Determination of Total Alkaloid

Total Alkaloid content was determined according to procedures described by (Nantongo *et al.*, 2018). Firstly, 200 cm^3 of 10% acetic acid in ethanol was prepared in a 250 cm^3 beaker, and 2.50 g of the sample was added. Then, it was allowed to stand for four hours. Thereafter, the extract was concentrated at 90 °C to a quarter of the original volume on a water bath and followed by the drop wise addition of 15 drops of concentrated NH_4OH to extract until a cloudy fume is formed. After 3 hours, the supernatant was discarded and the precipitate was washed with 20 cm^3 of NH_4OH and filtered using pre-weighed (w1) Whatman 1. Then, filter paper containing precipitate was dried in the oven at 60 °C for 30 minutes and weighed (w2) after allowing them to cool for a few minutes. Finally, the percentage of total alkaloid content was calculated gravimetrically as follows.

$$\text{Total Alkaloid}(\%) = \frac{\text{W2} - \text{W1}}{\text{Weight of sample}} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..4$$

Where, W_2 = weight of filter paper and precipitate, W_1= weight of filter paper

3.5.2. Determination of Antioxidant Activities of Honey

3.5.2.1. DPPH (2, 2-diphenyl-1- picrylhydrazyl) Scavenging Activity

DPPH free radical scavenging activity (inhibition %) of the methanol extract was performed according to the method described by (Chua *et al.*, 2013). Firstly, 0.004% solution of the DPPH radical solution in methanol was prepared. Then, 4 mL of this solution was mixed with 1 mL of a series of concentrations (0.5, 5, 10, 15, and 20 mg/mL) of honey sample extract in methanol (98.8%). Then, the mixtures were mixed and kept in the dark at room temperature for 30 minutes. After thirty minutes, the absorbance of the honey sample was read at 517 nm by UV-Vis spectrophotometer (T80, Ltd, UK). DPPH in methanol and methanol served as a positive control and a blank, respectively. Then, 0.4 mg/mL of the concentration was used to calculate the percentage of DPPH scavenging activity of the honey sample. The ability of the extract to scavenge DPPH free radicals scavenging activity was calculated and the IC_{50} value was calculated from the linear regression equation of the standard curve drawn against the concentration and percentage of antioxidant activity.

$$\text{Inhibition (\%)} = \frac{(A_{bc} - A_{bs})}{A_{bc}} * 100 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots..5$$

Where: A_{bc} = control absorbance (DPPH radical + methanol), Abs = sample absorbance (DPPH radical + sample)

3.5.2.2. Ferric Reducing Antioxidant Power (FRAP) Determination

Ferric Reducing Antioxidant Power is one of the assays to measure the antioxidant capacity of food samples by iron sulfate. The reducing power of the honey samples was determined based on the method described (Mahmood *et al.*, 2021). The FRAP reagent was prepared by mixing 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution with 300 mM acetate buffer (pH 3.6) and 20 mM iron (III) chloride hexahydrate. An aliquot of 200 µL of honey solution was mixed with 1.5 mL of FRAP reagent, and incubated at 37°C in a water bath for 4 mins. The absorbance of the mixture was then measured at 593 nm (distilled water as blank) using a UV- Vis spectrophotometer and a series of ferrous sulfate (FeSO4) standard (0.1, 0.2, 0.4, 0.6, 0.8, and 1mM, $y = 1.0709x + 0.169$, $R^2 = 0.9838$, $p < 0.001$) were used to estimate FRAP.

3.5.3. Determination of In Vitro Antibacterial Activities of Honey Samples

Honey Samples Preparation

The honey samples were prepared by diluting with sterile distilled water to obtain 50%, 75%, and 100% honey solutions. These solutions were then used to evaluate their activities against selected microorganisms (S. *aureus*-ATCC-25923, gram-positive, and *E. coli*-ATCC-25922, gram-negative).

Test microorganisms used for the current study

The two bacterial strains (*S. aureus*-ATCC-25923, gram-positive, and *E. coli*-ATCC-25922, gram-negative) were sourced from the Ethiopian Public Health Institute (EPHI) for this study. To prepare the inoculum, 2–3 colonies were carefully selected from a 24-hour-old culture grown on basal (Nutrient Agar) media. These colonies were then suspended in 5 mL of saline solution (0.85%). The suspended inoculum underwent agitation using a vortex for 15 seconds to ensure thorough mixing. Subsequently, the turbidity of the suspension was measured utilizing a McFarland densitometer. To achieve a standard 0.5 McFarland density, adjustments were made by adding either colony material or saline solution to the microbial stock solution (Andrews, 2006)**.**

3.5.3.1. Agar well diffusion assay

Mueller-Hinton agar medium was meticulously prepared in sterile petri dishes and then subjected to a 24-hour sterility check at 37°C in an incubator. Once confirmed sterile, the medium was evenly spread on the agar surface using a sterile swab, with the surface now primed for the introduction of the prepared bacterial inoculum solution.

Sterile filter paper discs were prepared and impregnated with various concentrations of honey solutions (50%, 75%, and 100%) by inoculating each disc with 60 microliters (60µL) of the respective honey solution. Then impregnated filter paper discs with concentrations of honey solutions (50%, 75%, and 100%) were meticulously placed on the surface of the swabbed agar plates, utilizing sterile forceps to ensure appropriate distances between each disc. Subsequently, the prepared plates were carefully incubated at 37°C for 24 hours to facilitate microbial growth and interaction with the introduced agents. Following incubation, the diameters of the inhibition zones surrounding each filter paper disc were methodically measured using a caliper, in millimeters, and the corresponding results were meticulously recorded for comprehensive analysis (Moussa *et al.* 2012).

3.5.3.2. Minimum Inhibition Concentration (MIC)

To determine the minimum inhibitory concentration (MIC) of the honey samples with concentrations of 50%, 75%, and 100%, 9 mL of Mueller Hinton Broth media were individually prepared for each honey sample while ensuring their respective concentrations were accurately represented. The sterility of each broth-honey solution was then meticulously validated. Then, 18–24 hour *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) bacterial cultures were used, and carefully prepared inoculum suspensions were used to attain a standardized turbidity equal to 0.5 McFarland. Following this, 100 microliters of each concentration of honey sample were introduced into separate test tubes, and to each of these, 1 mL of the standardized inoculum suspensions was carefully pipetted. Additionally, a negative control tube containing only broth was included for comparative purposes. The resulting tubes were then thoroughly vortexed and subsequently incubated for 24 hours at 37°C to enable the interaction of the bacterial cultures with the honey concentrations. Finally, the tubes were visually inspected, and the concentration of honey that resulted in clear tubes, signifying inhibition of bacterial growth, was identified as the minimum inhibitory concentration. The MIC of the honey samples was determined using the broth dilution method, which involved assessing the lowest concentration of honey capable of inhibiting bacterial growth, as indicated by the absence of turbidity in the test tubes as compared to the control tubes(Boateng & Diunase, 2015).

3.5.3.3. Minimum Bactericidal Concentration (MBC)

A dilution showing no visible growth during the MIC test was sub-cultured onto a fresh Mueller Hinton Agar plate by streaking using a sterile inoculating loop and incubated at 37°C for 24 h. The lowest concentration of the extracts showing no growth on the Muller Hinton Agar plate was recorded as MBC (Yalemwork *et al.*, 2013).

3.6. Data Analysis

Two-way analysis of variance (ANOVA) was conducted using Minitab software version 19 to determine the bioactive components, and antioxidant activities of *Apis mellifera* and *Meliponula beccarii* honey. SAS software version 9.3 was used to analyze the in vitro antibacterial activities of both honey samples. The mean comparison was performed using Tukey's test, and statistical significance was accepted at $\alpha \leq 0.05$ level. Correlation between bioactive contents, antioxidant, and in vitro antimicrobial activities was conducted using Pearson's correlation method.

General Linear Model (GLM):

$$Y = \mu + \alpha i + \beta j + (\alpha\beta)\, ij + \varepsilon ijk$$

Where: Y: The dependent variable (bioactive components, antioxidant activities, or in vitro antibacterial activities of honey)

μ: Overall mean effect

αi: Effect of bee species (i = 1 for *Apis mellifera,* i = 2 for *Meliponula beccarii*)

βj: Effect of value chain (j = 1, 2, 3,4, for farmers Gera, Gera Chira local collectors, retailers Agaro and retailers Jimma)

(αβ)ij: Interaction effect between bee species (i) and value chain (j)

εijk: Error term (represents random variation)

4. RESULTS AND DISCUSSIONS

4.1. Bioactive Components of *Apis mellifera* and *Meliponula beccari* honey collected from different value chain

4.1.1. Total Phenolic Content

The total phenolic content (TPC) of the honey sample across the bee species and the value chain presented in (Table 2). The TPC ranges from 25.52 ± 0.58 to 102.81 ± 0.52 mg GAE/100 g. Significantly higher value of TPC was recorded from *Meliponula beccarii* honey collected from farmer Gera whereas the lowest value is from *Apis mellifera* honey retailed at Jimma. The total phenolic content of the *Apis mellifera* honey ranged from 25.52 ± 0.58 to 39.38 ± 0.59 mg GAE/100g. The total phenolic content of the *Meliponula beccarii* honey samples in this study ranged from 47.35 ± 0.44 to 102.81 ± 0.52 mg GAE/100g.

The variability of phenolic content observed in this study could be due to a difference in the phenolic profiles of the honey, attributed to divergences storing honey for a long period, and the (Miranda *et al.,* 2023). Additionally, a distinct study revealed the various honey compositions made by various bee species with identical botanical origins (Castro-Vázquez *et al.,* 2008). The possible reason may be due to the process of honey production by the bees and also the storage of the honey (Pauliuc & Dranca, 2020).Phenolic is the largest group of phytochemicals that exist in plants and are incorporated into the honey through nectar or pollen from plants visited by the honeybee (Güneş *et al.,* 2017).

The total phenolic compound content of the *Meliponula beccarii* honey in this study was similar to honey samples from Spain Combarros-Fuertes *et al.* (2019) who reported a TPC range between 23.1 and 158 mg GAE /100 g of honey. The honey value chain, bee species, and the regions where the samples were taken could all be to blame for this. The finding of this study is higher than the work of Galhardo *et al.* (2021) who reported that the total phenolic contents of stingless honey samples ranged from 11.39 to 61.27 mgGAE/100 g in Western Paraná, Southern Brazil. The total phenolic content of *Apis mellifera* honey obtained in this study was closer to or within the reported ranges of phenolic (23.33 - 69.33 mg GAE/100g) for Ethiopians honey (Adgaba *et al.*, 2020), 10.3 - 98.0 mg GAE/100 g for state of Santa Catarina, Brazilian honey (Biluca *et al.*, 2016) and

25-50.9 mg GAE/100 g for Roraima Brazilian honey (Pontis *et al.,* 2014). It is postulated that the bee species and sources are responsible for these differences*(Ak et al.,* 2021).

4.1.2. Total Flavonoid Contents

Table 2 shows the result of the total flavonoid content of *Apis mellifera* and *Meliponula beccarii* honey collected from different value chains. Significantly higher value of TFC is recorded from *Meliponula beccarii* honey collected from farmer Gera whereas the lowest value is from *Apis mellifera* honey retailed at Jimma. The total flavonoid content of the *Apis mellifera* honey ranged from 13.17 ± 0.35 to 32.82 ± 0.35 mg CE/100 g. The total flavonoid content of the *Meliponula beccarii* honey samples in this study ranged from 16.98 ± 0.55 to 117.94 ± 0.54 mg CE/100 g.

It was observed that the TFC showed significant differences between the bee species and value chains. The reason for more declines in the total flavonoid at retailers Jimma for both types of honey could be because of the degradation of flavonoids. Heat and sunlight can reduce the quality of honey, following brief high- or low-level exposure over long periods (Grigoryan, 2016). The biosynthesis of phenolic compounds, flavonoids, and non-flavonoids, is regulated by different enzymes, depending on the plant species, its needs, and the oxidative stress to which they are subjected (Dezmirean *et al.,* 2017). The fermentation and chemical reactions during storage can also degrade both the polyphenols and flavonoids (Shamsudin *et al.,* 2019).

The total flavonoid content obtained in this study for *Apis mellifera* honey was comparable to the reported ranges of 42.03 ± 1.49 and 31.07 ± 1.31 CEQ /100g for *Schefflera abyssinica* and polyfloral Ethiopian honey respectively (Hailu & Belay, 2020). The range of values for the total flavonoid content reported here was in agreement with those previously found in another Ethiopian honey was 15.1- 42.6 mg catechin equivalent (CE)/100 g (Sime *et al.,* 2015). The total flavonoid content obtained in this study for *Apis mellifera* honey was closer to or within the reported ranges of 7.97 - 44.99 mg CE/100 g Southern Brazilian honey (Galhardo *et al.,* 2021). This finding is considerably higher than that of 1.65 - 5.93 mg CE/100 g Spanish honey (Combarros-Fuertes *et al.,* 2019) and 2.68 mg CE/100 g Cuban polifloral honey (Alvarez-Suarez *et al.,* 2018)

Table 2: Bioactive components of *Apis mellifera* and *Meliponula beccarii* honey

Bee species and VChain		Parameters				
		TPC	**TFC**	**TAC**	**Vit- C**	**Beta-Car**
Meliponula beccarii	FG	102.81 ± 0.52[a]	117.94 ± 0.54[a]	4.40 ± 0.40[h]	42.09 ± 0.25[a]	5.59 ± 0.03[a]
	GCLC	94.98 ± 1.28[b]	109.25 ±0.36[b]	5.33 ± 0.83[e]	25.58 ± 0.16[b]	2.62 ± 0.02[b]
	RA	55.13 ± 0.81[c]	18.54 ± 0.35[e]	7.2 ± 0.40 [d]	18.91 ± 0.41[c]	2.38 ± 0.08[c]
	RJ	47.35 ± 0.44[d]	16.98 ± 0.55[f]	8.67 ± 1.01[c]	10.66 ± 0.32[e]	0.88 ± 0.17[d]
Apis mellifera	FG	39.38 ± 0.59[e]	32.82 ± 0.35[c]	4.67 ± 0.46[g]	13.73 ± 0.95[d]	0.65 ± 0.01[e]
	GCLC	37.88 ± 1.92[f]	22.7 ± 0.21[d]	5.2 ± 0.40[f]	9.29 ± 0.41[f]	0.59 ± 0.07[e]
	RA	36.96 ± 1.32[g]	15.32 ± 0.36[g]	9.2 ± 0.69[b]	5.96 ± 0.26[g]	0.19 ± 0.01[f]
	RJ	25.52 ± 0.58[h]	13.17 ± 0.35[h]	12 ± 0.80[a]	3.88 ± 0.25[h]	0.15 ± 0.02[f]

Note; FG (Farmer Gera); GCLC (Gera Chira Local Collectors); RA (Retailer Agaro); RJ (Retailer Jimma); TPC = Total phenolic contents (mgGAE/100 g, GAE = Gallic acid equivalent); TFC= Total flavonoid contents (mg CE/100 g, CE = Catechin equivalent); TAC (total alkaloid content) (Percentages); Vitamin C (mg/100g) and Beta - Carotene (mg/100g). Data are presented as Means ± SD; Mean values across the column with different superscript letters indicate a significant difference at $p < 0.05$.

TFC of *Meliponula beccarii* honey in this study was higher than that of 12.41- 17.67 mg CE/100 g Malaysian honey (Izzati *et al.,* 2021); 10.70–25.71 mg CE/100 g Malaysian honey (Shamsudin *et al.,* 2019) and 4.19 mg CE/100 g Cuban honey (Alvarez-Suarez *et al.,* 2018). The finding for *Meliponula beccarii* honey is also consistent with the findings of Nweze *et al.* (2017) and Souza *et al.* (2021) who reported honey possesses the best TFC when compared with honey from *Apis mellifera* and all the bioactive compounds present in *Apis* honey can be found in stingless bee honey. Another recent study conducted by Miranda *et al.,* (2023) also demonstrated that the flavonoid content of *Melipona beaches* honey from a deciduous forest of Yucatan, Mexico was 36 to 55 mg CE/100 g, the storage conditions where the honey stored directly impacted the flavonoid content of the honey. The role of the flavonoids group is vital for the aroma and antioxidant potential of honey(Izzati *et al.,* 2021). Flavonoids are low-molecular-weight phenolic compounds that are responsible for the aroma and antioxidant activity of honey (Moniruzzaman *et al.*, 2012).

In terms of TFC, *Meliponula beccarii* honey with 16.98 ± 0.55- 117.94 ± 0.54 mg CE/100 g yielded a significantly higher TFC as compared to *Apis mellifera* honey 13.17 ± 0.35 - 32.82 ± 0.35 mg CE/100 g. *Meliponula beccarii* honey contains a higher level of flavonoid potential than honey produced by *Apis mellifera* honey (Alvarez-Suarez *et al.,* 2018). *Meliponula beccarii* honey's higher flavonoid potential could be attributed to specific foraging behavior, gut metabolism, and the unique floral sources visited (Ofijan, 2022). The result showed that the total flavonoid content of the sampled honey is significantly affected by the bee species and the value chain. Therefore, *Meliponula beccarii* honey samples exhibited significantly higher TFC compared to *Apis mellifera* honey.

4.1.3. Total Alkaloid Content

The total alkaloid content (TAC) of the honey sample was significantly affected by the bee species and value chain (Table 2). As influenced by the bee species and value chain, the TAC ranges from 4.40 ± 0.40 to 12 ± 0.80 %. Significantly higher value of TAC is recorded from *Apis mellifera* honey retailed at Jimma whereas the lowest value is from *Meliponula beccarii* honey collected from farmer Gera. TAC of the *Apis mellifera* honey ranged from 4.67 ± 0.46 to 12 ± 0.80 % whereas that of *Meliponula beccarii* honey ranged from 4.40 ± 0.40 to 8.67 ± 1.01 %.

The TAC was found to exhibit significant differences among the bee species and different value chains. The reason why total alkaloid content was significantly affected by these could be due the interplay of bee species, value chain practices such as storage containers and storage conditions (Tsagkaris *et al.* 2021; Miranda *et al.*, 2023) can all impact the stability and concentration of alkaloids in honey. The total alkaloid content of *Meliponula beccarii* honey was higher than the values reported by Igbang *et al.* (2018) study carried out in Biase, southern Nigeria. The total alkaloid content of *Apis mellifera* honey was lower than the values reported by Mahani *et al.* (2022) of honey from various provinces in Indonesia. This value is in line with the value reported by Adalina *et al.* (2020) that various types of honey monofloral (rubber honey, kapok honey, and mango honey) from *A. mellifera* bees originating from Java islands contain alkaloids. Overall, the total alkaloid content of *Meliponula beccarii* was more preferable and less affected across the value chain than *Apis mellifera*.

4.1.4. Beta Carotene Content

Table 2 shows the result of the beta carotene content of *Apis mellifera* and *Meliponula beccarii* honey collected from the different value chains. The result showed that the beta carotene content of the sample honey is significantly affected by the bee species and the value chain. Significantly higher value of beta carotene content is recorded from *Meliponula beccarii* honey collected from farmer Gera whereas the lowest value is from *Apis mellifera* honey retailed at Jimma. The beta carotene content of the *Apis mellifera* honey ranged from 0.15 ± 0.02 to 0.65 ± 0.01 mg/100g, whereas that of *Meliponula beccarii* honey ranged from 0.88 ± 0.17 to 5.59 ± 0.03 mg/100g.

In this study variation of beta carotene was noted throughout the value chain and bee species. This could be due to bee species contributing to honey production and their foraging behavior and dietary preferences can influence the beta carotene of honey(Ahmed, 2015). The composition of carotenoids in honey is dependent on a value chain that includes processing methods, storage place, and storage duration (Bueno-Costa *et al.*, 2016) which could be the cause of large ranges observed in the β-carotene values. The difference in total carotenoid content in honey is strongly influenced by the flower sources (Boussaid *et al.*, 2018), who showed that honey from citrus flowers had the highest total carotenoid content (4.72 mg/100g) while the lowest content (1.16 mg/100g) was found in honey from rosemary flower. Differences in seasons can impact the carotenoid content. Specifically, the dry season and varied bee forage contribute to higher carotenoid levels(Mahmood *et al.,* 2021). *Meliponula beccarii* honey has higher beta-carotene contents than *Apis mellifera* honey, the darker the honey, the greater its antioxidant potential (Hunter *et al.,* 2021).

The range of values for the beta carotene reported here was in agreement with those previously found in Cuban honey from A. *mellifera* (Alvarez-Suarez *et al.*, 2010; Alvarez-Suarez *et al.*, 2012; Alvarez-Suarez *et al.,* 2018). The beta carotene content obtained in this study was higher than Indonesian honey from A. *mellifera* values reported by Satriadi *et al.* (2023). The beta carotene content of *Meliponula beccarii* honey is similar contents to those reported by Bueno-Costa *et al.* (2016) which obtained contents ranging between 0.56 and 6.19 mg/100g in honey samples from Brazil.

Beta carotenoids in this study were in line with (Alvarez-Suarez *et al.,* 2010; Keng *et al.,* 2017) analyzed beta carotene content of Malaysian Stingless Kelulut honey and Cuban honey was 1.17-5.57 mg/100g and 1.55 - 3.09 mg/100g. Meanwhile, a study by Jimenez *et al.* (2016) on *Scaptotrigona mexicana* honey showed a very low total carotenoid content of 0.56 mg/100g was lower than the current study. Therefore, *Meliponula beccarii* honey samples from retailer Chira and farmer Gera showed significantly higher beta-carotene content compared to *Apis mellifera* honey.

4.1.5. Vitamin C Content

The vitamin C content of the honey sample was significantly affected by the bee species and value chain (Table 2). As influenced by the interaction of bee species and value chain, the vitamin C content of the honey sample ranges from 3.88 ± 0.25 to 42.09 ± 0.25 mg/100g. Significantly higher value of vitamin C content is recorded from *Meliponula beccarii* honey collected from farmer Gera whereas the lowest value is from *Apis mellifera* honey retailed at Jimma.

The Vitamin C content was found to exhibit significant differences among the bee species and different value chains. Vitamin C content in honey is significantly influenced by factors within the value chain, including the foraging environment, processing methods, and storage conditions (Machado *et al.,* 2018). Vitamin C content variation is associated with the oxidation of ascorbic acids to dehydroascorbic acid, and then to diketogulonic, and oxalic acids during storage (Burdurlu *et al.*, 2006). This is most likely due to the processing and storage of honey samples along the value chain.

Studies on honey samples collected from Northern Ethiopia have consistently reported similar vitamin C content, ranging from 23.74 ± 2.85 mg/100 g to 40.11 ± 3.39 mg/100 g of honey(Yayinie *et al.*, 2022). However, the results of the honey samples collected in different regions of Ethiopia were significantly higher than these research findings; it is from 72.7 ± 7.6 to 174.0 ± 9.0 mg /100 g honey (Sime *et al.,* 2015). The vitamin C content of *Meliponula beccarii* honey were closer to or within the reported ranges of 0.34 – 75.9 mg/100g of different Spanish honey samples Combarros-Fuertes *et al.* (2019).

The present finding is higher than the values reported by Alvarez-Suarez *et al.* (2018), 8.84 mg/100g from Cuban polifloral honey. The current finding is consistent with the finding of Satriadi *et al.* (2023) who reported that the Vitamin C content of *Meliponula beccarii* honey was 14.08 mg/100g. The vitamin C content of *Apis mellifera* honey is consistent with the finding of Bahar *et al.* (2022) 7.26 –19.69 mg/100g honey from different origins in Turkey. Vitamin C (Ascorbic acid) is provided naturally in foods including honey (Le *et al.,* 2011). Vitamin C is one of the antioxidants needed in the human body, it helps protect the body from the damaging effects of free radicals(Satriadi *et al.,* 2023). Generally, *Meliponula beccarii* honey samples exhibited significantly higher Vitamin C content compared to *Apis mellifera* honey.

4.2. Antioxidant Activities of *Apis mellifera* and *Meliponula beccarii* Honey Collected from Different Value Chain

The antioxidant power of honey has been regarded as an eligible parameter that can be coupled with other measurements to evaluate the honey quality. Since a single technique cannot assess the antioxidant activity, distinct antioxidant tests (DPPH radical scavenging activity, DPPH by 50% (IC_{50}), and ferric reducing antioxidant power (FRAP) were used to evaluate the antioxidant ability of *Apis mellifera* and *Meliponula beccarii* honey collected from different value chain in our study. Table 3 presents the antioxidant capacities of *Apis mellifera* and *Meliponula beccarii* honey collected from different value chains. The result showed that DPPH (*2, 2-diphenyl-1- picrylhydrazyl*), free radical scavenging activities), ferric reducing antioxidant power and concentration of honey solution required to mitigate the initial concentration of DPPH by 50% (IC_{50}) of the sample were significantly affected by the bee species and value chain of honey.

4.2.1. DPPH *(2, 2-Diphenyl-1- Picrylhydrazyl*) Scavenging Activity

Table 3 shows the result of DPPH (*2, 2-diphenyl-1- picryl hydroxyl*) scavenging activities of *Apis mellifera* and *Meliponula beccarii* honey collected from different value chains. The result showed that the DPPH scavenging activities of the sample honey is significantly affected by the bee species and value chain. Significantly higher value of DPPH scavenging activities is recorded from *Meliponula beccarii* honey collected from farmer Gera whereas the lowest value is from *Apis mellifera* honey retailed at Jimma. The DPPH scavenging activities of the *Apis mellifera* honey samples in this study ranged from 14.71 ± 0.33 to 25.91 ± 0.26% whereas that *Meliponula beccarii* honey samples ranged from 26.84 ± 0.71 to 35.28 ± 0.53%.

In this study, the effect of bee species and value chain on DPPH scavenging activity was more noticeable. This could be due to the reduction in bioactive compounds over time, which may happen by the oxidation under favorable conditions(Can *et al.*, 2015). The DPPH scavenging activity was developed by utilizing free radicals to assess the antioxidant activity of a compound (Shamsudin *et al.*, 2019). It measures the scavenging capacity of antioxidants, with the reduction of odd electron nitrogen in DPPH by acquiring hydrogen atoms from antioxidants to the corresponding hydrazine (Kedare & Singh, 2011). The DPPH radical scavenging effect can provide the overall hydrogen/electron donating activity of honey as well, like other dietary foods (Gül & Pehlivan, 2018). This is predicated on quantifying antioxidants' capacity to reduce DPPH radicals.

The decreasing absorbance is also accompanied by a discoloration of the DPPH purple color (Alves *et al.*, 2013). Blasa *et al*. (2006) and Salonen *et al.* (2017) argued that light-colored honey possessed lower antioxidant activity by comparison to darker-colored honey, although the differences between the free radical scavenging activities. Therefore DPPH is a free radical, which is stable at room temperature and produces a violet solution in alcohol. It is reduced in the presence of an antioxidant molecule(Pontis *et al.*, 2014).

The DPPH% values (18.4 ± 1.6 to 58.9 ± 2.5%) reported by Sime *et al.* (2015) for Ethiopian honey are consistent with the findings of this study for *Apis mellifera* and *Meliponula beccarii* honey. In previous Ethiopian studies by Hailu & Belay (2020) and Hawine (2020), DPPH% ranged from 41.75% to 45.94% and 37.93 ± 1.14% to 44.43 ± 0.97%, which were higher than the values reported in this study. The range values for the DPPH scavenging activity percentages of *Meliponula beccarii* honey were in line with mono-floral honey types from Turkey and various botanical origins from Malaysia (Gül & Pehlivan, 2018; Shamsudin *et al.,* 2022). The range of values for the DPPH scavenging activity percentages reported here was in agreement with those previously found in Cuban honey from *Melipona beecheii* (Alvarez-Suarez *et al.,* 2018). The values for the DPPH scavenging activity percentages of *Apis mellifera* reported here were in agreement with the finding of those previously found in Cuban honey from *Melipona beecheii* honey (Alvarez-Suarez *et al.,* 2018). The finding for *Apis mellifera* honey was closer to or within the reported ranges of the northeast region of Iran(Salehnezhad & Chermahini, 2019). Therefore, the DPPH scavenging activity of *Apis mellifera* and *Meliponula beccarii* honey samples varied significantly based on the bee species and value chain, with *Meliponula beccarii* honey exhibiting the highest antioxidant activity than *Apis mellifera* honey.

Table 3: Antioxidant activities of *Apis mellifera* and *Meliponula beccarii* honey

Bee species and VChain		Parameters		
		DPPH%	**IC_{50}**	**FRAP**
Meliponula beccarii	FG	35.28 ± 0.53[a]	9.27 ± 0.25[h]	387 ± 0.09[a]
	GCLC	33.75 ± 0.71[a]	9.42 ± 0.09[g]	371 ± 0.01[b]
	RA	27.57 ± 0.77[b]	23.19 ± 1.41[f]	334 ± 0.01[d]
	RJ	26.84 ± 0.71[b]	29.49 ± 7.83[b]	283 ± 0.04[h]
Apis mellifera	FG	25.91 ± 0.26[bc]	24.71 ± 1.34[e]	338 ± 0.04[c]
	GCLC	24.38 ± 0.79[c]	26.11 ± 1.08[d]	299 ± 0.03[e]
	RA	22.07 ± 0.92[d]	28.76 ± 0.85[c]	298 ± 0.03[f]
	RJ	14.71 ± 0.33[e]	62.76 ± 5.08[a]	291 ± 0.01[g]

Note; FG (Farmer Gera); GCLC (Gera Chira Local Collectors); RA (Retailer Agaro); RJ (Retailer Jimma); DPPH ((Free radical scavenging activity) *2,2-diphenyl-1- picrylhydrazyl* (percentage)); FRAP (Ferric reducing antioxidant power (µmol $Fe_2SO_4 .7H_2O$/100 g honey)); IC_{50} (concentration of honey solution required to mitigate the initial concentration of DPPH by 50%(mg/ml)). Data are presented as Means ± SD; Mean values do not share the same letter/s across the same column with distinct superscript letters indicating a significant difference at $p < 0.05$.

4.2.2. Half Maximal Inhibitory Concentration

The IC_{50} value of honey samples was significantly affected ($p < 0.05$) by the bee species and the value chain (Table 3). As influenced by the bee species and value chain, the IC_{50} value ranges from 9.27 ± 0.25 to 62.76 ± 5.08 mg/ml. Significantly higher value of IC_{50} is recorded from *Apis mellifera* honey retailed at Jimma whereas the lowest value is from *Meliponula beccarii* honey collected from farmer Gera. The IC_{50} value of *Apis mellifera* honey ranged from 24.71 ± 1.34 to 62.76 ± 5.08 mg/ml whereas that of *Meliponula beccarii* honey samples ranged from 9.27 ± 0.25 to 29.49 ± 7.83 mg/ml.

The results show that the IC_{50} of *Meliponula beccarii* honey samples was significantly lower than *Apis mellifera* honey. This indicates that *Meliponula beccarii* honey is a better free radical scavenger at lower concentrations as compared to *Apis mellifera* honey. The variations in the IC_{50} values of the honeys might be due to the differences in the phenolic contents and different types of phenolic compounds present (Idris *et al.,* 2011), since each phenolic acid has different scavenging activity(Aljadi & Kamaruddin, 2004; Küçük *et al.,* 2007). Similarly, variation in the IC_{50} value of honey depends on intrinsic and extrinsic factors, including place of origin, bee species, season, and storage conditions along the value chain (Ak *et al.,* 2021). IC_{50} is defined as the concentration of honey causing the decrease of initial DPPH concentration by 50% (Chis *et al.,* 2016). It is the amount of antioxidants required to scavenge 50 % of the free radicals (Lewoyehu & Amare, 2019)**.** A lower IC_{50} value in honey indicates a greater ability to neutralize free radicals and honey with a low value of IC_{50} has greater antioxidant activity than honey with a high value of IC_{50} (Pontis *et al.,* 2014). Therefore, it is worth mentioning that the IC_{50} values in the DPPH assay are inversely proportional to the antioxidant capacities in honey samples(Alonso *et al.,* 2002).

The finding of *Apis mellifera* and *Meliponula beccarii* honey was lower than the report of Hailu & Belay, (2020) for other Ethiopian *Schefflera abyssinica* and *polyfloral honey* (134.60 ± 8.66 -152.84 ± 8.25 mg/ml). IC_{50} values *Apis mellifera* honey samples values are higher than those reported by Chis *et al.* (2016) for Romanian honeydew honey. The result of this study is lower than that reported by Krpan *et al.* (2009) and Lewoyehu & Amare, (2019) for honey samples from selected districts of the Amhara and Tigray Regions, Ethiopia, and for honey samples from different locations across Croatia, respectively. This study's IC50 values for *Apis mellifera* honey align with, or are within the range of, values reported for Brazilian *Apis mellifera* honey (Bastos *et al.,* 2009). IC_{50} values of *Meliponula beccarii* honey samples values are higher than those reported by Pontis *et al.* (2014) for Roraima State, Brazilian honey. This finding is in agreement with the report of Shamsudin *et al.* (2022) who reported that the IC_{50} value of *Meliponula beccarii* honey varied between 11.27 and 24.09 mg/ml value. Overall, *Meliponula beccarii* honey samples exhibited greater antioxidant activity based on their lower IC_{50} values compared to *Apis mellifera* honey samples.

4.2.3. Ferric Reducing Antioxidant Power (FRAP)

Table 3 shows the result of ferric reducing antioxidant power value of *Apis mellifera* and *Meliponula beccarii* honey collected from different value chains. The result showed that the FRAP value of the sampled honey is significantly affected ($p < 0.05$) by the bee species and the value chain. Significantly higher value of FRAP is recorded from *Meliponula beccarii* honey collected from Gera whereas the lowest value is from *Meliponula beccarii* honey retailed at Jimma. The FRAP value of the *Apis mellifera* honey ranged from 291 ± 0.01 to 338 ± 0.04 µmol Fe_2SO_4 $.7H_2O$/100 g. The FRAP value of the *Meliponula beccarii* honey samples in this study ranged from 283 ± 0.04 to 387 ± 0.09 µmol Fe_2SO_4 $.7H_2O$/100 g.

It was discovered that the FRAP value varied significantly between the different value chains and bee species. The quantity of the components responsible for the FRAP value of honey varies widely according to the floral and geographical origin of honey (Gheldof & Engeseth, 2002). Processing, handling, and storage of honey at the value chain affect the antioxidant activity of honey only to a minor degree (Turkmen *et al.,* 2006). To assess phenolic compounds' antioxidant capacity to convert ferric ions (Fe3+) into ferrous ions (Fe2+), the ferric reducing antioxidant power (FRAP) analysis was carried out (Shamsudin *et al.,* 2022). FRAP is based on bioactive compounds' ability to reduce yellow ferric *tripyridyl triazine* complex (Fe3þ-TPTZ) to blue ferrous complex (Fe2þ-TPTZ) in the presence of electron donors (Da Silva *et al.,* 2023)**.** FRAP is a simple, direct test widely used for the determination of antioxidant activity in many different substances, including honey (Taormina *et al.,* 2001; Aljadi & Kamaruddin, 2004; Beretta *et al.,* 2005; Blasa *et al.,* 2006). It gives a direct estimation of the antioxidants or reductants present in a sample based on its ability to reduce the ferric to ferrous (Fe3+/Fe2+) couple.

The FRAP value of *Apis mellifera* honey samples was closer to or within the reported ranges of Ethiopian mono-floral honey (Adgaba *et al.*, 2020) and Malaysian honey (Moniruzzaman *et al.*, 2013) reported 225.4 – 465.7 FRAP (µmol Fe(II)/100 g) and 452.79 µmol Fe(II)/100 g of honey, respectively. These values are higher than those reported by Galhardo *et al.* (2021) and Shamsudin *et al.* (2022) for Brazilian honey and Malaysian honey respectively. The FRAP value of *Meliponula beccarii* honey is higher than those reported by Shamsudin *et al.* (2022) and Chua *et al.* (2013) for Malaysian honey. The FRAP values obtained in this study for *Meliponula beccarii* honey were closer to or within the reported ranges of raw Malaysian stingless bee honey (Nuratiqah *et al.*, 2020), who reported FRAP value of the four honey samples ranged from 385.97 to 624.72 µmol Fe/L Fe(II).

FRAP values in the honey samples analyzed could be attributed to the different amounts and types of phenolic compounds (Moniruzzaman *et al.*, 2013) presented in the honey samples as the samples used in this study belonged to different bee species and different value chains. Therefore, honey from different bee types and value chains possesses distinct antioxidant activity (Carina *et al.*, 2017). Generally, the FRAP values of *Apis mellifera* and *Meliponula beccarii* honey varied significantly based on bee species and value chain, with *Meliponula beccarii* showing higher FRAP values compared to *Apis mellifera* honey.

4.3. Antibacterial Activities of *Apis mellifera* and *Meliponula beccarii* Honey Collected from Different Value Chain

The in vitro antibacterial activities of *Apis mellifera* and *Meliponula beccarii* honey samples collected from different value chains are presented in Table 4. The findings revealed that bee species and its source along the value chain had a significant impact on the in vitro antibacterial activity. Honey samples exhibited antibacterial capacity against gram-positive and gram-negative bacteria strains.

4.3.1. Agar Well Diffusion Assay

The in vitro antibacterial activity of honey samples was significantly affected by the interaction of bee species and the value chain (Table 4). As influenced by bee species and value chain, the in vitro antibacterial activity of honey sample by well diffusion method ranges from 3.00 ± 3.00 to 27.67 ± 1.53 mm and from 3.00 ± 2.00 to 26.00 ± 2.65 mm by

100% honey concentration against *E.coli* and *S. aureus* respectively. Significantly higher inhibition zone in diameter is recorded from *Meliponula beccarii* honey retailed at Gera from farmer whereas the lowest value is from *Apis mellifera* honey retailed at Jimma by 100% honey concentration against *E.coli* and *S. aureus* respectively. No antibacterial activities were observed from *Apis mellifera* honey retailed at Jimma against *E.coli and S. aureus.*

The mean inhibition zone of *Meliponula beccarii* and *Apis mellifera* honey samples decreases along the value chain against *E.coli* and *S. aureus* bacteria. Honey's antibacterial power weakens as it travels through the processing and storage chain due to dilution, heat, filtration, improper storage, and potentially being blended with honey of varying potency. In short, the value chain can diminish honey's natural antibacterial properties. However, honey samples from *Meliponula beccarii* had stronger antibacterial activity than honey samples from *Apis mellifera* against the tested pathogenic bacteria. The lack of antibacterial properties shown by the *Apis mellifera* honey may be influenced by the processing or storage conditions of this honey (Julika *et al.*, 2022). Honey antibacterial activity is also associated with bioactive contents and antioxidant activities, as much as multiple compounds originated from the nectar of plants, pollen, propolis, and from the bee species itself (Salonen *et al.*, 2017).

Table 4: Mean Inhibition zone of *Apis mellifera* and *Meliponula beccarii* honey

Bee spec. & VChain		**Inhibition zones in mm**					
		Bacteria and concentration of honey					
		E.coli			***S. aureus***		
		100%	75%	50%	100%	75%	50%
Meliponula beccarii	FG	27.67 ± 1.53[a]	24.00 ± 3.00[a]	20.00 ± 2.65[a]	26.00 ± 2.65[a]	19.67 ± 4.16[a]	17.00 ± 3.60[a]
	GCLC	24.67 ± 2.52[b]	23.00 ± 3.00[b]	18.67 ± 5.51[b]	22.33 ± 4.51[b]	19.33 ± 3.21[a]	15.33 ± 3.05[b]
	RA	24.33 ± 1.52[c]	20.67 ± 0.57[c]	17.00 ± 1.00[c]	22.00 ± 3.61[b]	18.67 ± 2.52[b]	13.33 ± 4.16[c]
	RJ	6.33 ± 1.57[g]	4.00 ± 3.00[g]	2.67 ± 2.52[g]	5.67 ± 2.08[c]	2.67 ± 2.08[c]	0.67 ± 1.15[d]
	FG	15.33 ± 3.51[d]	12.00 ± 2.65[d]	9.33 ± 2.52[d]	5.26 ± 2.00[c]	1.67 ± 1.53[d]	0.56 ± 0.57[d]
	GCLC	12.67 ± 4.51[e]	10.00 ± 4.58[e]	7.33 ± 4.16[e]	5.00 ± 2.00[c]	1.00 ± 1.73[d]	0.33 ± 0.45[d]
	RA	10.33 ± 3.05[f]	9.00 ± 1.00[f]	7.00 ± 1.00[f]	3.67 ± 1.53[d]	1.00 ± 1.73[d]	0.00 ± 0.00[d]
Apis mellifera	RJ	3.00 ± 3.00[h]	1.67 ± 2.88[h]	0.00 ± 0.00[h]	3.00 ± 2.00[d]	1.00 ± 1.00[d]	0.00 ± 0.00[d]

Note; FG (-Farmer Geera); GCLC (Gera Chira Local Collectors); RA (Retailer Agaro); RJ(Retailer Jimma); E. *coli (Escherichia coli); S. aureus (Staphylococcus aureus);* Data are presented as Means ± SD; Mean values do not share the same letter/s across same column with distinct superscript letters indicate a significant difference at $p < 0.05$.

The mean inhibition zone from both types of honey samples in this study was less than the result reported by Yalemwork *et al.* (2013) and Boateng & Diunase, (2015) by 100% honey concentration using agar well diffusion method. A study on Cameroonian *A. mellifera* market honey samples against S. *aureus* and E.*coli* showed a relatively higher diameter of inhibition at 75% honey concentration Boateng & Diunase, (2015) compared to the result obtained in this study. Alvarez-Suarez *et al.* (2010) also reported that honey samples from stingless bees (*M. beecheii*) had a higher antimicrobial effect compared to honey samples of the sting bees *(A. mellifera*).

Gram-negative bacteria (*E.coli*) were found to be sensitive to the honey samples compared to the gram-positive bacteria *(S. aureus)* (Alvarez-Suarez *et al.,* 2010; Valdés-Silverio *et al.,* 2018). Gram-negative bacteria were more susceptible to the antibacterial action than gram-positive ones. Honey's various components, like hydrogen peroxide and methylglyoxal, might have easier access to disrupt the membranes of gram-negative bacteria due to their simpler structure (Follo *et al.,* 2013). Honey can disrupt bacterial membranes, inhibit protein synthesis, and even generate reactive oxygen species that damage bacterial cells(Nolan *et al.,* 2019). Gram-positive bacteria have a thick peptidoglycan wall, allowing a greater tolerance to high osmotic pressure (Pillet *et al.,* 2016).In line with Nishio *et al.* (2016), this study using an agar well diffusion assay found *Meliponula beccarii* honey to be more effective against gram-negative bacteria compared to gram-positive bacteria.

Higher antimicrobial activities of stingless bee honey from apiaries of Trinidad and Tobago against both gram-negative and gram-positive bacteria compared to honey from *Apis mellifera* (Brown *et al.,* 2020; Ng *et al.,* 2020; Domingos *et al.,* 2021). In a study by Ng *et al.* (2020) on the antibacterial activity of honeydew honey of a stingless bee, It was discovered that *E. Coli* was more vulnerable than *S. aureus*. In a comparable manner, Domingos *et al.* (2021) reported the strong antibacterial activity of stingless bee honey from the Amazon against a range of common gram-positive and gram-negative bacteria.

Besides, the honey sources, honey bee species, concentration of honey samples, and geographical areas from where the samples were collected could also bring about disparities in the results (Ofijan, 2023). Variations in the chemical makeup of the various bee species may result in variations in their antibacterial properties. The main components of honey that have antimicrobial activities are H_2O_2 and acids which are toxic to bacteria, and their amount depends on the presence of glucose oxidase enzyme (Brudzynski, 2006; Naama, 2009). Honey contains a high concentration of this enzyme, bacteria cannot respond normally to proliferative signals, and their growth remains arrested even when the honey is used in diluted forms (Brudzynski *et al.,* 2011). The peroxide group like H_2O_2 reacts with cell components such as cell walls and DNA inducing oxidative stress (Alzahrani *et al.,* 2012; Brudzynski *et al.,* 2012). However, when a honey sample is heated or exposed to sunlight during processing, purification, or storage, its enzymatic activity is rendered inactive (Ofijan *et al.,* 2022). Therefore, *Meliponula beccarii* honey had stronger antibacterial activity than *Apis mellifera* honey, likely due to differences in bee species and value chain (due to factors like storage and dilution) that affect the honey's bioactive content.

4.3.2. Minimum Inhibition Concentration

Table 5 shows the result of the minimum inhibitory concentration of *Apis mellifera* and *Meliponula beccarii* honey collected from different value chains. Honey samples from *Meliponula beccarii* had shown relatively lower minimum inhibitory concentration against both the tested bacteria compared to *Apis mellifera* honey samples. The study revealed that both the *Meliponula beccarii* and *Apis mellifera* (farmers Gera, retailers Gera Chira, and retailers Agaro) honey demonstrated inhibitory activity against *E. coli,* at least in the concentration of 50%. Retailers Jimma samples of honey from *Apis mellifera* have no minimum inhibitory concentration against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria. The *Meliponula beccarii* honey demonstrated inhibitory activity against *S. aureus* at least in the concentration of 50% compared to *Apis mellifera* honey. Based on a visual observation before incubation, the solution was clear because no bacterial growth occurred. After incubation, the solution was observed to be both clear and clouded. A straightforward solution shows inhibitory ability against bacterial growth. Hence, a clouded solution could not inhibit the growth of the test bacteria.

The differences in the MIC between the two types of honey were attributed to the bioactive compounds contained in the honey (Suhartatik *et al.,* 2023). There are variations in the chemical components of honey due to both internal and external influences; for instance, the soil condition and geographical location may influence the bioactive content (Shugaba, 2012). Thus, the less effective antibacterial activities of *Apis mellifera* honey samples in this study might be due to adulteration and exposure to light (Ofijan *et al.*, 2022).

Yalemwork *et al.* (2013) showed that stingless bee *tazma* honey was more effective against certain bacteria than *Apis mellifera* white Tigray honey. Specifically, their *tazma* honey inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at concentrations of 50%. The findings of this study were in agreement with other studies that showed honey exhibited a greater inhibitory effect on *E. coli* with a larger inhibition zone and lower MIC than *S. aureus* (Naama, 2009; Tuksitha *et al.*, 2018). This finding is in line with the findings by Suhartatik *et al.* (2023) which stated that honey from the *Apis mellifera* (wildflowers) and the *Apis mellifera* (kapok flowers) honey demonstrated inhibitory activity against *E. coli, Salmonella typhi,* and *S. aureus* at least in the concentration of 50%, the *Apis dorsata* honey demonstrated inhibitory activity against *E. coli* and *Salmonella typhi*. Generally, this study found that *Meliponula beccarii* honey is more effective against bacteria than *Apis mellifera* honey by inhibiting at lowest concentration, likely due to differences in bioactive compounds caused by factors like bee species and value chain.

Table 5: Minimum Inhibition Concentration of *Apis mellifera* and *Meliponula beccarii* honey

Bee spec. and VChain		**Bacteria and Concentration of Honey**					
		E.coli			***S. aureus***		
		100%	75%	50%	100%	75%	50%
Meliponula beccarii	FG	–	–	–	–	–	–
	GCLC	–	–	–	–	–	–
	RA	–	–	–	–	–	–
	RJ	–	+	+	+	+	+
Apis mellifera	FG	–	–	–	+	+	+
	GCLC	–	–	–	+	+	+
	RA	–	–	–	+	+	+
	RJ	+	+	+	+	+	+

FG (Farmer Geera); GCLC (Gera Chira Local Collectors); RA (Retailer Agaro); RJ (Retailer Jimma); Positive (+): Turbidity indicating growth; Negative (–): No turbidity indicating absence of growth

4.3.3. Minimum Bactericidal Concentration

The observed differences in Minimum Bactericidal Concentration (MBC) between *Meliponula beccarii* and *Apis mellifera* honey highlight the variation in potency depending on the bee species and potentially the value chain of the honey (Table 6). Honey samples from *Meliponula beccarii* had the lowest minimum bactericidal concentration against most of the test bacteria compared to the *Apis mellifera* honey samples. Minimum bactericidal concentration is the lowest concentration of honey that kills bacteria. The lower MBC value indicates that the lower concentration of honey is required to kill gram-negative (*E.coli*) and gram-positive bacteria (*S. aureus*). Both *E. coli* and *S. aureus* were the most susceptible to *Meliponula beccarii* honey samples at the lowest concentration of honey except honey from retailer Jimma *Meliponula beccarii* honey demonstrated a lower MBC (more potent) against both *E. coli* and S. aureus compared to *Apis mellifera honey*, which only showed activity against *E. coli* and at a higher concentration (100%).

This aligns with previous studies by Yalemwork *et al.* (2013) and Nwankwo *et al.* (2014) who reported even lower MBCs for both bacteria strains. However, it's important to consider that these studies might have involved different honey sources or processing methods, potentially affecting the final potency. Overall, *Meliponula beccarii* honey was more potent against bacteria (lower MBC) than *Apis mellifera* honey, possibly due to bee species or value chain.

Table 6: MBC of *Apis mellifera* and *Meliponula beccarii* honey

Bee species and VChain		**Bacteria and concentration of honey**					
		E.coli			***S. aureus***		
		100%	75%	50%	100%	75%	50%
Meliponula beccarii	FG	–	–	–	–	–	–
	GCLC	–	–	–	–	–	–
	RA	–	–	–	–	–	–
	RJ	_	+	+	+	+	+
Apis mellifera	FG	–	–	–	+	+	+
	GCLC	–	–	–	+	+	+
	RA	–	–	–	+	+	+
	RJ	+	+	+	+	+	+

Note: FG (Farmer Geera); GCLC (Gera Chira Local Collectors); RA (Retailer Agaro); RJ (Retailer Jimma); Positive (+): Indicating growth; Negative (–): Indicating absence of growth

4.4. Correlation between Bioactive Components, Antioxidant Activities, and Antibacterial Activities

The correlation results between bioactive components, antioxidants, and antibacterial activities calculated for honey samples collected from different value chains are shown in Table 7. In a study by Ratner (2009), correlations closer to 1 (positive or negative) indicate strong relationships. In these cases, one parameter tends to move in the same direction (positive) or opposite direction (negative) as the other parameter with high consistency. This suggests a potentially powerful underlying connection between them. Moderate correlations (around 0.3 to 0.7) show a noticeable trend, but the link is weaker. Other factors might be influencing the parameters alongside their connection. Weak correlations (around 0.1 to 0.3) suggest minimal or unclear relationships. Finally, a correlation of 0 indicates no linear association between the two parameters. Changes in one do not seem to predict changes in the other in a linear way.

There is a strong positive correlation ($p < 0.05$) ($r = 0.951$) between total phenolic content (TPC) and total flavonoid content (TFC). This suggests that honey rich in phenolic, measured by TPC, also tends to be high in flavonoids, measured by TFC (Rom *et al.*, 2022). The high correlation value and likely very low P-value (based on the correlation) further support this strong correlation. In essence, honey with abundant TPC likely has high TFC as well, as both measures indicate the presence of these beneficial phenolic compounds which contribute to the antioxidant properties of honey(Suleiman *et al.*, 2020).

Similarly, there is strong a correlation between Vitamin C and TPC: $r = 0.941$, DPPH%: $r = 0.902$ at ($p < 0.05$). This suggests that honey with high vitamin C content tends to be richer in other antioxidants and exhibit greater antibacterial activity(Czernicka *et al.*, 2024). While the correlations between Vitamin C and FRAP ($r = 0.551$($p < 0.05$)) and inhibition of *S. aureus* at various concentrations (*S. aureus* 100%: $r = 0.875$, *S. aureus* 75%: $r = 0.828$, *S. aureus* 50%: $r = 0.862$) are positive, they are weaker compared to the correlations observed with other parameters like TPC ($r = 0.941$) and DPPH% ($r = 0.902$). The lower correlation with FRAP suggests that other antioxidant compounds might play a more significant role in the specific type of antioxidant activity it measures. Similarly, the positive correlations with *S. aureus* inhibition indicate a potential influence of Vitamin C on this antibacterial effect, but other factors likely contribute as well, given the moderate strength of the relationships(Otmani *et al.*, 2021).

Table 7: Correlation Matrix between Bioactive Contents, Antioxidant Capacity, and In Vitro Antibacterial Activities

Parameters	TPC	TFC	TAC	Beta	Vit- C	DPPH %	IC50	FRAP	*E.coli*100	*E.coli*75	*E.coli*50	*S.aur*100	*S.aur* 75
TFC	0.951												
TAC	-0.582	-0.561											
Beta-Car	0.911	0.840	-0.496										
Vitamin C	0.941	0.895	-0.594	0.982									
DPPH %	0.914	0.856	-0.742	0.838	0.902								
IC50	-0.774	-0.683	0.806	-0.654	-0.736	-0.926							
FRAP	0.447	0.533	-0.694	0.432	0.551	0.598	-0.516						
*E.coli*100%	0.811	0.729	-0.639	0.811	0.843	0.904	-0.793	0.583					
E.coli 75%	0.823	0.749	-0.632	0.795	0.828	0.895	-0.787	0.571	0.988				
E. coli 50%	0.811	0.733	-0.636	0.789	0.820	0.882	-0.785	0.555	0.970	0.981			
S. aur 100%	0.871	0.758	-0.451	0.882	0.875	0.839	-0.673	0.416	0.923	0.921	0.916		
S. aur 75 %	0.853	0.729	-0.422	0.840	0.828	0.804	-0.644	0.372	0.899	0.909	0.902	0.983	
S. aur 50 %	0.878	0.780	-0.447	0.870	0.862	0.828	-0.652	0.421	0.894	0.903	0.895	0.986	0.983

Where: TPC (total phenolic content); TFC (total flavonoid); Vit –C (vitamin C); Beta-car, (beta carotene content) ; DPPH (2, 2-diphenyl -1-picryhdrazyl) ; IC_{50}, (half maximal inhibitory concentration); FRAP (Ferric reducing antioxidant power); Saur(*Staphylococcus aureus*); *E.coli* (*Escherichia coli*)

There is also a strong positive correlation between *E. coli* and *S. aureus* inhibition. These high correlation coefficients (*S. aureus* 100% and *E.coli* 100%: r = 0.923 ($p < 0.05$)) suggest that honey effective against *E. coli* at higher concentrations (*E.coli* 100%) is likely also effective against *S. aureus* at higher concentrations (*S. aureus* 100%). This strong positive correlation, along with likely very low P-values (based on the correlation values), indicates that these honey might possess broad-spectrum antibacterial activity, meaning they can inhibit a wide range of bacteria(Kačániová *et al.*, 2022).

A strong negative correlation between IC_{50} and most other parameters (except FRAP), indicates an inverse relationship. This means lower IC_{50} values (which signify higher antioxidant activity) tend to be associated with higher values of other antioxidant parameters (DPPH%, TAC). The strength of this inverse relationship is evident from the strong negative correlation coefficients (mostly below -0.7). A strong negative correlation exists between IC_{50} and DPPH% (r = -0.926). This implies that these honey possess strong free radical scavenging activity (measured by DPPH %) and require less concentration to achieve it (reflected by the lower IC50 value), indicating their overall high antioxidant potential. The correlations between FRAP and most other parameters are generally weaker, with no values exceeding r = 0.7 (considered a strong positive correlation). This suggests that FRAP might be measuring a distinct aspect of antioxidant activity in honey. Different antioxidant assays target various mechanisms of free radical scavenging. FRAP could be more sensitive to specific types of free radicals or pathways not as effectively captured by DPPH% or TAC(Cianciosi *et al.*, 2018).

This research found a strong positive correlation between total phenols (TPC) and total flavonoids (TFC), similar to a previous study on Sicilian (Italy) monofloral honey *(*Attanzio *et al.*, 2016) which reported a correlation coefficient of (r = 0.919). The research finding is higher than the study of (Albu *et al.*, 2022) who reported a strong correlation between total phenol content and total flavonoid content (r =0.76) of Romanian monofloral honey. The research findings were in agreement with Beretta *et al.* (2005) report, which highlighted a strong correlation between total phenol content and total antioxidant activity (rPC/FRAP = 0.885). A strong correlation between Vitamin C and TPC, DPPH in this study is higher than reported by (Alexandra *et al.*, 2021). The strong negative correlation between IC_{50} and DPPH% in this study is lower than that of (Gorjanović *et al.*, 2013). Strong correlation results between bioactive components, antioxidants, and antibacterial activities of honey samples findings in this study are comparable with other studies, including those by (Alvarez-Suarez *et al.*, 2010; Boussaid *et al.*, 2018; Gül & Pehlivan, 2018; Zapata-Vahos *et al.*, 2023).

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The results of the current study indicated that the *Meliponula beccarii* honey had higher total phenolic, total flavonoid, vitamin C, and beta-carotene content and also showed higher percent DPPH scavenging activity and ferric-reducing antioxidant power than *Apis mellifera* honey. However, *Apis mellifera* honey had higher alkaloid content. *Meliponula beccarii* honey showed higher antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* based on their inhibition zones in diameter. But, *Apis mellifera* honey only showed antibacterial activity against *Escherichia coli.* These indicated that the honey value chain (honey producers, collectors, and sellers) and bee species affected the antibacterial activities of honey. Therefore, it can be concluded that bee species and value chain affected the bioactive components, antioxidant activity, and antibacterial activity of the honey.

5.2. Recommendations

Based on the finding, the following points were recommended

- Antimicrobial assays need to work on extracts of bioactive compounds.
- Further research using rodent and human models is important to further verify the honeys
- The limitation of this study in describing samples based on floral origin, which is a major factor in honey, and is a state-of-the-art description of honey
- Implement quality control measures and standards along the honey value chain to ensure consistent levels of bioactive components and antioxidant activities in honey products.

6. REFERENCES

Abd Jalil, M. A., Kasmuri, A. R., & Hadi, H. (2017). Stingless bee honey, the natural wound healer: A review. *Skin Pharmacology and Physiology*, *30*(2), 66–75. https://doi.org/10.1159/000458416

Abdi, G. G. (2023). *Assessment of Honey Handling Practices Along the Supply Chain From Gera District to Jimma Town, Southwestern Ethiopia*.

Adalina, Y., Kusmiati, E., & Pudjiani, M. (2020). Phytochemical test and physical-chemical properties of rubber honey from three types of bees (Apis mellifera, Apis dorsata, and Trigona Itama). *IOP Conference Series: Materials Science and Engineering*, *935*(1). https://doi.org/10.1088/1757-899X/935/1/012007

Adgaba, N., (2007). Atlas of pollen grains of major honeybee flora of Ethiopia. *Holetta bee research centre, Holetta, Ethiopia*.

Adgaba, N., Al-Ghamdi, A. A., Getachew, A., Tadesse, Y., Belay, A., Ansari, M. J., Radloff, S. E., & Sharma, D. (2017). Characterization of honey by their botanical and geographical origins based on physico-chemical properties and chemo-metrics analysis. *Journal of Food Measurement and Characterization*, *11*(3), 1106–1117. https://doi.org/10.1007/s11694-017-9487-4

Adgaba, N., Al-ghamdi, A., Sharma, D., Tadesse, Y., Alghanem, S. M., Ali, K., Javed, M., Khalid, G., & Mohamed, A. (2020). Saudi Journal of Biological Sciences Physico-chemical, antioxidant, and anti-microbial properties of some Ethiopian mono-floral honey. *Saudi Journal of Biological Sciences*, *27*(9), 2366–2372. https://doi.org/10.1016/j.sjbs.2020.05.031

Afrin, S., Haneefa, S. M., Fernandez-Cabezudo, M. J., Giampieri, F., Al-Ramadi, B. K., & Battino, M. (2020). Therapeutic and preventive properties of honey and its bioactive compounds in cancer: An evidence-based review. *Nutrition Research Reviews*, *33*(1), 50–76. https://doi.org/10.1017/S0954422419000192

Afroz R, & PJ, T. E. Z. W. and L. (2016). Molecular Pharmacology of Honey. *Clinical and Experimental Pharmacology*, *06*(03). https://doi.org/10.4172/2161-1459.1000212

Ahmed, M.-E. K. (2015). Scientific Papers. *Sudan Medical Journal*, *51*(2), 64–68. https://doi.org/10.12816/0019153

Ajibola, A. (2015). Money and health. *Nature*, *217*(5131), 797. https://doi.org/10.1038/217797a0

Ak, Z., Ns, M. I., F, T. A., Mn, I., & Hm, Y. (2021). *Quality Characteristics, Antioxidant and Anticancer Potential of Stingless Bee Honey and Honeybee Honey from Similar Environmental Conditions*. *20*(4), 29–36.

Al-Kafaween, M. A., Alwahsh, M., Mohd Hilmi, A. B., & Abulebdah, D. H. (2023). Physicochemical Characteristics and Bioactive Compounds of Different Types of Honey and Their Biological and Therapeutic Properties: A Comprehensive Review. In *Antibiotics* (Vol. 12, Issue 2). https://doi.org/10.3390/antibiotics12020337

Al-Mamary, M., Al-Meeri, A., & Al-Habori, M. (2002). Antioxidant activities and total phenolics of different types of honey. *Nutrition Research*, *22*(9), 1041–1047.

https://doi.org/10.1016/S0271-5317(02)00406-2
Albu, A., Radu-Rusu, R. M., Simeanu, D., Radu-Rusu, C. G., & Pop, I. M. (2022). Phenolic and Total Flavonoid Contents and Physicochemical Traits of Romanian Monofloral Honeys. *Agriculture (Switzerland)*, *12*(9), 1–15. https://doi.org/10.3390/agriculture12091378
Alexandra Bodó , Lilla Radványi , Tamás K″oszegi, M. K. 1, & , Rita Csepregi , Dávid U. Nagy, Á. F. and M. K. (2021). *and Mineral Content*.
Aljadi, A. M., & Kamaruddin, M. Y. (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry*, *85*(4), 513–518. https://doi.org/10.1016/S0308-8146(02)00596-4
Alkadi, H. (2022). A Review on Free Radicals and Antioxidants. *Infectious Disorders - Drug Targets*, *20*(1), 16–26. https://doi.org/10.2174/22123989oteznmziwtcvy
Almasaudi, S. (2021). The antibacterial activities of honey. *Saudi Journal of Biological Sciences*, *28*(4), 2188–2196. https://doi.org/10.1016/j.sjbs.2020.10.017
Alonso, Á. M., Guillén, D. A., Barroso, C. G., Puertas, B., & García, A. (2002). Determination of the antioxidant activity of wine byproducts and its correlation with polyphenolic content. *Journal of Agricultural and Food Chemistry*, *50*(21), 5832–5836. https://doi.org/10.1021/jf025683b
Alvarez-Suarez, José M., Gasparrini, M., Forbes-Hernández, T. Y., Mazzoni, L., & Giampieri, F. (2014). The composition and biological activity of honey: A focus on manuka honey. *Foods*, *3*(3), 420–432. https://doi.org/10.3390/foods3030420
Alvarez-Suarez, José M., Giampieri, F., Brenciani, A., Mazzoni, L., Gasparrini, M., González-Paramás, A. M., Santos-Buelga, C., Morroni, G., Simoni, S., Forbes-Hernández, T. Y., Afrin, S., Giovanetti, E., & Battino, M. (2018). Apis mellifera vs Melipona beecheii Cuban poly floral honey: A comparison based on their physicochemical parameters, chemical composition, and biological properties. *Lwt*, *87*, 272–279. https://doi.org/10.1016/j.lwt.2017.08.079
Alvarez-Suarez, José M., Giampieri, F., Damiani, E., Astolfi, P., Fattorini, D., Regoli, F., Quiles, J. L., & Battino, M. (2012). Radical-scavenging Activity, Protective Effect Against Lipid Peroxidation and Mineral Contents of Monofloral Cuban Honeys. *Plant Foods for Human Nutrition*, *67*(1), 31–38. https://doi.org/10.1007/s11130-011-0268-7
Alvarez-Suarez, Jose M., González- Paramás, A. M., Santos-Buelga, C., & Battino, M. (2010). Antioxidant characterization of native monofloral Cuban honey. *Journal of Agricultural and Food Chemistry*, *58*(17), 9817–9824. https://doi.org/10.1021/jf1018164
Alvarez-Suarez, Jose M., Tulipani, S., Díaz, D., Estevez, Y., Romandini, S., Giampieri, F., Damiani, E., Astolfi, P., Bompadre, S., & Battino, M. (2010). Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content, and other chemical compounds. *Food and Chemical Toxicology*, *48*(8–9), 2490–2499. https://doi.org/10.1016/j.fct.2010.06.021
Alves, A., Ramos, A., Gonçalves, M. M., Bernardo, M., & Mendes, B. (2013). Antioxidant activity, quality parameters, and mineral content of Portuguese monofloral honey. *Journal of Food Composition and Analysis*, *30*(2), 130–138.

https://doi.org/10.1016/j.jfca.2013.02.009

Alzahrani, H. A., Alsabehi, R., Boukraâ, L., Abdellah, F., Bellik, Y., & Bakhotmah, B. A. (2012). The antibacterial and antioxidant potency of floral honey from different botanical and geographical origins. *Molecules, 17*(9), 10540–10549. https://doi.org/10.3390/molecules170910540

Alzahrani, H. A., Boukraa, L., Bellik, Y., Abdellah, F., Bakhotmah, B. A., Kolayli, S., & Sahin, H. (2012). Evaluation of the antioxidant activity of three varieties of honey from different botanical and geographical origins. *Global Journal of Health Science*, *4*(6), 191–196. https://doi.org/10.5539/gjhs.v4n6p191

Andrews, J. M. (2006). BSAC standardized disc susceptibility testing method (version 5). *Journal of Antimicrobial Chemotherapy*, *58*(3), 511–529. https://doi.org/10.1093/jac/dkl277

Ashagrie Tafere, D. (2021). Chemical composition and uses of Honey: A Review. *Journal of Food Science and Nutrition Research, 04*(03), 194–201. https://doi.org/10.26502/jfsnr.2642-11000072

Attanzio, A., Tesoriere, L., Allegra, M., & Livrea, M. A. (2016). Monofloral honey by Sicilian black honeybee (Apis mellifera ssp. sicula) have high reducing power and antioxidant capacity. *Heliyon*, *2*(11). https://doi.org/10.1016/j.heliyon.2016.e00193

Aurongzeb, M., Rashid, Y., & Kamran Azim, M. (2019). Antifungal activity of unifloral and multifloral Pakistani honeys against clinically isolated Candida species. *J. Biochem. Mol. Biol*, *52*(3), 105–117.

Ávila, S., Beux, M. R., Ribani, R. H., & Zambiazi, R. C. (2018). Stingless bee honey: Quality parameters, bioactive compounds, health-promotion properties, and modification detection strategies. *Trends in Food Science and Technology*, *81*, 37–50. https://doi.org/10.1016/j.tifs.2018.09.002

Ávila, S., Lazzarotto, M., Hornung, P. S., Teixeira, G. L., Ito, V. C., Bellettini, M. B., Beux, M. R., Beta, T., & Ribani, R. H. (2019). Influence of stingless bee genus (Scaptotrigona and Melipona) on the mineral content, physicochemical and microbiological properties of honey. *Journal of Food Science and Technology*, *56*(10), 4742–4748. https://doi.org/10.1007/s13197-019-03939-8

Aysun, H. (2009). Floating-point arithmetic in COBOL. *Communications of the ACM*, *5*(5), 269–271. https://doi.org/10.1145/367710.367739

Bacayo, M. F. D. C., Shafi, S. F., Choon, W. C., Fattepur, S., Nilugal, K. C., Khan, J., Asmani, F., & Yusuf, E. (2019). *A Comparative Study of the Antimicrobial Activity of Wild (Tualang) Honey and Artificial Honey Against Methicillin-resistant Staphylococcus Aureus, Streptococcus pneumoniae and Klebsiella pneumoniae. Bromo 2018*, 111–117. https://doi.org/10.5220/0008358301110117

Bahar, S., Korkmaz, S., & Korkmaz, B. İ. O. (2022). Color and vitamin C equivalent antioxidant capacity of honey from different origins in Turkey. *ACTA Apicola Brasilica*, *10*, e9649. https://doi.org/10.18378/aab.10.9649

Barros, L., Calhelha, R. C., Vaz, J. A., Ferreira, I. C. F. R., Baptista, P., & Estevinho, L. M. (2007). Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. *European Food Research and Technology*, *225*(2), 151–156. https://doi.org/10.1007/s00217-006-0394-x

Bastos, D. H. M., Dos Santos, M. C. M., Mendonça, S., & Torres, E. A. F. S. (2009). Antioxidant capacity and phenolic content of stingless bee honey from Amazon in comparison to Apis bee honey. *Acta Horticulturae*, *841*(August 2009), 483–485. https://doi.org/10.17660/actahortic.2009.841.64

Becerril-sánchez, A. L., Quintero-salazar, B., Dublán-garcía, O., & Escalona-buendía, H. B. (2021). Phenolic compounds in honey and their relationship with antioxidant activity, botanical origin, and color. *Antioxidants*, *10*(11), 1–23. https://doi.org/10.3390/antiox10111700

Beretta, G., Granata, P., Ferrero, M., Orioli, M., & Facino, R. M. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta*, *533*(2), 185–191. https://doi.org/10.1016/j.aca.2004.11.010

Bian, Y., Zhang, Y., Zhou, Y., Li, G. H., & Feng, X. S. (2022). Progress in the Pretreatment and Analysis of Flavonoids: An Update since 2013. *Separation and Purification Reviews*, *51*(1), 11–37. https://doi.org/10.1080/15422119.2020.1801469

Bicudo de Almeida-Muradian, L., Monika Barth, O., Dietemann, V., Eyer, M., Freitas, A. da S. de, Martel, A. C., Marcazzan, G. L., Marchese, C. M., Mucignat-Caretta, C., Pascual-Maté, A., Reybroeck, W., Sancho, M. T., & Gasparotto Sattler, J. A. (2020). Standard methods for Apis mellifera honey research. *Journal of Apicultural Research*, *59*(3), 1–62. https://doi.org/10.1080/00218839.2020.1738135

Biluca, F. C., Braghini, F., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2016). Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (Meliponinae). *Journal of Food Composition and Analysis*, *50*, 61–69. https://doi.org/10.1016/j.jfca.2016.05.007

Biluca, F. C., da Silva, B., Caon, T., Mohr, E. T. B., Vieira, G. N., Gonzaga, L. V., Vitali, L., Micke, G., Fett, R., Dalmarco, E. M., & Costa, A. C. O. (2020). Investigation of phenolic compounds, antioxidant and anti-inflammatory activities in stingless bee honey (Meliponinae). *Food Research International*, *129*(June 2019), 108756. https://doi.org/10.1016/j.foodres.2019.108756

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M. P., Albertini, M. C., & Piatti, E. (2006). Raw Millefiori honey is packed full of antioxidants. *Food Chemistry*, *97*(2), 217–222. https://doi.org/10.1016/j.foodchem.2005.03.039

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M. P., & Piatti, E. (2007). Honey flavonoids as protection agents against oxidative damage to human red blood cells. *Food Chemistry*, *104*(4), 1635–1640. https://doi.org/10.1016/j.foodchem.2007.03.014

Boateng, J., & Diunase, K. N. (2015). Comparing the antibacterial and functional properties of Cameroonian and manuka kinds of honey for potential wound healing-have we come full cycle in dealing with antibiotic resistance? *Molecules*, *20*(9), 16068–16084. https://doi.org/10.3390/molecules200916068

Bogdanov, S., Jurendic, T., Sieber, R., & Gallmann, P. (2008). Honey for nutrition and health: A review. *Journal of the American College of Nutrition*, *27*(6), 677–689. https://doi.org/10.1080/07315724.2008.10719745

Borsato, D. M., Prudente, A. S., Döll-Boscardin, P. M., Borsato, A. V., Luz, C. F. P., Maia, B. H. L. N. S., Cabrini, D. A., Otuki, M. F., Miguel, M. D., Farago, P. V., &

Miguel, O. G. (2014). Topical Anti-inflammatory activity of monofloral honey of Mimosa cabrilla Provided by *melipona marginata* during winter in Southern Brazil. *Journal of Medicinal Food*, *17*(7), 817–825. https://doi.org/10.1089/jmf.2013.0024

Boudiar, I., Bouacha, M., Besnaci, S., Kouch, M., Abdi, A., & Schweitzer, P. (2022). *High-Performance Anion-Exchange Chromatography With Pulsed Amperometric Detection (Hpaec – Pad) and Physicochemical Analysis of Honey : Evaluation of the Composition and the Quality*. *23*(3), 217–229.

Boulanouar Bakchiche, Mounir Habati, Ahmed Benmebarek, A. G. (2017). Total Phenolic, Flavonoid Contents and Antioxidant Activities of Honey and Propolis Collected from the Region of Laghouat (South of Algeria). *International Journal of Pharmacognosy & Chinese Medicine*, *1*(2), 91–97. https://doi.org/10.23880/ipcm-16000110

Boussaid, A., Chouaibi, M., Rezig, L., Hellal, R., Donsì, F., Ferrari, G., & Hamdi, S. (2018). Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. *Arabian Journal of Chemistry*, *11*(2), 265–274. https://doi.org/10.1016/j.arabjc.2014.08.011

Braghini, F., Biluca, F. C., Schulz, M., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2022). Stingless bee honey: a precious but unregulated product - reality and expectations. *Food Reviews International*, *38*(S1), 683–712. https://doi.org/10.1080/87559129.2021.1884875

Brown, E., O'Brien, M., Georges, K., & Suepaul, S. (2020). Physical characteristics and antimicrobial properties of Apis mellifera, Frieseomelitta nigra, and Melipona favosa bee honey from apiaries in Trinidad and Tobago. *BMC Complementary Medicine and Therapies*, *20*(1), 85. https://doi.org/10.1186/s12906-020-2829-5

Brudzynski, K. (2006). Effect of hydrogen peroxide on antibacterial activities of Canadian honey. *Canadian Journal of Microbiology*, *52*(12), 1228–1237. https://doi.org/10.1139/W06-086

Brudzynski, K. (2020). A current perspective on hydrogen peroxide production in honey. A review. *Food Chemistry*, *332*, 127229. https://doi.org/10.1016/j.foodchem.2020.127229

Brudzynski, K., Abubaker, K., & Miotto, D. (2012). Unraveling a mechanism of honey antibacterial action: Polyphenol/H 2O 2-induced oxidative effect on bacterial cell growth and DNA degradation. *Food Chemistry*, *133*(2), 329–336. https://doi.org/10.1016/j.foodchem.2012.01.035

Brudzynski, K., Abubaker, K., St-Martin, L., & Castle, A. (2011). Re-examining the role of hydrogen peroxide in bacteriostatic and bactericidal activities of honey. *Frontiers in Microbiology*, *2*(OCT), 1–9. https://doi.org/10.3389/fmicb.2011.00213

Bucekova, M., Jardekova, L., Juricova, V., Bugarova, V., Di Marco, G., Gismondi, A., Leonardi, D., Farkasovska, J., Godocikova, J., Laho, M., Klaudiny, J., Majtan, V., Canini, A., & Majtan, J. (2019). Antibacterial activity of different blossom honey: New findings. *Molecules*, *24*(8). https://doi.org/10.3390/molecules24081573

Bueno-Costa, F. M., Zambiazi, R. C., Bohmer, B. W., Chaves, F. C., Silva, W. P. da, Zanusso, J. T., & Dutra, I. (2016). Antibacterial and antioxidant activity of honey from the state of Rio Grande do Sul, Brazil. *Lwt*, *65*, 333–340.

https://doi.org/10.1016/j.lwt.2015.08.018
Burdurlu, H. S., Koca, N., & Karadeniz, F. (2006). Degradation of vitamin C in citrus juice concentrates during storage. *Journal of Food Engineering*, *74*(2), 211–216. https://doi.org/10.1016/j.jfoodeng.2005.03.026
Can, Z., Yildiz, O., Sahin, H., Akyuz Turumtay, E., Silici, S., & Kolayli, S. (2015). An investigation of Turkish honey: Their physicochemical properties, antioxidant capacities, and phenolic profiles. *Food Chemistry*, *180*, 133–141. https://doi.org/10.1016/j.foodchem.2015.02.024
Carina, F., Gois, D., Schulz, M., Braghini, F., Valdemiro, L., França, H., Rodrigues, E., Vitali, L., Amadeu, G., Borges, D. L. G., Carolina, A., Costa, O., & Fett, R. (2017). *Journal of Food Composition and Analysis Phenolic compounds, antioxidant capacity and bioaccessibility of minerals of stingless bee honey (Meliponinae)*. *63*(July), 89–97. https://doi.org/10.1016/j.jfca.2017.07.039
Castro-Vázquez, L., Díaz-Maroto, M. C., González-Viñas, M. A., De La Fuente, E., & Pérez-Coello, M. S. (2008). Influence of storage conditions on chemical composition and sensory properties of citrus honey. *Journal of Agricultural and Food Chemistry*, *56*(6), 1999–2006. https://doi.org/10.1021/jf072227k
Cebrero, G., Sanhueza, O., Pezoa, M., Báez, M. E., Martínez, J., Báez, M., & Fuentes, E. (2020). Relationship among the minor constituents, antibacterial activity and geographical origin of honey: A multifactor perspective. *Food Chemistry*, *315*, 126296. https://doi.org/10.1016/j.foodchem.2020.126296
Chettri, U., & Kumari, S. (2020). A Review on Prebiotic Importance of Stingless Bee Honey and its Ethnomedicinal and Therapeutic Potential. *Article in International Journal of Pharmaceutical Sciences Review and Research*, *63*(2), 150–156. www.globalresearchonline.net
Chidi, H., Chidi, O. H., & Odo, P. E. (2017). *Meliponiculture for Sustainable Economy MELIPONICULTURE FOR SUSTAINABLE ECONOMY*. *Article Info ABSTRACT*. *November*. https://www.researchgate.net/publication/320922565
Chis, A. M., Purcarea, C., Dzugan, M., & Teusdea, A. (2016). *Comparative Antioxidant Content and Antioxidant Activity of Selected Romanian and Polish Honeydew Honey*. *April 2017*.
Chowdhury, H. M. H., Kubra, K., & Ahmed, R. R. (2015). Screening of antimicrobial, antioxidant properties, and bioactive compounds of some edible mushrooms cultivated in Bangladesh. *Annals of Clinical Microbiology and Antimicrobials*, *14*(1), 1–6. https://doi.org/10.1186/s12941-015-0067-3
Chua, L. S., Rahaman, N. L. A., Adnan, N. A., & Eddie Tan, T. T. (2013). Antioxidant activity of three honey samples in relation to their biochemical components. *Journal of Analytical Methods in Chemistry*, *2013*. https://doi.org/10.1155/2013/313798
Cianciosi, D., Forbes-Hernández, T. Y., Afrin, S., Gasparrini, M., Reboredo-Rodriguez, P., Manna, P. P., Zhang, J., Lamas, L. B., Flórez, S. M., Toyos, P. A., Quiles, J. L., Giampieri, F., & Battino, M. (2018). Phenolic compounds in honey and their associated health benefits: A review. *Molecules*, *23*(9), 1–20. https://doi.org/10.3390/molecules23092322
Clearwater, M. J., Revell, M., Noe, S., & Manley-Harris, M. (2018). Influence of

genotype, floral stage, and water stress on floral nectar yield and composition of mānuka (Leptospermum scoparium). *Annals of Botany*, *121*(3), 501–512. https://doi.org/10.1093/AOB/MCX183

Combarros-Fuertes, P., Estevinho, L. M., Dias, L. G., Castro, J. M., Tomás-Barberán, F. A., Tornadijo, M. E., & Fresno-Baro, J. M. (2019). Bioactive Components and Antioxidant and Antibacterial Activities of Different Varieties of Honey: A Screening Before Clinical Application. *Journal of Agricultural and Food Chemistry*, *67*(2), 688–698. https://doi.org/10.1021/acs.jafc.8b05436

Cui, Z. H., He, H. L., Wu, S. Bin, Dong, C. L., Lu, S. Y., Shan, T. J., Fang, L. X., Liao, X. P., Liu, Y. H., & Sun, J. (2021). Rapid screening of essential oils as substances that enhance antibiotic activity using a modified well diffusion method. *Antibiotics*, *10*(4). https://doi.org/10.3390/antibiotics10040463

Czernicka, M. A., Sowa-Borowiec, P., Dudek, T., Cichoński, J., Puchalski, C., & Chrzanowski, G. (2024). Antioxidant Capacity of Honey Enriched by Wildflowers. *Applied Sciences*, *14*(5), 2018. https://doi.org/10.3390/app14052018

Da Silva Cruz, L. F., Lemos, P. V. F., Santos, T. de S., Tavares, P. P. L. G., Nascimento, R. Q., Almeida, L. M. R., de Souza, C. O., & Druzian, J. I. (2023). Storage conditions significantly influence the stability of stingless bee (Melipona scutellaris) honey. *Journal of Apicultural Research*, *62*(3), 530–541. https://doi.org/10.1080/00218839.2021.1950971

Da Silva, P. M., Gauche, C., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2016). Honey: Chemical composition, stability, and authenticity. *Food Chemistry*, *196*, 309–323. https://doi.org/10.1016/j.foodchem.2015.09.051

De Araújo, F. G., de Araújo, N. O., de Oliveira, V. R. L., Leite, R. H. de L., Dos Santos, F. K. G., & Aroucha, E. M. M. (2023). Physicochemical and bioactive properties of Apis and stingless bee (Meliponini) honey from Brazilian Caatinga. *Acta Scientiarum - Animal Sciences*, *45*, 1–8. https://doi.org/10.4025/actascianimsci.v45i1.59799

Degaga, A. H. (2017). Identification of honey source bee floras during major and minor honey harvesting seasons in Jimma zone, Southwest. *Journal of Environment and Earth Science*, *7*(3), 25–32.

Deng, J., Liu, R., Lu, Q., Hao, P., Xu, A., Zhang, J., & Tan, J. (2018). Biochemical properties, antibacterial and cellular antioxidant activities of buckwheat honey in comparison to manuka honey. *Food Chemistry*, *252*(August 2017), 243–249. https://doi.org/10.1016/j.foodchem.2018.01.115

Dezmirean, D. S., Mărghitaş, L. A., Chirilă, F., Copaciu, F., Simonca, V., Bobiş, O., & Erler, S. (2017). Influencia del origen geográfico, la fuente vegetal y las sustancias polifenólicas en las propiedades antimicrobianas del propóleos contra patógenos humanos y de las abejas melíferas. *Journal of Apicultural Research*, *56*(5), 588–597. https://doi.org/10.1080/00218839.2017.1356205

Domingos, S. C. B., Clebis, V. H., Nakazato, G., de Oliveira, A. G., Takayama Kobayashi, R. K., Peruquetti, R. C., Pereira, C. D., Santa Rosa, M. T., & dos Santos Medeiros, L. (2021). Antibacterial activity of honey from Amazonian stingless bees of Melipona spp. and its effects on bacterial cell morphology. *Journal of the Science of Food and Agriculture*, *101*(5), 2072–2077. https://doi.org/10.1002/jsfa.10828

Duarte, A. W. F., Dos Santos Vasconcelos, M. R., De Menezes, A. P. D., Da Silva, S. C., Oda-Souza, M., & López, A. M. Q. (2012). Composition and antioxidant activity of honey from Africanized and stingless bees in Alagoas (Brazil): A multivariate analysis. *Journal of Apicultural Research*, *51*(1), 23–35. https://doi.org/10.3896/IBRA.1.51.1.04

Dubero Sime, Minaleshawa Atlabachew, M. R.-A. and T. Z. (2015). *Total phenols and antioxidant activities of natural honey and propolis collected from different geographical regions of Ethiopia*. *29*(2), 163–172.

Elsayed Azab, A., A Adwas, Almokhtar, Ibrahim Elsayed, A. S., A Adwas, A., Ibrahim Elsayed, Ata Sedik, & Quwaydir, F. A. (2019). Oxidative stress and antioxidant mechanisms in the human body. *Journal of Applied Biotechnology & Bioengineering*, *6*(1), 43–47. https://doi.org/10.15406/jabb.2019.06.00173

Escriche, I., Kadar, M., Juan-Borrás, M., & Domenech, E. (2014). Suitability of antioxidant capacity, flavonoids, and phenolic acids for floral authentication of honey. Impact of industrial thermal treatment. *Food Chemistry*, *142*, 135–143. https://doi.org/10.1016/j.foodchem.2013.07.033

Eteraf-Oskouei, T., & Najafi, M. (2013). Traditional and modern uses of natural honey in human diseases: A review. *Iranian Journal of Basic Medical Sciences*, *16*(6), 731–742.

Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M., & Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples : Different contributions of the entire honey and phenolic extract. *Food Chemistry*, *114*(4), 1438–1443. https://doi.org/10.1016/j.foodchem.2008.11.028

Follo, C., Ozzano, M., Montalenti, C., Santoro, M. M., & Isidoro, C. (2013). Knockdown of cathepsin D in zebrafish fertilized eggs determines congenital myopathy. *Bioscience Reports*, *33*(2), 371–378. https://doi.org/10.1042/BSR20120100

Galhardo, D., Garcia, R. C., Schneider, C. R., Braga, G. C., Chambó, E. D., de França, D. L. B., & Ströher, S. M. (2021). Physicochemical, bioactive properties and antioxidant of apis mellifera l. Honey from Western Paraná, Southern Brazil. *Food Science and Technology (Brazil)*, *41*(June), 247–253. https://doi.org/10.1590/fst.11720

Galhardo, D., Garcia, R. C., Schneider, C. R., Braga, G. C., Chambó, E. D., Luckmann, D., França, B. De, & Ströher, S. M. (2021). *Physicochemical, bioactive properties and antioxidant of Apis mellifera L . honey from western Paraná, Southern Brazil*. *2061*(June), 247–253.

Geeraert, L., Aerts, R., Berecha, G., Daba, G., De Fruyt, N., D’hollander, J., Helsen, K., Stynen, H., & Honnay, O. (2020). Effects of landscape composition on bee communities and coffee pollination in Coffea arabica production forests in southwestern Ethiopia. *Agriculture, Ecosystems and Environment*, *288*(September 2019). https://doi.org/10.1016/j.agee.2019.106706

Gheldof, N., & Engeseth, N. J. (2002). Antioxidant capacity of honey from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry*, *50*(10), 3050–3055. https://doi.org/10.1021/jf0114637

Gorjanović, S. Ž., Alvarez-Suarez, J. M., Novaković, M. M., Pastor, F. T., Pezo, L., Battino, M., & Sužnjević, D. Ž. (2013). Comparative analysis of the antioxidant activity of honey of different floral sources using recently developed polarographic and various spectrophotometric assays. *Journal of Food Composition and Analysis*, *30*(1), 13–18. https://doi.org/10.1016/j.jfca.2012.12.004

Grecka, K., Kús, P. M., Worobo, R. W., & Szweda, P. (2019). Study of the anti-staphylococcal potential of honey produced in Northern Poland. *Molecules*, *23*(2), 1–23. https://doi.org/10.3390/molecules23020260

Grigoryan, K. (2016). Safety of Honey. In *Regulating Safety of Traditional and Ethnic Foods*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-800605-4/00012-8

Gül, A., & Pehlivan, T. (2018). Antioxidant activities of some monofloral honey types produced across Turkey. *Saudi Journal of Biological Sciences*, *25*(6), 1056–1065. https://doi.org/10.1016/j.sjbs.2018.02.011

Güneş, M. E., Şahin, S., Demir, C., Borum, E., & Tosunoğlu, A. (2017). Determination of phenolic compounds profile in chestnut and floral honey and their antioxidant and antimicrobial activities. *Journal of Food Biochemistry*, *41*(3). https://doi.org/10.1111/jfbc.12345

Gupta, D. (2015). Methods for determination of antioxidant capacity: A review. *International Journal of Pharmaceutical Sciences and Research*, *6*(2), 546–566. https://doi.org/10.13040/IJPSR.0975-8232.6(2).546-66

Habib, H. M., Al Meqbali, F. T., Kamal, H., Souka, U. D., & Ibrahim, W. H. (2014). Bioactive components, antioxidants, and DNA damage the inhibitory activities of honey from arid regions. *Food Chemistry*, *153*, 28–34. https://doi.org/10.1016/j.foodchem.2013.12.044

Hailu, D., & Belay, A. (2020). Melissopalynology and antioxidant properties are used to differentiate Schefflera abyssinica and polyfloral honey. *PLoS ONE*, *15*(10 October), 1–22. https://doi.org/10.1371/journal.pone.0240868

Hau-Yama, N. E., Magaña-Ortiz, D., Oliva, A. I., & Ortiz-Vázquez, E. (2020). Antifungal activity of honey from stingless bee Melipona beecheii against Candida albicans. *Journal of Apicultural Research*, *59*(1), 12–18. https://doi.org/10.1080/00218839.2019.1665247

Hawine Debela. (2020). Physicochemical Properties and Biofunctional Activities of Two Monofloral Honeys From Southwest Ethiopia : the Case of Jimma Zone.Msc. Thesis. *Addis Abeba Science and Technology University*.

Hossain, M. L., Lim, L. Y., Hammer, K., Hettiarachchi, D., & Locher, C. (2022). A Review of Commonly Used Methodologies for Assessing the Antibacterial Activity of Honey and Honey Products. *Antibiotics*, *11*(7). https://doi.org/10.3390/antibiotics11070975

Hunter, M., Ghildyal, R., D'Cunha, N. M., Gouws, C., Georgousopoulou, E. N., & Naumovski, N. (2021). The bioactive, antioxidant, antibacterial, and physicochemical properties of a range of commercially available Australian honey. *Current Research in Food Science*, *4*, 532–542. https://doi.org/10.1016/j.crfs.2021.08.002

Ibrahimi, H., & Hajdari, A. (2020). Phenolic and flavonoid content, and antioxidant activity of honey from Kosovo. *Journal of Apicultural Research*, *59*(4), 452–457.

https://doi.org/10.1080/00218839.2020.1714194

Idris, Y. M. A., Mariod, A. A., & Hamad, S. I. (2011). Physicochemical properties, phenolic contents and antioxidant activity of Sudanese honey. *International Journal of Food Properties*, *14*(2), 450–458. https://doi.org/10.1080/10942910903243673

Igbang, O. J., Abang, O., & Eneji. (2018). Selected Antinutrients and Proximate Compositions of Honey (Apis mellifera) Sample Collected from Biase, Southern Senatorial District of Cross River State, Nigeria. *Biochemistry & Pharmacology: Open Access*, *07*(04). https://doi.org/10.4172/2167-0501.1000261

Ikeda, K., Tsujimoto, K., Uozaki, M., Nishide, M., Suzuki, Y., Koyama, A. H., & Yamasaki, H. (2011). Inhibition of multiplication of herpes simplex virus by caffeic acid. *International Journal of Molecular Medicine*, *28*(4), 595–598. https://doi.org/10.3892/ijmm.2011.739

Inhan-Garip, A., Aksu, B., Akan, Z., Akakin, D., Ozaydin, A. N., & San, T. (2011). Effect of extremely low-frequency electromagnetic fields on growth rate and morphology of bacteria. *International Journal of Radiation Biology*, *87*(12), 1155–1161. https://doi.org/10.3109/09553002.2011.560992

Irish, J., Carter, D. A., Shokohi, T., & Blair, S. E. (2006). Honey has an antifungal effect against Candida species. *Medical Mycology*, *44*(3), 289–291. https://doi.org/10.1080/13693780500417037

Irshad, M., Zafaryab, M., Singh, M., & Rizvi, M. M. A. (2012). Comparative Analysis of the Antioxidant Activity of Cassia Fistula Extracts. *International Journal of Medicinal Chemistry*, *2012*, 1–6. https://doi.org/10.1155/2012/157125

Istasse, T., Jacquet, N., Berchem, T., Haubruge, E., Nguyen, B. K., & Richel, A. (2016). Extraction of honey polyphenols: Method development and evidence of cis isomerization. *Analytical Chemistry Insights*, *2016*(11), 49–57. https://doi.org/10.4137/aci.s39739

Izzati, S. R. S., Nur Syahidah, R., Asmaliza, A. G., Huat, J. T. Y., Nadiawati, A., & Norhayati, N. (2021). Composition and functional properties of stingless bee honey : A review. *Malaysian Journal Of Applied Sciences*, *6*(1), 111–127.

Jemberie, W., Negash, W., Alemu, K., Tarekegn, A., Brhan, M., & Raja, N. (2020). Stingless bee Meliponula Cockerell (Hymenoptera: Apidae: Meliponini) ground nest architecture and traditional knowledge on the use of honey in the Amhara Region, Northwest Ethiopia. *Israel Journal of Entomology*, *50*(1), 147–162. https://doi.org/10.5281/zenodo.4588315

Jimenez, M., Beristain, C. I., Azuara, E., Mendoza, M. R., & Pascual, L. A. (2016). Physicochemical and antioxidant properties of honey from Scaptotrigona mexicana bee. *Journal of Apicultural Research*, *55*(2), 151–160. https://doi.org/10.1080/00218839.2016.1205294

Julika W. N., A. Ajit, Naila, A., N.F. Nabilah, N. A. Aliyah, & A. Z. Sulaiman. (2022). The antibacterial properties of stingless bee honey in Malaysia and its effects as a food preservative. *The Maldives National Journal of Research*, *10*(2), 63–72. https://doi.org/10.62338/gby1xe83

Kačániová, M., Borotová, P., Galovičová, L., Kunová, S., Štefániková, J., Kowalczewski, P. Ł., & Šedík, P. (2022). Antimicrobial and Antioxidant Activity of Different Honey

Samples from Beekeepers and Commercial Producers. *Antibiotics*, *11*(9). https://doi.org/10.3390/antibiotics11091163

Kebede Nigussie, Subramanian, P. A., & Mebrahtu, G. (2012). Physicochemical analysis of Tigray honey: An attempt to determine major quality markers of honey. *Bulletin of the Chemical Society of Ethiopia*, *26*(1), 127–133. https://doi.org/10.4314/bcse.v26i1.14

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, *48*(4), 412–422. https://doi.org/10.1007/s13197-011-0251-1

Kek, S. P., Chin, N. L., Yusof, Y. A., Tan, S. W., & Chua, L. S. (2018). Classification of the entomological origin of honey based on its physicochemical and antioxidant properties. *International Journal of Food Properties*, *20*(3), S2723–S2738. https://doi.org/10.1080/10942912.2017.1359185

Kempf, M., Wittig, M., Schönfeld, K., Cramer, L., Schreier, P., & Beuerle, T. (2011). Pyrrolizidine alkaloids in food: Downstream contamination in the food chain caused by honey and pollen. *Food Additives and Contaminants - Part A*, *28*(3), 325–331. https://doi.org/10.1080/19440049.2010.521771

Keng, C. B., Haron, H., Abdul Talib, R., & Subramaniam, P. (2017). Physical Properties, Antioxidant Content and Anti-Oxidative Activities of Malaysian Stingless Kelulut (Trigona spp.) Honey. *Journal of Agricultural Science*, *9*(13), 32. https://doi.org/10.5539/jas.v9n13p32

Khansaritoreh, E., Salmaki, Y., Akbari Azirani, T., Henareh, F., Alizadeh, K., Ramezani, E., Zarre, S., Beckh, G., & Behling, H. (2021). The sources and quality of Iranian honey. *Heliyon*, *7*(4), e06651. https://doi.org/10.1016/j.heliyon.2021.e06651

Kishore, R. K., Halim, A. S., Syazana, M. S. N., & Sirajudeen, K. N. S. (2011). Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. *Nutrition Research*, *31*(4), 322–325. https://doi.org/10.1016/j.nutres.2011.03.001

Kıvrak, Ş., & Kıvrak, İ. (2017). Assessment of phenolic profile of Turkish honey. *International Journal of Food Properties*, *20*(4), 864–876. https://doi.org/10.1080/10942912.2016.1188307

Koche, D., Shirsat, R., & Kawale, M. (2016). An Overview of Major Classes of Phytochemicals: Their Types and Role in Disease Prevention. *Hislopia Journal*, *9*(1/2), 1–11.

Krpan, M., Marković, K., Šarić, G., Skoko, B., HrušKar, M., & VahčIć, N. (2009). Antioxidant activities and total phenolics of acacia honey. *Czech Journal of Food Sciences*, *27*(SPEC. ISS.), 245–248. https://doi.org/10.17221/1112-cjfs

Küçük, M., Kolayli, S., Karaoğlu, Ş., Ulusoy, E., Baltaci, C., & Candan, F. (2007). Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry*, *100*(2), 526–534. https://doi.org/10.1016/j.foodchem.2005.10.010

Kumsa, T. and, & Takele, D. (2014). Assessment of the Effect of Seasonal Honeybee Management on Honey Production of Ethiopian Honeybee (Apis mellifera) in Modern Beekeeping in Jimma Zone. *Greener Journal of Plant Breeding and Crop*

Science, *2*(3), 067–075. https://doi.org/10.15580/gjpbcs.2014.3.010314013
Lachman, J., Orsák, M., Hejtmánková, A., & Kovářová, E. (2010). Evaluation of antioxidant activity and total phenolics of selected Czech honey. *Lwt*, *43*(1), 52–58. https://doi.org/10.1016/j.lwt.2009.06.008
Le, V., Vera, S., & Gonz, A. V. (2011). *Vitamin C and Sugar Levels as Simple Markers for Discriminating Spanish Honey Sources*. *76*(3). https://doi.org/10.1111/j.1750-3841.2011.02041.x
Lewoyehu, M., & Amare, M. (2019). Comparative Assessment on Selected Physicochemical Parameters and Antioxidant and Antimicrobial Activities of Honey Samples from Selected Districts of the Amhara and Tigray Regions, Ethiopia. *International Journal of Food Science*, *2019*. https://doi.org/10.1155/2019/4101695
Lusby, P. E., Coombes, A. L., & Wilkinson, J. M. (2005). Bactericidal activity of different honeys against pathogenic bacteria. *Archives of Medical Research*, *36*(5), 464–467. https://doi.org/10.1016/j.arcmed.2005.03.038
Mahani M., E. Wulandari¸ E. Lembong, L. H. R. A. (2022). Correlation of Alkaloid Content and Taste of Honey From Various Provinces in Indonesia. *International Journal of Pharmacy and Pharmaceutical Sciences*, *14*(12), 16–21. https://doi.org/10.22159/ijpps.2022v14i12.46169
Machado De-Melo, A. A., Almeida-Muradian, L. B. de, Sancho, M. T., & Pascual-Maté, A. (2018). Composición y propiedades de la miel de Apis mellifera: una revisión. *Journal of Apicultural Research*, *57*(1), 5–37. https://doi.org/10.1080/00218839.2017.1338444
Mahmood, A. L., Lani, M. N., Hassan, Z., Razak, S. B. A., & Ahmad, F. T. (2021). Antioxidant and antimicrobial properties of Indo-Malayan stingless bee (Heterotrigona itama) honey from different seasons and distribution of flowers. *Food Research*, *5*(2), 498–507. https://doi.org/10.26656/fr.2017.5(2).546
Małgorzata Brodowska, K. (2017). European Journal of Biological Research Natural flavonoids: classification, potential role, and application of flavonoid analogs. *European Journal of Biological Research*, *7*(2), 108–123. http://dx.doi.org/10.5281/zenodo.545778
Mark, M. D., Kirupha, S. D., Elango, S., & Vadodaria, K. (2021). Fabrication of nanofibrous membrane using stingless bee honey and curcumin for wound healing applications. *Journal of Drug Delivery Science and Technology*, *63*, 102271. https://doi.org/10.1016/j.jddst.2020.102271
Massoura, M. (2019). *An investigation into the antibacterial mechanism of honey by Maria Massoura A thesis submitted to the University of Birmingham for the degree of Doctor of Philosophy*. *September*.
Matsuura, H. N., & Fett-Neto, A. G. (2015). Plant Alkaloids: Main Features, Toxicity, and Mechanisms of Action. *Plant Toxins*, *July*, 1–15. https://doi.org/10.1007/978-94-007-6728-7_2-1
Mavric, E., Wittmann, S., Barth, G., & Henle, T. (2008). Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (Leptospermum scoparium) honey from New Zealand. *Molecular Nutrition and Food Research*, *52*(4), 483–489. https://doi.org/10.1002/mnfr.200700282

Meo, S. A., Al-Asiri, S. A., Mahesar, A. L., & Ansari, M. J. (2017). Role of honey in modern medicine. *Saudi Journal of Biological Sciences*, *24*(5), 975–978. https://doi.org/10.1016/j.sjbs.2016.12.010

Minden-Birkenmaier, B. A., & Bowlin, G. L. (2018). Honey-based templates in wound healing and tissue engineering. *Bioengineering*, *5*(2). https://doi.org/10.3390/bioengineering5020046

Miranda, I. R., Ancona, D. B., & Ordóñez, Y. M. (2023). Influence of phenolic compounds and flavonoids on the color and antioxidant activity of Melipona beecheii honey from the deciduous forest of Yucatan, Mexico. *Emirates Journal of Food and Agriculture*, *35*(12), 1–10. https://doi.org/10.9755/ejfa.2023.3198

Mirończuk-Chodakowska, I., Witkowska, A. M., & Zujko, M. E. (2018). Endogenous non-enzymatic antioxidants in the human body. *Advances in Medical Sciences*, *63*(1), 68–78. https://doi.org/10.1016/j.advms.2017.05.005

Molan, P. C. (2006). Using honey in wound care. *International Journal of Clinical Aromatherapy*, *3*(2), 21–24. http://hdl.handle.net/10289/2030

Moniruzzaman, M., Benhanifia, M., Sulaiman, S. A., Gan, S. H., & Centre, H. G. (2012). *Physicochemical and Antioxidant Properties of Algerian Honey*. *Ii*, 11199–11215. https://doi.org/10.3390/molecules170911199

Moniruzzaman, M., Sulaiman, S. A., Khalil, M. I., & Gan, S. H. (2013). Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honey: A comparison with manuka honey. *Chemistry Central Journal*, *7*(1), 1–12. https://doi.org/10.1186/1752-153X-7-138

Moussa, A., Noureddine, D., Saad, A., Abdelmelek, M., & Abdelkader, B. (2012). Antifungal activity of four honeys of different types from Algeria against pathogenic yeast: Candida albicans and Rhodotorula sp. *Asian Pacific Journal of Tropical Biomedicine*, *2*(7), 554–557. https://doi.org/10.1016/S2221-1691(12)60096-3

Muhammad, A., Odunola, O. A., Gbadegesin, M. A., Adegoke, A. M., Olugbami, J. O., & Uche, N. S. (2015). Modulatory role of Acacia honey from north-west Nigeria on sodium arsenite-induced clastogenicity and oxidative stress in male Wistar rats. *Natural Product Research*, *29*(4), 321–326. https://doi.org/10.1080/14786419.2014.940945

Naama, R. T. A.-. (2009). Evaluation of the in-vitro inhibitory effect of honey on some microbial isolate. *African Journal of Bacteriology Research*, *1*(6), 064–067. http://www.academicjournals.org/journal/JBR/article-abstract/EDC25D78969

Nantongo, J. S., Odoi, J. B., Abigaba, G., & Gwali, S. (2018). Variability of phenolic and alkaloid content in different plant parts of Carissa edulis Vahl and Zanthoxylum chalybeum Engl. *BMC Research Notes*, *11*(1), 1–5. https://doi.org/10.1186/s13104-018-3238-4

Ngaini, Z., Hussain, H., Kelabo, E. S., Wahi, R., & Farooq, S. (2023). Chemical profiling, biological properties and environmental contaminants of stingless bee honey and propolis. *Journal of Apicultural Research*, *62*(1), 131–147. https://doi.org/10.1080/00218839.2021.1948745

Nishio, E. K., Ribeiro, J. M., Oliveira, A. G., Andrade, C. G. T. J., Proni, E. A., Kobayashi, R. K. T., & Nakazato, G. (2016). Antibacterial synergic effect of honey from two stingless bees: Scaptotrigona bipunctata Lepeletier, 1836, and S. postica Latreille, 1807. *Scientific Reports*, *6*(February), 1–8. https://doi.org/10.1038/srep21641

Nolan, V. C., Harrison, J., & Cox, J. A. G. (2019). Dissecting the antimicrobial composition of honey. *Antibiotics,* *8*(4), 1–16. https://doi.org/10.3390/antibiotics8040251

Nordin, A., Sainik, N. Q. A. V., Chowdhury, S. R., Saim, A. Bin, & Idrus, R. B. H. (2018). Physicochemical properties of stingless bee honey from around the globe: A comprehensive review. *Journal of Food Composition and Analysis*, *73*, 91–102. https://doi.org/10.1016/j.jfca.2018.06.002

Nur Eszaty, F. E., Mohamed, N. M. A., Saiful, I. A. R., Norjihada, I. I., Norhana, J., Nurliyana, A. Z., Mohamad Ikhwan, J., Sagadevan, S., & Nadirul, H. M. N. (2022). A Review on Recent Progress of Stingless Bee Honey and Its Hydrogel-Based Compound for Wound Care Management. *Molecules*, *27*, 1–20.

Nuratiqah, S., Yaacob, S., Wahab, R. A., Huyop, F., Lani, M. N., & Zin, N. M. (2020). Morphological alterations in gram-positive and gram-negative bacteria exposed to minimal inhibitory and bactericidal concentration of raw Malaysian stingless bee honey. *Biotechnology & Biotechnological Equipment*, *34*(1), 575–586. https://doi.org/10.1080/13102818.2020.1788421

Nwankwo, C. M., Ezekoye, C. C., & Igbokwe, S. O. (2014). Phytochemical screening and antimicrobial activity of apiary honey produced by a honey bee (Apis mellifera) on clinical strains of Staphylococcus aureus, Escherichia coli, and Candida albicans. *African Journal of Biotechnology*, *13*(23), 2367–2372. https://doi.org/10.5897/ajb2013.13570

Nweze, J. A., Okafor, J. I., Nweze, E. I., & Nweze, J. E. (2017). Evaluation of physicochemical and antioxidant properties of two stingless bee honey: A comparison with Apis mellifera honey from Nsukka, Nigeria. *BMC Research Notes*, *10*(1), 4–9. https://doi.org/10.1186/s13104-017-2884-2

Ofijan T, Muleta, D., & Desalegn, A. (2022). In vitro, antimicrobial properties of apis mellifera L. and Meliponulla beccarii L. honeys from Kellem and West Wollega Zones, Western Ethiopia. *International Journal of Food Properties*, *25*(1), 65–75. https://doi.org/10.1080/10942912.2021.2019761

Ofijan Tesfaye. (2023). Evaluating the Antioxidant Properties of Unifloral Honey (Apis mellifera L.) from Ethiopia. *International Journal of Food Science*, *2023*. https://doi.org/10.1155/2023/7664957

Omar, S., Mat-Kamir, N. F., & Sanny, M. (2019). Antibacterial activity of Malaysian produced stingless-bee honey on wound pathogens. *Journal of Sustainability Science and Management*, *14*(3), 67–79.

Otmani, A., Amessis-ouchemoukh, N., Birinci, C., Yahiaoui, S., Kolayli, S., Rodríguez-Flores, M. S., Escuredo, O., Carmen, M., & Ouchemoukh, S. (2021). Food Bioscience Phenolic compounds and antioxidant and antibacterial activities of Algerian honeys. *Food Bioscience*, *42*(June), 101070. https://doi.org/10.1016/j.fbio.2021.101070

Özcan, M. M., & Al Juhaimi, F. (2015). La miel como fuente de antioxidantes naturals.

Journal of Apicultural Research, *54*(3), 145–154. https://doi.org/10.1080/00218839.2016.1144976

Pasarin, D., & Rovinaru, C. (2018). Sources of Carotenoids and Their Uses As Animal Feed Additives - a Review. *Scientific Papers-Series D-Animal Science*, *61*(2), 74–85.

Patricia, V., Oliverio, V., Triny, L., & Favián, M. (2015). Meliponini biodiversity and medicinal uses of pot-honey from El Oro province in Ecuador. *Emirates Journal of Food and Agriculture*, *27*(6), 502–506. https://doi.org/10.9755/ejfa.2015.04.079

Pauliuc, D., & Dranca, F. (2020). *Individual Phenolics and Physicochemical Parameters Suitability for Romanian Honey Authentication*.

Pillet, F., Formosa-Dague, C., Baaziz, H., Dague, E., & Rols, M. P. (2016). Cell wall as a target for bacteria inactivation by pulsed electric fields. *Scientific Reports*, *6*(September 2015), 1–8. https://doi.org/10.1038/srep19778

Ponnuchamy, R., Bonhomme, V., Prasad, S., Das, L., Patel, P., Gaucherel, C., Pragasam, A., & Anupama, K. (2014). Honey pollen: Using melissopalynology to understand foraging preferences of bees in tropical south India. *PLoS ONE*, *9*(7). https://doi.org/10.1371/journal.pone.0101618

Pontis, J. A., da Costa, L. A. M. A., da Silva, S. J. R., & Flach, A. (2014). Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. *Food Science and Technology*, *34*(1), 69–73. https://doi.org/10.1590/S0101-20612014005000015

Poquet, L., Clifford, M. N., & Williamson, G. (2009). Bioavailability of Flavanols and Phenolic Acids. *Plant Phenolics and Human Health: Biochemistry, Nutrition, and Pharmacology*, 51–89. https://doi.org/10.1002/9780470531792.ch2

Prithviraj Karak. (2019). Biological Activities of Flavonoids: an Overview. *International Journal of Pharmaceutical Sciences and Research*, *10*(4), 1567–1574. https://doi.org/10.13040/IJPSR.0975-8232.10(4).1567-74

Purbafrani, A., Ghazizade Hashemi, S. A., Bayyenat, S., Moghaddam, H. T., & Saeidi, M. (2014). The benefits of honey in the Holy Quran. *International Journal of Pediatrics*, *2*(3), 67–73. https://doi.org/10.22038/ijp.2014.3417

Rababah, T. M., Al-Omoush, M., Brewer, S., Alhamad, M., Yang, W., Alrababah, M., Al-Ghzawi, A. A. M., Al-U'datt, M., Ereifej, K., Alsheyab, F., Esoh, R., & Almajwal, A. (2014). Total phenol, antioxidant activity, flavonoids, anthocyanins, and color of honey as affected by floral origin found in the arid and semiarid Mediterranean areas. *Journal of Food Processing and Preservation*, *38*(3), 1119–1128. https://doi.org/10.1111/jfpp.12071

Ranneh, Y., Akim, A. M., Hamid, H. A., Khazaai, H., Fadel, A., Zakaria, Z. A., Albujja, M., & Bakar, M. F. A. (2021). Honey and its nutritional and anti-inflammatory value. *BMC Complementary Medicine and Therapies*, *21*(1), 1–17. https://doi.org/10.1186/s12906-020-03170-5

Ranneh, Y., Ali, F., Zarei, M., Akim, A. M., Hamid, H. A., & Khazaai, H. (2018). Malaysian stingless bee and Tualang honey: A comparative characterization of total antioxidant capacity and phenolic profile using liquid chromatography-mass spectrometry. *Lwt*, *89*, 1–9. https://doi.org/10.1016/j.lwt.2017.10.020

Ratner, B. (2009). The correlation coefficient: Its values range between 1/1, or do they.

Journal of Targeting, Measurement and Analysis for Marketing, *17*(2), 139–142. https://doi.org/10.1057/jt.2009.5

Robbins, R. J. (2003). Phenolic acids in foods: An overview of analytical methodology. *Journal of Agricultural and Food Chemistry*, *51*(10), 2866–2887. https://doi.org/10.1021/jf026182t

Rom, D., Alencar, S. M., Bueno-silva, B., De, C., Franchin, M., Durrer, R., Carvalho, P. De, Sousa, E. De, & Ferreira, A. (2022). *Antimicrobial Activity of Honey against Oral Microorganisms : Current Reality, Methodological Challenges and Solutions*. 1–17.

Rosli, F. N., Hazemi, M. H. F., Akbar, M. A., Basir, S., Kassim, H., & Bunawan, H. (2020). Stingless bee honey: Evaluating its antibacterial activity and bacterial diversity. *Insects*, *11*(8), 1–13. https://doi.org/10.3390/insects11080500

Roubik, Patricia Vit, Silvia R.M. Pedro, D. W. (2013). *A legacy of stingless bees*.

Salehnezhad, S., & Chermahini, S. H. (2019). *Characterization of Apitherapy Honeys for Medical Applications Characterization of Apitherapy Honeys for Medical Applications*. *October*. https://doi.org/10.26717/BJSTR.2019.22.003751

Salonen, A., Virjamo, V., Tammela, P., Fauch, L., & Julkunen-Tiitto, R. (2017). Screening bioactivity and bioactive constituents of Nordic unifloral honey. *Food Chemistry*, *237*, 214–224. https://doi.org/10.1016/j.foodchem.2017.05.085

Samarghandian, S., Farkhondeh, T., & Samini, F. (2017). Honey and health: A review of recent clinical research. *Pharmacognosy Research*, *9*(2), 121–127. https://doi.org/10.4103/0974-8490.204647

Saranraj, P., Sivasakthi, S., & Feliciano, G. D. (2016). Pharmacology of Honey: A Review. *Advances in Biological Research*, *10*(4), 271–289. https://doi.org/10.5829/idosi.abr.2016.10.4.104104

Satriadi, T., Aryadi, M., Susilawati, Fitriani, A., & Badaruddin. (2023). Proximate Analysis and Antioxidant Activity of Kelulut (Heterotrigona itama) Honey. *Tropical Journal of Natural Product Research*, *7*(July), 3388–3391.

Seca, A. M. L., & Moujir, L. (2020). Natural compounds: A dynamic field of applications. *Applied Sciences (Switzerland)*, *10*(11). https://doi.org/10.3390/app10114025

Shahidi, F., & Zhong, Y. (2010). Novel antioxidants in food quality preservation and health promotion. *European Journal of Lipid Science and Technology*, *112*(9), 930–940. https://doi.org/10.1002/ejlt.201000044

Shamsudin, S., Selamat, J., Abdul Shomad, M., Ab Aziz, M. F., & Haque Akanda, M. J. (2022). Antioxidant Properties and Characterization of Heterotrigona itama Honey from Various Botanical Origins according to Their Polyphenol Compounds. *Journal of Food Quality*, *2022*. https://doi.org/10.1155/2022/2893401

Shamsudin, S., Selamat, J., Sanny, M., Abd. Razak, S. B., Jambari, N. N., Mian, Z., & Khatib, A. (2019). Influence of origins and bee species on physicochemical, antioxidant properties and botanical discrimination of stingless bee honey. *International Journal of Food Properties*, *22*(1), 238–263. https://doi.org/10.1080/10942912.2019.1576730

Shamsudin, S., Selamat, J., Sanny, M., Shamsul Bahari, A. R., Jambari, N. N., & Khatib, A. (2019). Comparative characterization of physicochemical and antioxidant properties of processed Heterotrigona items honey from different origins and classification by chemometrics analysis. *Molecules*, *24*(21), 1–20. https://doi.org/10.3390/molecules24213898

Shugaba, A. (2012). Analysis of Biochemical Composition of Honey Samples from North-East Nigeria. *Biochemistry & Analytical Biochemistry*, *2*(3). https://doi.org/10.4172/2161-1009.1000139

Souza, E. C. A., Menezes, C., & Flach, A. (2021). Stingless bee honey (Hymenoptera, Apidae, Meliponini): a review of quality control, chemical profile, and biological potential. *Apidologie*, *52*(1), 113–132. https://doi.org/10.1007/s13592-020-00802-0

Stagos, D., Soulitsiotis, N., Tsadila, C., Papaeconomou, S., Arvanitis, C., Ntontos, A., Karkanta, F., Adamou-Androulaki, S., Petrotos, K., Spandidos, D. A., Kouretas, D., & Mossialos, D. (2018). Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. *International Journal of Molecular Medicine*, *42*(2), 726–734. https://doi.org/10.3892/ijmm.2018.3656

Stefan Bogdanov, Maˇrghitasß Liviu Al, Dezmirean Daniel, Adela Moise , Otilia Bobis, L. L. (2009). Physico-chemical and bioactive properties of different floral origin honey from Romania. *Food Chemistry*, *112*(4), 863–867. https://doi.org/10.1016/j.foodchem.2008.06.055

Suhartatik, N., Karyantina, M., & Lestari, E. (2023). The minimum inhibitory concentrations (MICs) of four types of honey against several bacteria. *IOP Conference Series: Earth and Environmental Science*, *1177*(1). https://doi.org/10.1088/1755-1315/1177/1/012031

Suleiman, M. H. A., ALaerjani, W. M. A., & Mohammed, M. E. A. (2020). Influence of altitudinal variation on the total phenolic and flavonoid content of Acacia and Ziziphus honey. *International Journal of Food Properties*, *23*(1), 2077–2086. https://doi.org/10.1080/10942912.2020.1842445

Taormina, P. J., Niemira, B. A., & Beuchat, L. R. (2001). Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. *International Journal of Food Microbiology*, *69*(3), 217–225. https://doi.org/10.1016/S0168-1605(01)00505-0

Thawabteh, A. M., Thawabteh, A., Lelario, F., Bufo, S. A., & Scrano, L. (2021). Classification, toxicity, and bioactivity of natural diterpenoid alkaloids. *Molecules*, *26*(13). https://doi.org/10.3390/molecules26134103

Tomczyk, M., Tarapatskyy, M., & Dżugan, M. (2019). The influence of geographical origin on honey composition was studied by Polish and Slovak honeys. *Czech Journal of Food Sciences*, *37*(4), 232–238. https://doi.org/10.17221/40/2019-CJFS

Tsagkaris, A. S., Koulis, G. A., Danezis, G. P., Martakos, I., Dasenaki, M., Georgiou, C. A., & Thomaidis, N. S. (2021). Honey authenticity: analytical techniques, state of the art and challenges. *RSC Advances*, *11*(19), 11273–11294. https://doi.org/10.1039/d1ra00069a

Tuksitha, L., Chen, Y. L. S., Chen, Y. L., Wong, K. Y., & Peng, C. C. (2018). Antioxidant and antibacterial capacity of stingless bee honey from Borneo (Sarawak). *Journal of Asia-Pacific Entomology*, *21*(2), 563–570. https://doi.org/10.1016/j.aspen.2018.03.007

Turkmen, N., Sari, F., Poyrazoglu, E. S., & Velioglu, Y. S. (2006). Effects of prolonged heating on antioxidant activity and color of honey. *Food Chemistry*, *95*(4), 653–657. https://doi.org/10.1016/j.foodchem.2005.02.004

Valdés-Silverio, L. A., Iturralde, G., García-Tenesaca, M., Paredes-Moreta, J., Narváez-Narváez, D. A., Rojas-Carrillo, M., Tejera, E., Beltrán-Ayala, P., Giampieri, F., & Alvarez-Suarez, J. M. (2018). Physicochemical parameters, chemical composition, antioxidant capacity, microbial contamination and antimicrobial activity of Eucalyptus honey from the Andean region of Ecuador. *Journal of Apicultural Research*, *57*(3), 382–394. https://doi.org/10.1080/00218839.2018.1426349

Vit, P., Medina, M., & Enríquez, M. E. (2004). Quality standards for medicinal uses of Meliponinae honey in Guatemala, Mexico, and Venezuela. *Bee World*, *85*(1), 2–5. https://doi.org/10.1080/0005772X.2004.11099603

Wan Yusuf, W. N., Wan Mohammad, W. M. Z., Gan, S. H., Mustafa, M., Abd Aziz, C. B., & Sulaiman, S. A. (2019). Tualang honey ameliorates viral load, and CD4 counts and improves quality of life in asymptomatic human immunodeficiency virus-infected patients. *Journal of Traditional and Complementary Medicine*, *9*(4), 249–256. https://doi.org/10.1016/j.jtcme.2018.05.003

Wang, T., Frandsen, H. L., Christiansson, N. R., Rosendal, S. E., Pedersen, M., & Smedsgaard, J. (2019). Pyrrolizidine alkaloids in honey: Quantification with and without standards. *Food Control*, *98*, 227–237. https://doi.org/10.1016/j.foodcont.2018.11.033

Wasihun, A. G., & Kasa, B. G. (2016). Evaluation of the antibacterial activity of honey against multidrug-resistant bacteria in Ayder Referral and Teaching Hospital, Northern Ethiopia. *SpringerPlus*, *5*(1). https://doi.org/10.1186/s40064-016-2493-x

Williams, R. J., Spencer, J. P. E., & Rice-Evans, C. (2004). Flavonoids: Antioxidants or signaling molecules? *Free Radical Biology and Medicine*, *36*(7), 838–849. https://doi.org/10.1016/j.freeradbiomed.2004.01.001

Wink, M. (2013). Evolution of secondary metabolites in legumes (Fabaceae). *South African Journal of Botany*, *89*, 164–175. https://doi.org/10.1016/j.sajb.2013.06.006

Yaacob, M., Rajab, N. F., Shahar, S., & Sharif, R. (2018). Stingless bee honey and its potential value: A systematic review. *Food Research*, *2*(2), 124–133. https://doi.org/10.26656/fr.2017.2(2).212

Yalemwork Ewnetu, Lemma, W., & Birhane, N. (2013). Antibacterial effects of Apis mellifera and stingless bees honey on susceptible and resistant strains of Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae in Gondar, Northwest. *BMC Complementary and Alternative Medicine*, *13*(1), 1. https://doi.org/10.1186/1472-6882-13-269

Yap, P., & Abu Bakar, M. F. (2014). Physicochemical, phytochemical, and antimicrobial properties of wild honey collected at mangrove and mountain areas in Sabah, Malaysian Borneo. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(7), 287–292.

Yayinie, M., Atlabachew, M., Tesfaye, A., Hilluf, W., Reta, C., & Alemneh, T. (2022). Polyphenols, flavonoids, and antioxidant content of honey coupled with chemometric method : geographical origin classification from Amhara region, Ethiopia.

International Journal of Food Properties, *25*(1), 76–92. https://doi.org/10.1080/10942912.2021.2021940

Zainol, M. I., Mohd Yusoff, K., & Mohd Yusof, M. Y. (2013). Antibacterial activity of selected Malaysian honey. *BMC Complementary and Alternative Medicine*, *13*. https://doi.org/10.1186/1472-6882-13-129

Zapata-Vahos, I. C., Henao-Rojas, J. C., Yepes-Betancur, D. P., Marín-Henao, D., Giraldo Sánchez, C. E., Calvo-Cardona, S. J., David, D., & Quijano-Abril, M. (2023). Physicochemical Parameters, Antioxidant Capacity, and Antimicrobial Activity of Honeys from Tropical Forests of Colombia: Apis mellifera and Melipona eburnea. *Foods*, *12*(5). https://doi.org/10.3390/foods12051001

Zulkhairi Amin, F. A., Sabri, S., Mohammad, S. M., Ismail, M., Chan, K. W., Ismail, N., Norhaizan, M. E., & Zawawi, N. (2018). Therapeutic properties of stingless bee honey in comparison with European bee honey. *Advances in Pharmacological Sciences*, *2018*. https://doi.org/10.1155/2018/6179596

APPENDICES

1. ANOVA tables of bioactive constituents of honey samples (**1A-E**)

A) Analysis of Variance for total phenolic content

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Bee Spec	1	9663.9	9663.87	8715.33	0.000
VChain of honey	3	4218.9	1406.30	1268.27	0.000
Bee Spec * VChain	3	3141.2	1047.05	944.28	0.000
Error	16	17.7	1.11		
Total	23	17041.7			

B) Analysis of Variance for total flavonoid content

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Bee Spec	1	11973.9	11973.9	75100.03	0.000
VChain of honey	3	13183.8	4394.6	27562.97	0.000
Bee Spec * VChain	3	15193.3	5064.4	31764.21	0.000
Error	16	2.6	0.2		
Total	23	40353.6			

C) Analysis of Variance for alkaloid content

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Bee Spec	1	11.207	11.2067	25.47	0.000
VChain of honey	3	35.807	11.9356	27.13	0.000
Bee Spec * VChain	3	105.460	35.1533	79.89	0.000
Error	16	7.040	0.4400		
Total	23	159.513			

D) Analysis of Variance for vitamin C content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Bee Spec	1	4504587	4504587	2697.69	0.000
VChain of honey	3	10447931	3482644	2085.67	0.000
Bee Spec * VChain	3	36937935	12312645	7373.75	0.000
Error	16	26717	1670		
Total	23	51917169			

E) Analysis of Variance for beta carotene

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Bee Spec	1	36.5136	36.5136	6347.78	0.000
VChain of honey	3	21.2852	7.0951	1233.45	0.000
Bee Spec * VChain	3	14.3113	4.7704	829.33	0.000
Error	16	0.0920	0.0058		
Total	23	72.2021			

2. ANOVA tables of the total antioxidant capacity of the honey sample as measured by assay (**2A-C**)

A) Analysis of Variance for DPPH scavenging activity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Bee Spec	1	496.172	496.172	1121.48	0.000
VChain of honey	3	337.081	112.360	253.96	0.000
Bee Spec * VChain	3	49.795	16.598	37.52	0.000
Error	16	7.079	0.442		
Total	23	890.127			

B) Analysis of Variance for IC_{50} Value

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Bee Spec	1	1888.5	1888.51	158.72	0.000
VChain of honey	3	3144.8	1048.25	88.10	0.000
Bee Spec * VChain	3	761.3	253.75	21.33	0.000
Error	16	190.4	11.90		
Total	23	5984.9			

C) Analysis of Variance for FRAP

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Bee Spec	1	0.000839	0.000839	1.93	0.183
VChain of honey	3	0.005894	0.001965	4.53	0.018
Bee Spec * VChain	3	0.058959	0.019653	45.28	0.000
Error	16	0.006944	0.000434		
Total	23	0.072637			

3. ANOVA tables of In vitro antimicrobial activities of honey (Agar well diffusion assay) against *S. Aureus* and *E. coli* bacteria**(3A-F)**

   A) Analysis of Variance for Zones of Inhibition against *S. Aureus* with 100% concentration of honey

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Repl	2	97.583333	48.791667	33.46	<.0001
Bee Spec	1	1320.166667	1320.166667	905.26	<.0001
VChain	3	408.166667	136.055556	93.30	<.0001
Bee Spec ***** VChain	3	341.500000	113.833333	78.06	<.0001

B) Analysis of Variance for zones of inhibition against *S. Aureus* with 75% concentration of honey

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Repl	2	73.000000	36.500000	22.88	<.0001
Bee Spec	1	1162.041667	1162.041667	728.44	<.0001
VChain	3	318.791667	106.263889	66.61	<.0001
Bee Spec * VChain	3	300.458333	100.152778	62.78	<.0001

C) Analysis of Variance for zones of inhibition against *S. Aureus* with 50%

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Repl	2	40.7500000	20.3750000	6.70	0.0091
Bee Spec	1	782.0416667	782.0416667	257.11	<.0001
VChain	3	257.7916667	85.9305556	28.25	<.0001
Bee Spec * VChain	3	239.4583333	79.8194444	26.24	<.0001

concentration of honey

D) Analysis of Variance for zones of inhibition against *E. coli* with 100% concentration of honey

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Repl	2	110.3333333	55.1666667	42.13	<.0001
Bee Spec	1	651.0416667	651.0416667	497.16	<.0001
VChain	3	298.1250000	99.3750000	75.89	<.0001
Bee Spec * VChain	3	806.1250000	268.7083333	205.20	<.0001

E) Analysis of Variance for zones of inhibition against *E. coli* with 75% concentration of honey

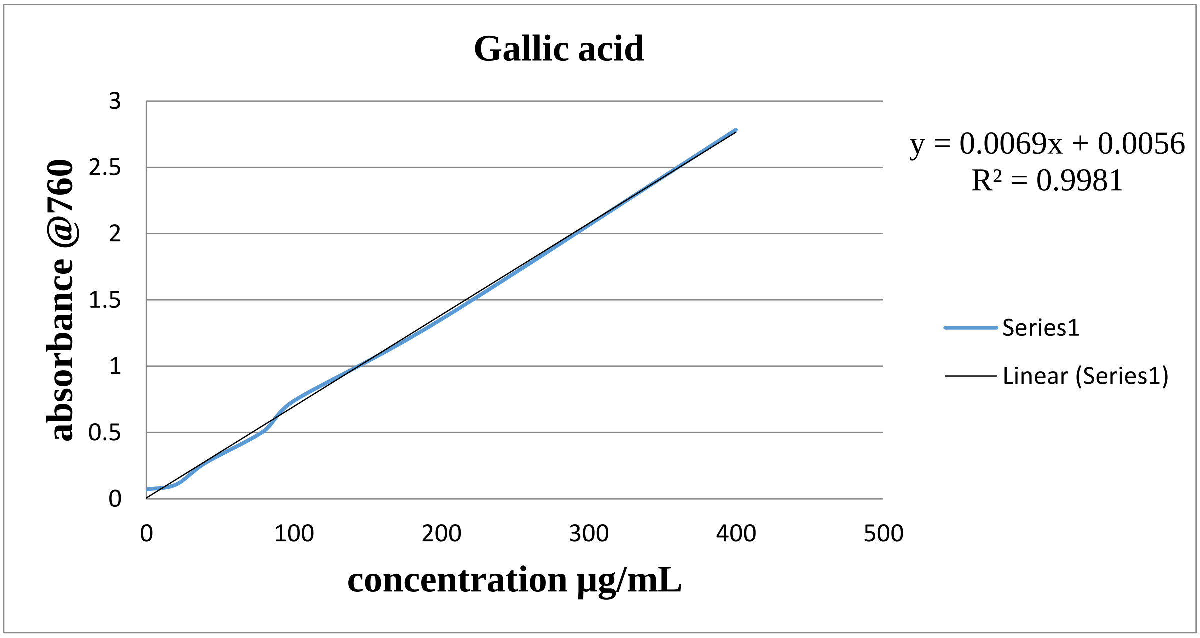
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Repl	2	98.5833333	49.2916667	22.44	<.0001
Bee Spec	1	570.3750000	570.3750000	259.68	<.0001
VChain	3	268.7916667	89.5972222	40.79	<.0001
Bee Spec ***** VChain	3	706.4583333	235.4861111	107.21	<.0001

F) Analysis of Variance for zones of inhibition against *E. coli* with 50% concentration of honey

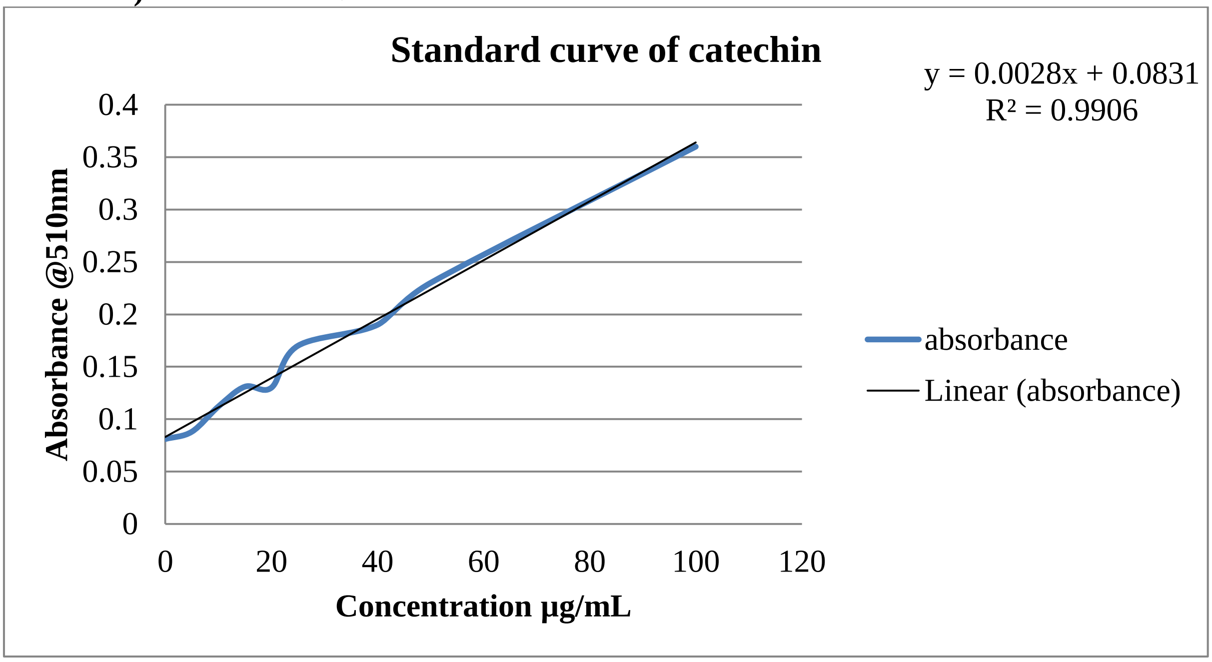
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Repl	2	75.2500000	37.6250000	8.31	0.0042
Bee Spec	1	450.6666667	450.6666667	99.49	<.0001
VChain	3	172.5000000	57.5000000	12.69	0.0003
Bee Spec ***** VChain	3	558.6666667	186.2222222	41.11	<.0001

1. Figures of standards of bioactive constituents, correlation of bioactive constituents, and antioxidant activities of honey samples (**1A-C**)

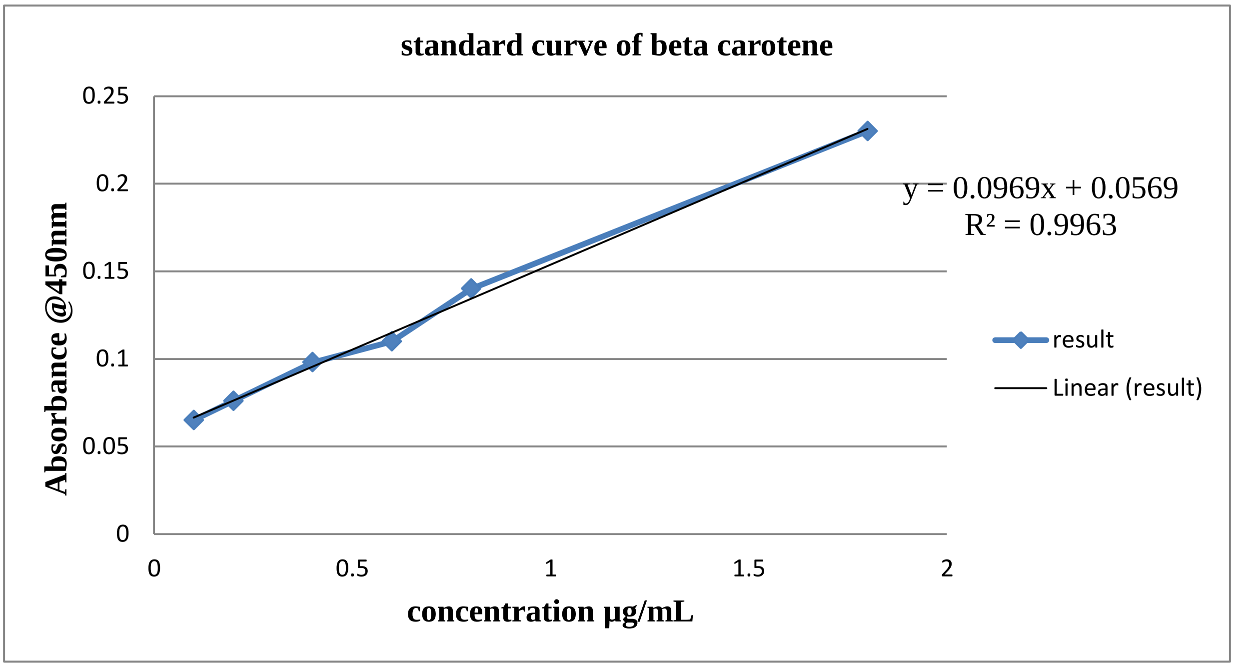
A) A standard curve of Gallic acid



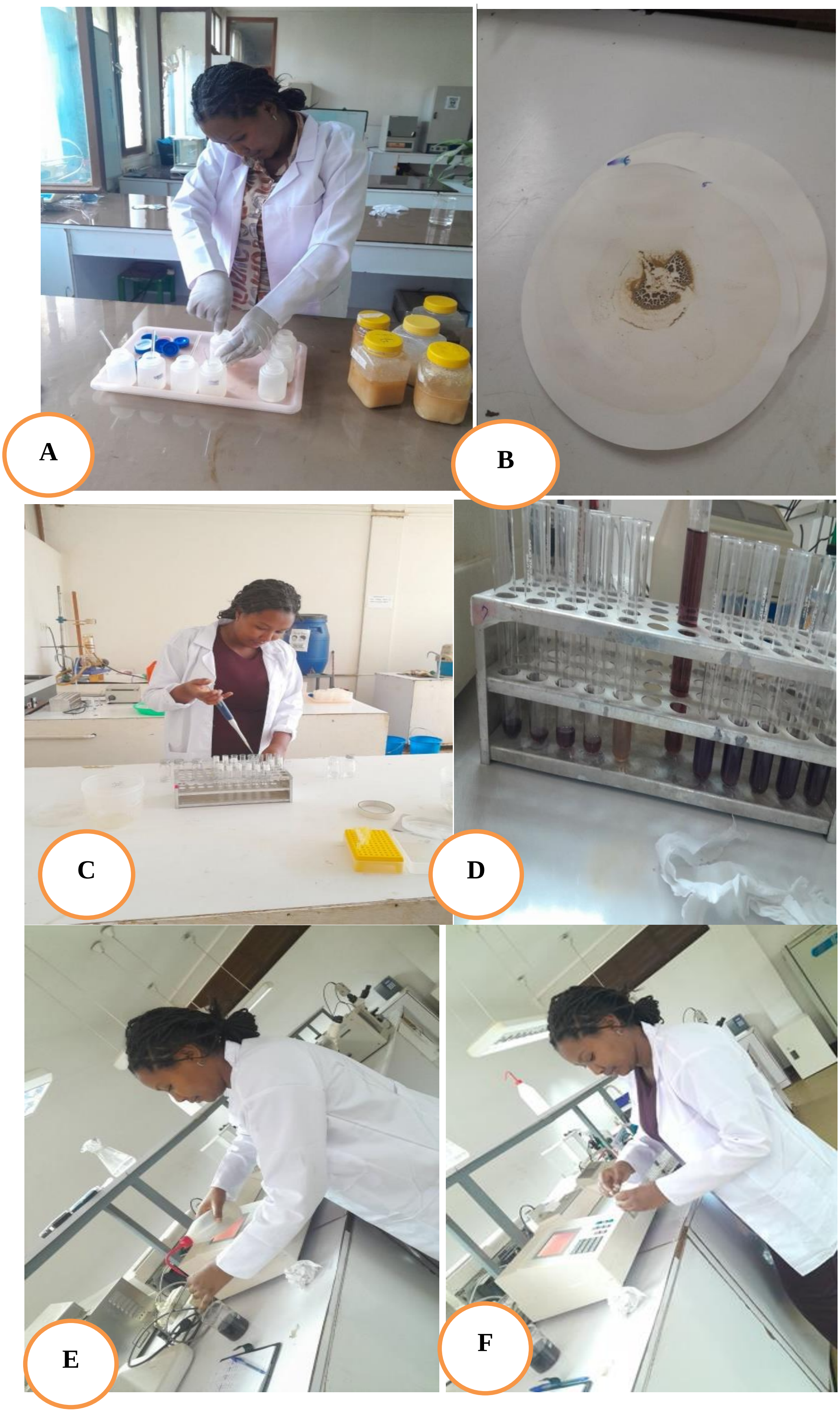
B) A standard curve of Catechin



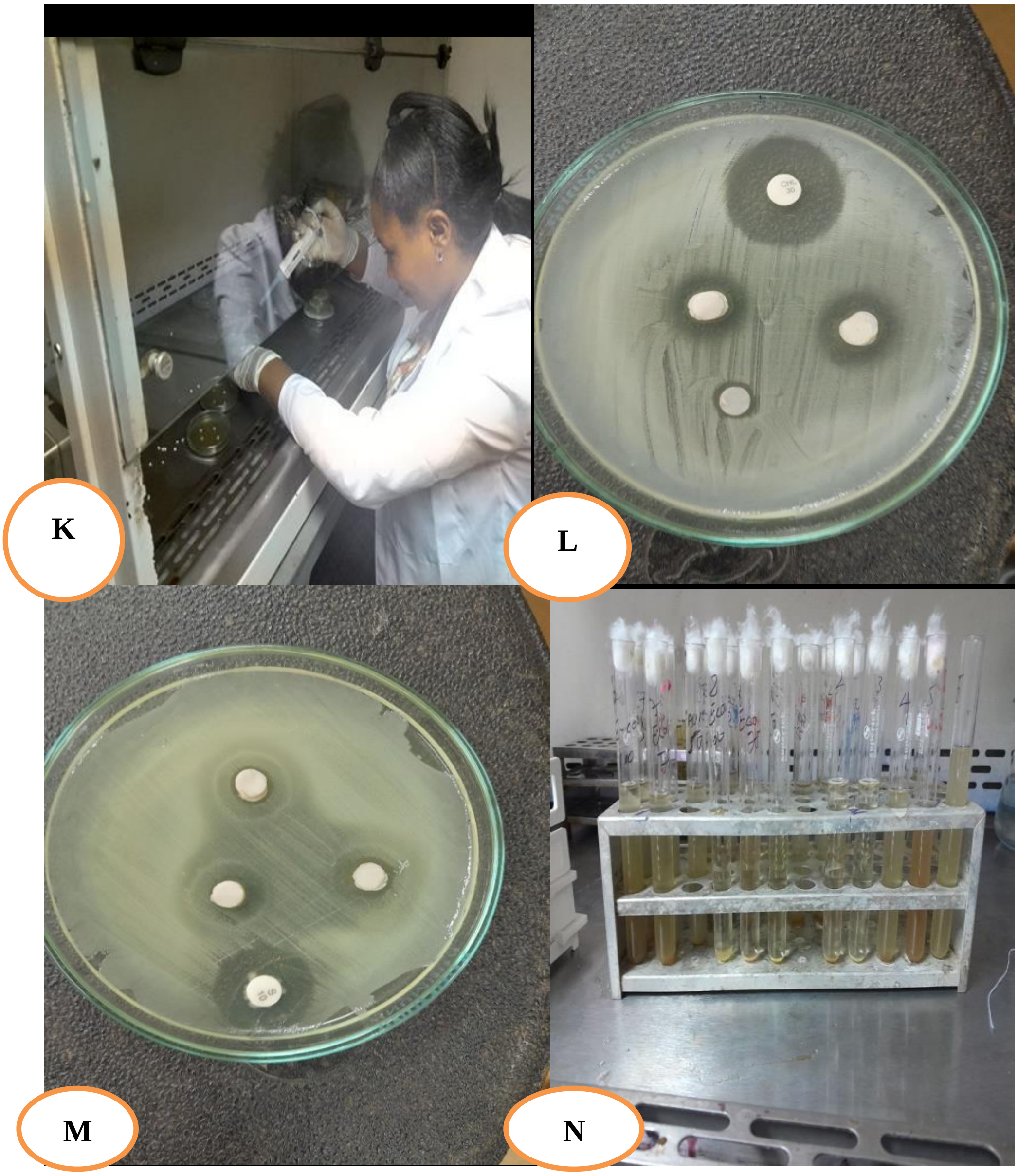
C) A standard curve of Beta carotene



2. Some photos taken during the laboratory analysis




G
H
I
J



A, preparation of honey for further analysis; B, alkaloid content of honey; C and D, *2, 2-diphenyl -1- picryhdrazyl* solution preparation and mixing with extract of honey sample; E, F, and G, during spectrophotometer reading of honey parameters; H and I, doing titration for vitamin C determination and J, K, L, M and N during antibacterial determination.