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Honeybees' foraging patterns and their relation to honey antimicrobial activity

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HONEYBEES' FORAGING PATTERNS AND THEIR RELATION TO HONEY ANTIMICROBIAL ACTIVITY

Isack Frank Rikohe

A Dissertation submitted in partial fulfillment of the requirements for the Master's in Biodiversity and Ecosystem Management of the Nelson Mandela African Institution of Science and Technology
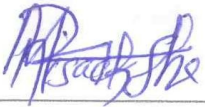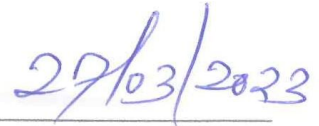
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ABSTRACT

Honeybees' existence is highly influenced by the availability of their preferred foraging plants. This study assessed honeybees' foraging patterns and their relationship to honey antimicrobial activity in Same District-Kilimanjaro, during the short and long rainy seasons of 2021/2022. The quadrats of 5 x 5 m (shrubs and forbs) nested with 1x1 m (grasses) were established along four transects of 5 km distance each to assess plant diversity and foraging patterns. The agar well diffusion method was employed for the antimicrobial assay. There was a significant difference in plant diversity between the rainy seasons ($t = 2.60$, $p = 0.01$ and $t = 2.27$, $p = 0.03$). *Grewia bicolor, Terminalia brownii, Ziziphus mucronata, Combretum schumannii,* and *Cordia monoica* were the most visited plants by 2761, 2528, 1966, 1163, and 662 visits, during the short rain season. During the long rainy season, *Acacia mellifera*, *Hoslundia opposita*, *Ocimum bacilicum*, and *Acalypha fruticosa* were the most visited by 1638, 788, 340, and 38 visits. Honey harvested during the short rain season had higher antimicrobial activities with zones of inhibition ranging between 10 mm - 19 mm. Besides, the most susceptible microorganisms were *Escherichia coli* and *Staphylococcus aureus*. The plant leaf extracts of *T. brownii*, *C. schumannii,* and *H. opposita* exhibited higher antimicrobial activities against tested microorganisms. Significant differences were observed in antimicrobial activities among honey ($F = 28.5$, $p = <0.001$) and plant extracts ($F = 15.9$, $p < 0.001$). A strong correlation was observed in antimicrobial activities between honey harvested during the short rainy season with *T. brownii* ($r = 0.836$, $p = 0.078$) and *C. monoica* ($r = 0.732$, $p = 0.159$). Honeybees' foraging patterns vary among the bloomed plant species across the rainy seasons; thus, honey's antimicrobial potential is highly influenced by floral sources and the harvesting season.

DECLARATION

I, Isack Frank Rikohe, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and that it has never been submitted nor been concomitantly submitted for the degree for degree award in any other institution.
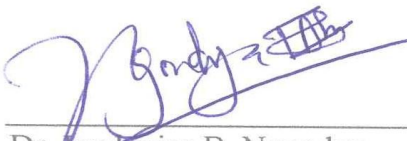
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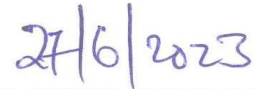Isack Frank Rikohe   Date

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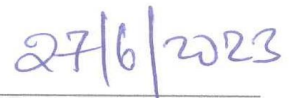Dr. Issakwisa B. Ngondya   Date

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CERTIFICATION

The undersigned certify that they have read the dissertation titled "*Honeybees' Foraging Patterns and their Relation to Honey Antimicrobial Activity*" and recommend for examination in Partial fulfillment for the requirements for the award of Master's in Biodiversity and Ecosystem Management of the Nelson Mandela African Institution of Science and Technology.

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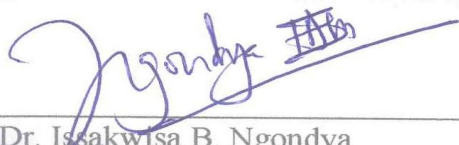Dr. Stephano H. Mlozi   Date

ACKNOWLEDGMENTS

DEDICATION

I dedicate this work to my parents, my mother, Ms. Rhoda Manyerere Matuma, my father, the late Mr. Rikohe Marwa Mbahi, and my family for their contribution, courage, and everlasting support from them to make this happen.

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LIST OF ABBREVIATIONS AND SYMBOLS

μL	Microliter
ATCC	American Type Culture Collection
DMSO	Dimethyl sulfoxide
Km	kilometer
MHA	Muller Hinton Agar
MIC	Minimum Inhibitory Concentration
mL	Milliliter
Mm	Millimeter
N	Total number of individuals in a population
NM-AIST	Nelson Mandela African Institution of Science and Technology
PDA	Potato Dextrose Agar
PS	Plant species
PSI	Pounds per square inch
ZI	Zones of inhibitions

CHAPTER ONE

INTRODUCTION

1.1   Background of the Problem

Honeybees (*Apis mellifera*) are eusocial insects of the genus Apis distributed worldwide (Quezada-Euán *et al*., 1996). Honeybees live in colonies while pursuing different activities based on age and sex (Sajwani *et al*., 2014). Globally, Honeybees has been very beneficial for its products and services to humans and ecosystems (Easton-Calabria *et al*., 2019). For example, they offer pollination services to angiosperm, which in turn ensure agricultural production and help to improve food security (Hung *et al*., 2018) and ecosystem health.

Honeybees customarily forage on various plant species, both flowering plants (nectar, pollen, and resins) and non-flowering plants (resins) around their home range (Aronne *et al*., 2012; Requier & Leonhardt, 2020). To secure more resources, honeybees can travel to different distances within the range of up to 7 km depending on fodders availability, quality and quantity, flower morphology, and nutritional requirements of the colony (Beekman & Ratnieks, 2000; Ghosh *et al*., 2020; Vere *et al*., 2017). Regardless of their dependence on plants delivered resources, honeybees show choices for certain plant species in a particular landscape (Aronne *et al*., 2012).

The plant materials that honeybees gather have a variety of purposes. For example, nectar, which is produced by specialized tissue called nectaries and is present in various plant parts, including flowers and leaves (Gotelli *et al*., 2017; Pacini *et al*., 2003), serves as the main raw material for the manufacture of honey (Sajwani *et al*., 2014). The composition of nectar is remarkably similar across all areas of production in a plant (Jones & Koptur, 2015; Manson *et al*., 2012). Thus, the composition of leaf extract and nectar from the same plant have been reported to share notable similarities in their secondary metabolites (Yamani *et al*., 2014). The plants foraged by honeybees have a significant relationship and contribute to honey's physicochemical and biological properties, including its antimicrobial properties (Balkanska *et al*., 2020; Mercan *et al*., 2007; Sherlock *et al*., 2010). Accordingly, plants with potential therapeutic properties foraged by honeybees account for the medicinal value of produced honey in the given area (Adgaba *et al*., 2020; Roby *et al*., 2020; Sherlock *et al*., 2010).

The information on flowering plants available in a particular area and the foraging pattern of honeybees towards those fodders aids their persistence (Mallinger & Prasifka, 2017). The knowledge of how foraged plants and the season of harvest affect honey's antimicrobial properties will be useful to the beekeeping industry because it can be applied both in the research area and

elsewhere. This study has highlighted essential honeybees' foraging patterns that shades a way for sustainable honeybee conservation in Same district northern Tanzania and providing information on the antimicrobial relation between honey and the most visited plant species across rain seasons.

1.2   Statement of the Problem

Microbial resistance of pathogenic microorganisms to synthetic antibiotics and antimycotics has increased scientists' interest in using natural products like honey as an alternative cure (Levy & Marshall, 2004; Mcloone *et al*., 2015; Molan, 2006). Honey has been used since ancient times as a medicinal product, and no bacteria have been reported so far as honey resistant (Dixon, 2003; Kuropatnicki *et al*., 2018). The floral sources that honeybees visit for foraging and the season of harvest has been demonstrated to have a significant relation to the antimicrobial properties of the produced honey (Balkanska *et al*., 2020; Mercan *et al*., 2007; Sherlock *et al*., 2010). For instance, in Tanzania, a study conducted by Luvanda and Lyimo (2018) on honey antimicrobial activity in the Central and Western zones, and the one conducted by Kakengi and Idani (2018) in Geita, Dodoma, Morogoro, Kisarawe, and Tabora revealed the variation in antimicrobial potential of produced honey across these geographical areas with different vegetations. Despite the fact that the floral sources and harvest season have a significant impact on the medicinal potential of produced honey, little is known about honeybees' foraging patterns in Northern Tanzania during the short and long rain seasons as well as how the foraged plants relate to honey's antimicrobial properties.

1.3   Rationale of the Study

Most studies on the antibacterial properties of honey reveal a strong correlation between plant sources and their impact on honey antimicrobial activity (Balkanska *et al*., 2020; Mercan *et al*., 2007; Sherlock *et al*., 2010). The reported variation is attributed to the fact that plants from which honeybees collect nectar and convert it into honey differ in their nectar composition, including secondary metabolites, which are as well influenced by geographical factors, seasonality, and other environmental-related factors (Prinsloo, 2018; Rodrigo *et al*., 2017). The foraging pattern of honeybees also differs accordingly to certain plants among the bloomed species across the seasons (Aronne *et al*., 2012; Ghosh *et al*., 2020; Urbanowicz *et al*., 2020). This situation, therefore, necessitates research in other potential beekeeping areas where honey can be produced in large quantities.

1.4 Research Objectives

1.4.1 General Objective

This study aimed to assess honeybees' (*Apis mellifera)* foraging patterns and evaluate its relation to honey antimicrobial potential in Same district, northern Tanzania.

1.4.2 Specific Objectives

To achieve the general objective, the study had three specific objectives as follows:

(i)   To determine flowering plants (grasses, forbs, and shrubs) diversity and honeybees' foraging pattern of available flowering plants around selected areas in Same district.

(ii)   To determine the antimicrobial activity of leaf extracts from honeybees' most visited flowering plants available at the study period from selected areas in Same district against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Candida albicans*.

(iii)   To determine the antimicrobial activity of honey samples collected from selected areas in Same district against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi,* and *Candida albicans*.

1.5 Research Questions

(i)   What is the diversity of flowering plants (shrubs, forbs, grasses) and the most visited flowering plants by honeybees in Same district?

(ii)   What is the antimicrobial activity of the preferred flowering plant's leaf extract?

(iii)   What is the antimicrobial activity of honey samples collected from the study sites?

1.6 Significance of the Study

This study highlights potential information and knowledge on honeybees' foraging pattern towards forages availability, which is advantageous to both the conservation of potential plants and bees that are essential for pollinating most crops and flowering plants (Hung *et al*., 2018; Keshlaf, 2014); the knowledge and information can be employed by the stakeholders to ensure a steady supply of forages to bees and guarantees their persistence. The findings of this study provide alternative treatment options using affordable and accessible natural products that reduce the

effects of the overuse of resisted synthetic antibiotics. The study provides helpful information on the floral source of honey, which is vital in local honey markets and hence improves the livelihood of beekeepers and the community at large. Also, the findings from this study can be used to map potential areas for beekeeping due to documentation of plants favored by honeybees.

1.7 Delineation of the Study

The study had some limitations firstly, the observation method used for data collection (foraging preference) may not have been able to capture all plant species visited by honeybees; therefore, methods such as DNA Metabarcoding and Melissopalynology can be used in future studies. Secondly, the study revealed the antimicrobial relationship between plants and produced honey in a particular season but did not determine which plant compounds exactly contribute to honey's antimicrobial activities, so individual plant Phyto-compounds may be determined in future studies. The study identified plant diversity and honeybee visits to those plants across the rainy seasons, but it did not focus on the effects of these seasons on honey quality and the health of the honeybee colonies, this can be explored further.

CHAPTER TWO

LITERATURE REVIEW

2.1   Overview of Foraging Ecology

Foraging by honeybees (*Apis mellifera*) is an important activity performed by worker bees aged above 21 days (Sajwani *et al*., 2014). Foraging guarantee colony survival by providing all the necessary nutrients per the colony's needs. Most foraged plants' materials are nectar, pollen, and resins (Sajwani *et al*., 2014). These plant-based resources are used by honeybees as essential raw materials in the production of hive products. For example, nectar is collected and used as the main raw material in the production of honey (Berenbaum & Calla, 2021); the composition of nectar helps attract pollinators to visit flowers (Aizenberg-Gershtein *et al*., 2013). Pollen is another essential plant material that worker honeybees collect from anthers of plant flowers; at the same time, they facilitate plant reproduction through pollination (Dukku, 2013; Kumar & Sharma, 2016). Honeybees rely on plant materials for survival and forage on various plants around their home range (Aronne *et al*., 2012); they travel different distances influenced by numeral factors, including fodders' availability to secure the required nutrients and food resources (Beekman & Ratnieks, 2000; Vere *et al*., 2017). Even though they forage on a variety of plant species, honeybees prefer some plants over others (Hawkins *et al*., 2015). Foraging repetitions/consistency of honeybees to certain plant species in a landscape reveals the concept of preferences and choices for certain species (Aronne *et al*., 2012).

2.2   Factors Contributing to Foraging Preferences

Several factors trigger foraging preference in honeybees (Gonzalez *et al*., 1995; Martin, 2004). Accordingly, the quality and quantity of floral resources are mentioned as significant factors that contribute to or define the preference of honeybees for particular plants on the landscape (Ghosh *et al*., 2020). The number of flowers per plant is another factor that contributes to foraging preferences. Bees and other pollinators are attracted to plants that produce many flowers to avoid excess use of energy during flying (Akter *et al*., 2017). In addition, the flower morphology, and arrangement impacts on honeybees' foraging behavior (Vere *et al*., 2017). Plants with favorable morphology will be more visited and preferred (Mallinger & Prasifka, 2017). The location of flowers, colors, and nectar guides can also affect honeybee foraging behavior, as bees prefer easily visible and apparent plants (Koethe *et al*., 2020).

It's important to determine which plants in a certain area honeybees prefer to forage on. This is significant, especially now that both managed and wild honeybee colonies are disappearing worldwide (Guilbaud *et al*., 2014; Hawkins *et al*., 2015). In most cases, these colony losses and honeybees decline are contributed by habitat degradation (Goulson *et al*., 2015). Knowing the forages that honeybees prefer can also help to conserve these potentially beneficial insects for the ecosystem (Mallinger & Prasifka, 2017). In turn, we can benefit from the products and services they offer in agriculture and conservation (Hung *et al*., 2018). The knowledge on the plants preferred by honeybees will stimulate the development of apiculture sector by ensuring the availability of resources for bees, especially during the flower scarcity period in the beekeeping calendar (Kumar & Sharma, 2016). Regardless of whether the foraging preference is critical for the booming beekeeping sector and honeybees' conservation, there is still a knowledge gap, especially in the Northern Tanzania, on available plants and the foraging pattern of honeybees.

2.3 Honey Composition and its Associated Antimicrobial Property

Honey is among other products produced by honeybees of different species, primarily stinging bees of the genus Apis and stingless bees of the genus Meliponin (Rao *et al*., 2016). Honey is a sweet substance generated naturally by honeybees from collected plant nectar or secretion of a living plant, which is altered by combining with their secretion, deposit, dehydrating, and stored in a comb to let it ripen or mature (Ball, 2007; Hadagali & Chua, 2014).

Honey is mainly composed of carbohydrate and water (Santos-Buelga & González-Paramás, 2017). Simple sugars, fructose, and glucose are the main carbohydrates found in honey (Khan *et al*., 2018). These sugars in honey contribute about 80% of all honey components (Alvarez-Suarez, 2017). Water is a component of honey; for well-ripened honey, water varies from 15% to 20% (Muruke, 2014). Other components of honey are proteins, minerals, amino acids, and vitamins (Alvarez-Suarez, 2017). In addition, honey is made of a mixture of enzymes introduced directly by honeybees in collected nectar and other enzymes like acid phosphate and catalase found naturally in collected nectar (Aurongzeb & Azim, 2011; Berenbaum & Calla, 2021). These enzymes are responsible for different conversational and biological reactions in honey. Amylase, for example, turns starch into simple sugar; invertase, converts sucrose to fructose and glucose; and glucose oxidase converts simple sugar, which is glucose, to gluconic acid and hydrogen peroxide (Berenbaum & Calla, 2021; El-sound, 2012). Honey has different compositions in terms of quantity and quality, which are caused by a variety of factors such as the botanical origin of the resources collected by honeybees, the effects of geographical area, seasonality, handling, and

manipulation of honey by individual beekeepers, storage, and climatic conditions of a particular area (Bogdanov *et al*., 2008).

2.4   Biological Activity of Honey

Honey has been reported with anti-inflammatory, immunomodulatory, antioxidant, antibacterial, anti-mutagenic, and anticancer characteristics, providing nutritional and therapeutic benefits to humans (Ahmed & Othman, 2013; Estevinho *et al*., 2008; Hadagali & Chua, 2014; Khan *et al*., 2007). Honey has been used for many purposes since prehistoric days, including food, storage, and medicine (Kuropatnicki *et al*., 2018; Saikaly & Khachemoune, 2017). Its popularity grew significantly after discovering its antibacterial properties in 1892 (Molan, 2016). The properties and composition of honey that contribute to its antimicrobial activities are high sugar content, hydrogen peroxide, and gluconic acid produced after activating the glucose oxidase enzyme (Kwakman & Zaat, 2012). After activating this enzyme, especially during the dilution of honey, it stimulates the breakdown of glucose sugar in honey to form hydrogen peroxide and gluconic acid, which offers antiseptic and bactericidal properties to honey (Al-Waili *et al*., 2011; Hadagali & Chua, 2014). Low pH is another factor that plays a significant role in the healing property of honey (Kwakman & Zaat, 2012). The nectar collected by honeybees contains Phyto-compounds, including flavonoids and phenolic acids, which act as an anti-inflammatory agent in honey (Ahmed & Othman, 2013; Nolan *et al*., 2019). Moreover, the antimicrobial peptide bee defensin-1 is also attributed to honey's antimicrobial properties (Mcloone *et al*., 2016). Another compound is methyl glycogen, formed after the non-enzymatic reaction of Dihydroxyacetone (DHA), primarily found in plant resources (Kwakman *et al*., 2011; Nolan *et al*., 2019).

2.5   Medicinal Use and Health Benefits of Honey

Different researchers reported on the medicinal use of honey that existed thousands of years back (Kwakman *et al*., 2011; Malone & Tsai, 2016; Namias, 2003). Honey is administered in treating wounds (Hadagali & Chua, 2014). Wound treatment with honey has proven more effective than conventional treatment (Mandal & Mandal, 2011). Honey inhibits the formation of scars, especially after wound healing and inflammation reduction (Abdallah & Hamed, 2019). Honey potentially improves skin health (Mcloone *et al.,* 2016). It provides beneficial probiotic microorganisms in the human body when consumed (Al-Waili *et al*., 2011; Jia *et al*., 2020). Some beneficial microorganisms have antimicrobial effects on foreign pathogens that invade the human body. For example, it has been reported that the Bacillus species, named A2, was found in honey, inhibited growth, and had lethal effects against *Escherichia coli* (Jia *et al*., 2020).

Honey improves the human immune system (Ahmed & Othman, 2013). It also plays a significant role in kidney and liver health by protecting against tissue damage, this is attributed to the presence of reactive oxygen species (Khan *et al*., 2018). Honey facilitates the human reproductive system by adjusting the amount of sperm and serum testosterone formed with their associated ability to fertilize (Khan *et al*., 2018); also administered purposively in hepatitis, influenza, and tuberculosis treatment (Khan *et al*., 2018). Honey has significant uses in treating burns (Aurongzeb & Azim, 2011), it also inhibits the development of cancer and its associated process; this is attributed much to the antioxidant feature of honey (Ahmed & Othman, 2013; Othman, 2012).

2.6   Honey Healing Process and Mechanism for Antimicrobial Activity

Some healing mechanisms and processes of honey still need scientific research to fill the information gap, especially in most African countries where honey has been used and proven to treat various diseases in different societies (Mokaya *et al*., 2020). Honey's medicinal properties are likewise debatable, owing to its many variables and unusual actions (Kwakman & Zaat, 2012). Wound therapy with honey is the most explored topic; numerous studies have shown that honey significantly impacts the healing of wounds and its biological implications due to its properties and compositions (Malone & Tsai, 2016; Molan, 2006; Saikaly & Khachemoune, 2017; Surg, 1988). The hygroscopic nature of honey helps reduce the amount of fluid available in wood for microbial growth (Al-Waili *et al*., 2011), as it can adsorb and absorb moisture around its environment (Mandal & Mandal, 2011). This mechanism is also attributed to the fact that honey is composed of a high amount of sugar (Aggad, 2014) and initiates osmolality effects on microbes found in the wound environment (Adgaba *et al*., 2020), which finally compromise microbial growth but also death due to hyperosmolality effects (Abdallah & Hamed, 2019). High viscosity is another healing property of honey; this helps avoid microbial infections in wood and accelerates fast healing by acting as a barrier (Hadagali & Chua, 2014; Kwakman & Zaat, 2012). Hydrogen peroxide and gluconic acid also affect wound healing, as they provide an acidic condition that stresses microbial survival by providing toxicity effects (Al-Waili *et al*., 2011).

Honey also helps to reduce or inhibit malodor formation, which is reported to be only treated by honey (Hadagali & Chua, 2014). Hadagali and Chua (2014) described how wound malignancy occurs because of bacterial metabolic activity toward protein tissue and amino acids, which produces sulfur, ammonia, and amines as byproducts. Honey prevents the development of odor-causing substances by providing sugar to microorganisms instead of amino acid protein. Methyl gluconic also helps in the treatment especially of microbial infections (Kwakman *et al*., 2011); the process is ascribed to its capacity to alter the structure of bacteria's flagella and fimbriae, as well

as interfere with cell membrane function and cause shrinkage, which eventually leads to the death of the bacterium (Nolan *et al*., 2019). Furthermore, there is explicit information demand on using honey as medicine due to the need for more scientific and laboratory baseline to validate. Scientific studies on produced honey should be considered to boost therapeutic honey markets and approval for clinical application (Mcloone *et al*., 2016; Mokaya *et al*., 2020).

2.7   Overview of Medicinal Plants and their Value

The kingdom Plantae generally contains a variety of species from different families, which are used for different purposes, such as food, medicine, flavor, and cosmetics (Akinnibosun & Edionwe, 2016; Beltrán *et al*., 2018; Gedikoğlu *et al*., 2019). Medicinal use of plants has been recognized and practiced since the era of our ancestors (Arulmozhi *et al*., 2018). The world health organization also recognized the contribution of plants as medicine, which reported that over 80% of the global population depends on plants for medicine (Atef *et al*., 2019). The medicinal use of plants varies from place to place, depending on indigenous knowledge of plant species or plant availability in such areas. In human treatment, it is reported that different from synthetic medicines, plants have fewer toxicity effects on human health when administered (Atef *et al*., 2019).

Findings from other studies show the medicinal effects of plants on a variety of diseases. For instance, Elansary *et al*. (2017) reported the traditional use of Eucalyptus species in treating sore throats, wounds, hemorrhages, colds, and dysentery. Burman *et al*. (2018) reported the use of medicinal plants in the treatment of inflammation and improved human digestion system. They also help in cancer treatment by inhibiting division of cancer cells (Akinnibosun & Edionwe, 2016), and improve heart health (Nikmaram *et al*., 2018). Moreover, medicinal plants are administered for teeth infections, asthma, and rheumatism (Gedikoğlu *et al*., 2019). The plant medicinal values differ among species (Akinnibosun & Edionwe, 2016; Mokaya *et al*., 2020). The variation in the medicinal value of plants is because plants vary in the quality and amount of their phytochemical compounds. These phytocompounds are found and vary in plant parts which are responsible for offering medicinal properties (Mlozi *et al*., 2020). Phytochemicals or biological compounds are formed as a result of secondary metabolism by a plant (Manandhar *et al*., 2019). These compounds are carotenoids, alkaloids, tannins, phenolic acids, flavonoids, terpenoids, chlorophyll, and saponin (Atef *et al*., 2019; Burman *et al*., 2018; Silva-Beltrán *et al*., 2015).

2.8   Medicinal Plants with Their Influences on Honey Healing Properties

The results from several research on plants and honeybees have revealed the significant contribution of plants to the physical-chemical and biological properties of honeybees' products

(Adgaba *et al*., 2020; Aggad, 2014; Jn *et al*., 2014). This is because honeybees collect nectar, pollen, and resins from plants, and convert them into hive useful products such as honey, and propolis, so their life depends on plant-delivered resources (Sajwani *et al*., 2014). Therefore, if honeybees prefer to collect or forage resources from medicinal plants, honey, and other hive-associated products will have high medicinal value compared to non-medicinal plant resources (Jn *et al*., 2014). Knowing potential medicinal plants that honeybees forage on can considerably help to conserve those plants, which in turn helps to maintain the existence of our important pollinators while benefiting honeybees' products with high medical value.

CHAPTER THREE

MATERIALS AND METHODS

3.1   Study Site Description

The study areas are located in Same district, Northern Tanzania in two wards of Vumari (study area I) and Kisiwani (study area II). The wards where beekeeping activities are highly practiced alongside boundaries of Mkomazi National Park were selected as the study sites selection purposefully based on beekeeping activities and the location of the wards from Mkomazi National Park. Also, the decision to select the wards with the aforementioned considerations was made after survey to the potential areas and consultation with the Mkomazi National Park authority and Same District Council. Generaly, the district is bordered to the north by Mwanga District, northeast by Kenya, southeast by Tanga region, and to the west by Manyara region (Fig. 1). The area experiences annual rainfall ranging from 1000 to 2000 mm, divided into two seasons: a short rainy season between November and January and a long rainy season from February to May (Prins & Loth, 1988). The main economic activity in the study areas is agriculture, in which people are involved in both commercial and food production agriculture; besides agriculture, tourism is among other growing economic activities (Mwanyoka & Lopa, 2016). In addition, Mkomazi national park and other protected areas, such as Pare Mountains, Chome, and Shengena forest reserves, prompt tourism activities in the district.
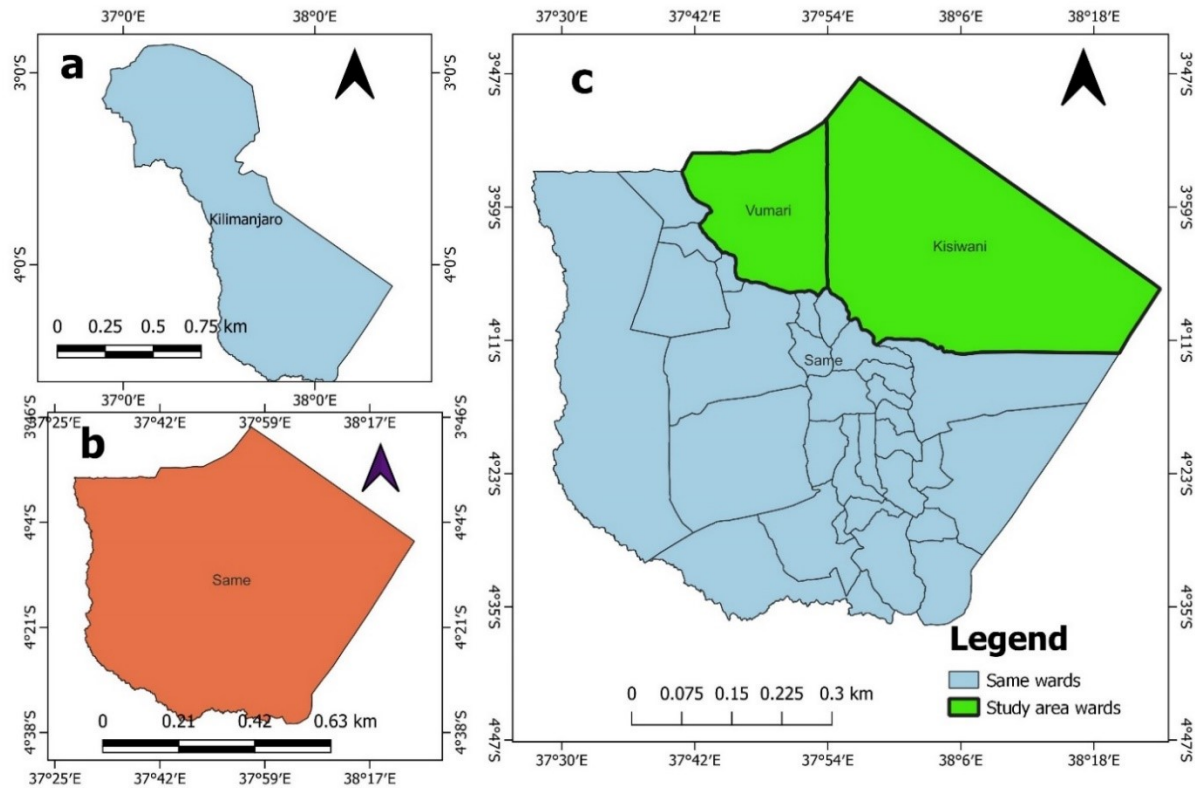
Figure 1: The diagrammatical representation of the study area, showing region (a), district (b), and wards (c) where the study was conducted in 2022

3.2 Assessment of Plant Diversity

Field observations were conducted to assess flowering plants' diversity and honeybees' foraging preferences during short (November 2021 - January 2022) and long (March - May 2022) rain seasons. The transect method was used for plant diversity assessment as per Ashton and Macintosh (2002), with minor modifications. Two study sites of about 40 Km apart with at least 30 occupied beehives were selected from Vumari and Kisiwani wards in Same district. In each site, two crosscutting transects of at least five (5) km each were established with beehives at the center; 20 points spaced at 0.5 km were established along the two transects. At each point, two quadrats of 5 m x 5 m (shrubs and forbs) nested with 1 m x 1 m (grasses) were systematically established on each side of the transect at 50 m from the transect to make 40 5 x 5 m and 40 1 x 1 m quadrats making a total of 80 quadrats (N = 80) at each study area per season. All shrubs, forbs, and grasses in these quadrats were identified and counted with the help of a botanist and field guide (Fig. 3). In this case, shrubs were defined regarding the growth form of multi-stemmed and height of less than 5 m (Opler *et al*., 1980; Zizka *et al*., 2014) (Fig. 2).
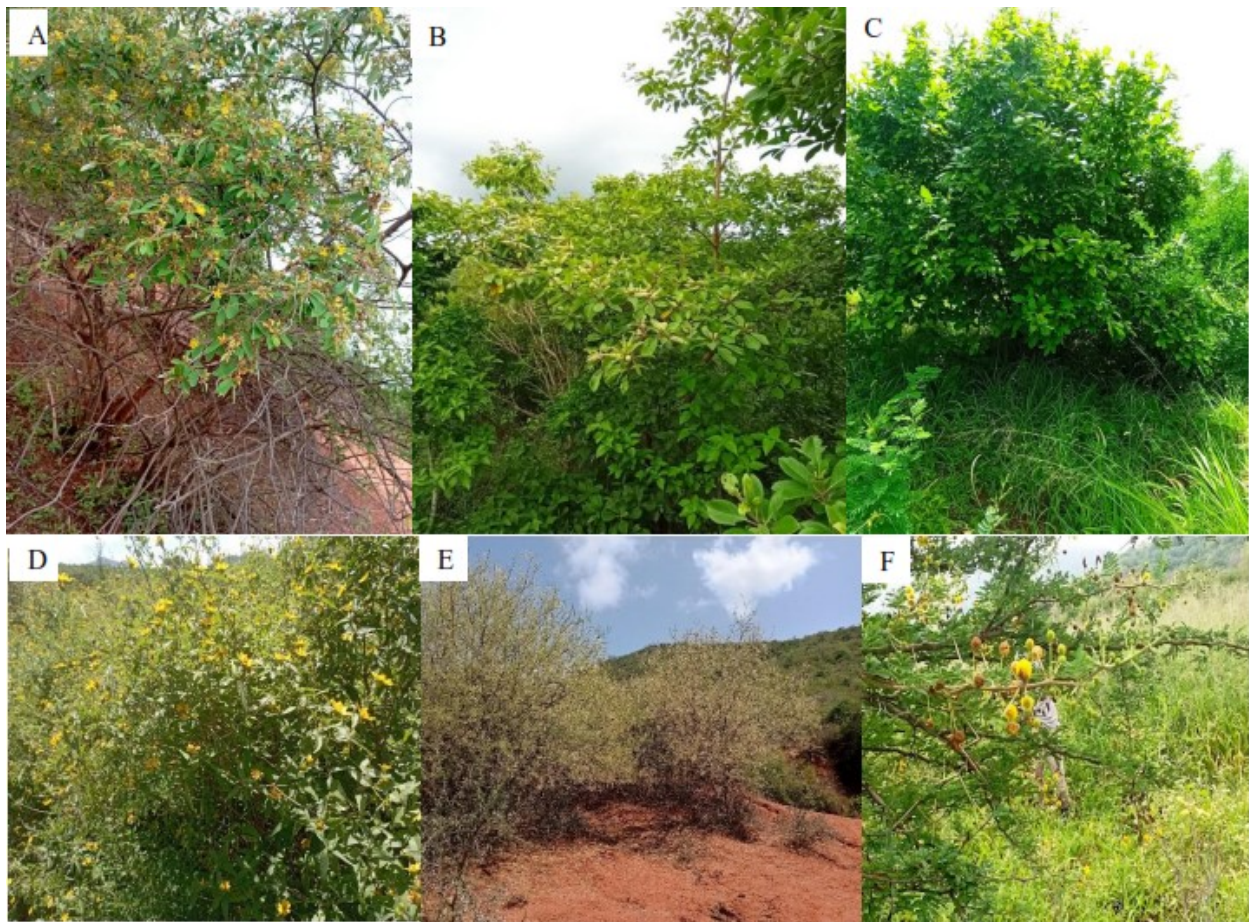
Figure 2: Some plants (shrubs) recorded during the study, **(A)** *Grewia bicolar*, **(B)** *Terminalia brownii* **(C)** *Ziziphus mucronata*, **(D)** *Aspilia mosambicensis*, **(E)** *Acacia mellifera*, **(F)** *Acacia nilotica*

Figure 3:   **Data collection in the study sites at various established quadrats during the short and long rain seasons of 2021–2022**

3.3   Assessment of Honeybees' Flower Visitations

The quadrats established for plant diversity assessment were used for honeybees' flower visitation by randomly selecting plots that contained plants with flowers during the study period (Fig. 4). The observation was conducted from 8:00 am to 11:00 am and 4:00 pm to 6:00 pm, which have been identified as the peak flower visitation hours for honeybees (Lázaro *et al*., 2013; Mallinger & Prasifka, 2017). The observation involved recording the number of honeybee visits per flower per time (Arroyo *et al.,* 1985). Four people were involved in counting the number of honeybees' visits to different plant flowers in a specific quadrat, the observation time at each quadrat of interest lasted for five (5) minutes (Abrol, 2006).

Figure 4: **Honeybees' visitations on flowers of different plant species; (A)** *Cordia monoica* **(B)** *Hoslundia opposita* **(C)** *Oxygonum sinuatum* **(D)** *Ocimum basilicum*

3.4   Plant Materials

The selection of plant species based on the most preferred plants by honeybees observed from the field; most of these plants were also reported in different studies as honeybees' preferred fodders in Northern Tanzania and elsewhere (Bareke & Addi, 2018; Mpondo *et al*., 2021). Fresh plant leaves of the selected seven plants, *Acacia mellifera*, *Ocimum sinuatum*, *Hoslundia opposita*, *Combretum schumannii*, *Grewia bicolor*, *Terminalia brownii*, and *Cordia monoica*, were collected directly from plants in two different study sites (S 03629791 E 9557445 and S 04.18873 E 038.03840) of Same district in Kilimanjaro. The voucher specimens of the plant materials collected are PS/NM-AIST/001, PS/NM-AIST/002, PS/NM-AIST/003, PS/NM-AIST/004, PS/NM-AIST/005, PS/NM-AIST/006, and PS/NM-AIST/007 that were deposited at NM-AIST. The collected fresh plant leaves were washed with distilled water and left for three weeks at room temperature to dry (Airaodion *et al*., 2019). The grinder was used to grind dried leaves to fine powder; the powder was then stored at room temperature before extraction.

3.5   Honey Sample

At the end of each rain season, the short rain season (January) and the long rain season (May); raw honey samples were harvested directly from five (5) randomly selected beehives at each study area. Honey samples were categorized according to season and area of harvest. Honey samples A and B were harvested from the same location (Vumali), similar to honey samples C and D (Kisiwani). The distance between the study areas (Vumali and Kisiwani) was about 40 Km apart. Honey samples A and D were harvested during the end of the short rain season, while the harvest for honey samples B and C was during the end of the long rain season. The collected honey samples from different honeybees' hives were filtered using double-sieve honey strainer filters and mixed to get one composite sample. Then the samples were stored in 50 mL falcon tubes and kept at a temperature of 20°C in the University of Dar es salaam food microbiology laboratory for antimicrobial assay.

3.6   Microorganisms and Sub-culturing

Five pathogenic microorganisms, including four bacteria and one fungus, were provided from the food microbiology laboratory at the University of Dar es salaam; the selected microorganisms were *Staphylococcus aureus* (ATCC 6538)*, Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 11775), *Salmonella typhi* (ATCC 14028), and *Candida albicans* (Laboratory isolate). The microorganisms were selected purposively to evaluate the antimicrobial potency of honey samples and plant extracts of honeybees' preferred fodders. The sub-culture was conducted where Potato Dextrose Agar (PDA) and Muller Hinton Agar (MHA) were used for fungi and bacteria, respectively.

3.7   Preparation of Inoculum

An overnight Nutrient agar/Potato dextrose agar culture of the test microorganisms was used to prepare the inocula. A loopful of cells from the stock cultures were transferred to test tubes containing Sabouraud dextrose broth for fungi and Nutrient broth for bacteria. The two were then incubated for 24 hours at 37°C and 25°C, respectively, without agitation to create the active cultures for the assays (Duraipandiyan *et al*., 2006) 0.2 mL of the culture was added to 5 mL of Sabouraud dextrose broth and nutrient broth, and it was then incubated until it attained the required turbidity of 0.5 McFarland solution at 600 nm and absorbance of 0.08 to 0.1, or 1.5×10^8 CFU/mL.

3.8 Plant Leaf Extraction

During the extraction of crude extracts, chromatographic methods were employed for all seven plant samples, as previously used by Mlozi *et al*. (2020), with minor modifications. Fine powder of plant leaves of 70 g were sequentially dissolved in 700 mL of petroleum ether, then ethanol. The mixture was shaken slightly and left for 48 hours at room temperature, followed by filtration, which was done using Whitman filter papers diameter of 125 mm. After filtration, the filtrates were subjected to a rotary evaporator at the temperature of 40°C, a speed of 100 RPM, to obtain crude extracts. Then the extracts were left at room temperature to allow evaporation of the remaining solvents. The dried crude extracts were stored at a temperature of 4°C for antimicrobial assay.

3.9 Preparation of Honey Samples and Crude Extracts for Antimicrobial Assay

During the preparation of the samples for antimicrobial assay, 100 mg of plant crude extracts were dissolved in 1 mL of Dimethyl sulfoxide (DMSO) to make a stock of 100 mg/mL, besides; for the honey sample, 90 mL of raw honey of each sample was dissolved in 10 mL of distilled water to make a stock of 90 % (vv). The vortex mixer was used to mix the crude extracts and honey samples, ensuring they dissolved completely in solvents. Then, the mixer was used for antimicrobial assay accordingly.

3.10 Antimicrobial Susceptibility Test Assay

In the antimicrobial assay, the agar well diffusion method was employed to test the antimicrobial activity of both plant extract and honey; at the same time, Fluconazole and Chloramphenicol were used as positive controls during experiments for fungi and bacteria, respectively.

3.11 Agar Well diffusion

The agar well diffusion method was used as described by Bello *et al*. (2022) with minor modifications Growth media (Muller Hinton Agar for bacteria and Potato Dextrose Agar for fungi) were prepared per manufacturer instruction, whereas 39 g of each nutrient agar was suspended separately in one litter of distilled water followed by slightly boiling to dissolve completely. The media were autoclaved at the temperature of 121°C and pressure of 15 pounds per square inch (psi) for 15 minutes and then allowed to cool in a sterilized fume hood chamber. Next, 20 mL of freshly sterilized prepared nutrient agars, Muller Hinton Agar, and Potato Dextrose Agar were added in a 9 cm diameter disposable Petri dishes and left for 5 minutes to solidify at room temperature. After the solidification of nutrient agar, inoculum prepared from 0.5 standard

McFarland for each microorganism was spread into the disposable Petri dishes using sterilized cotton swabs. During the preparation of the wells, stainless steel borer was used to punch the wells of 6 mm diameter. After the spread of microorganisms into Petri dishes with nutrient agar, a 50 µL for both plant extracts and honey samples was added to prepared wells, while 30 µL of fluconazole and chloramphenicol were added in separate wells in each plate as a positive control. The plant extracts, honey, fluconazole, and chloramphenicol were endorsed to defuse, followed by incubation for 24 hours at 37°C for bacterial strains and 48 hours at 27°C for fungi. This treatment of honey, plant extracts as well as fluconazole and chloramphenicol were done in triplicate. A transparent ruler calibrated in millimeters was used to measure the zone of inhibition's diameters. The three independent experiments were reported in mean and standard deviation.
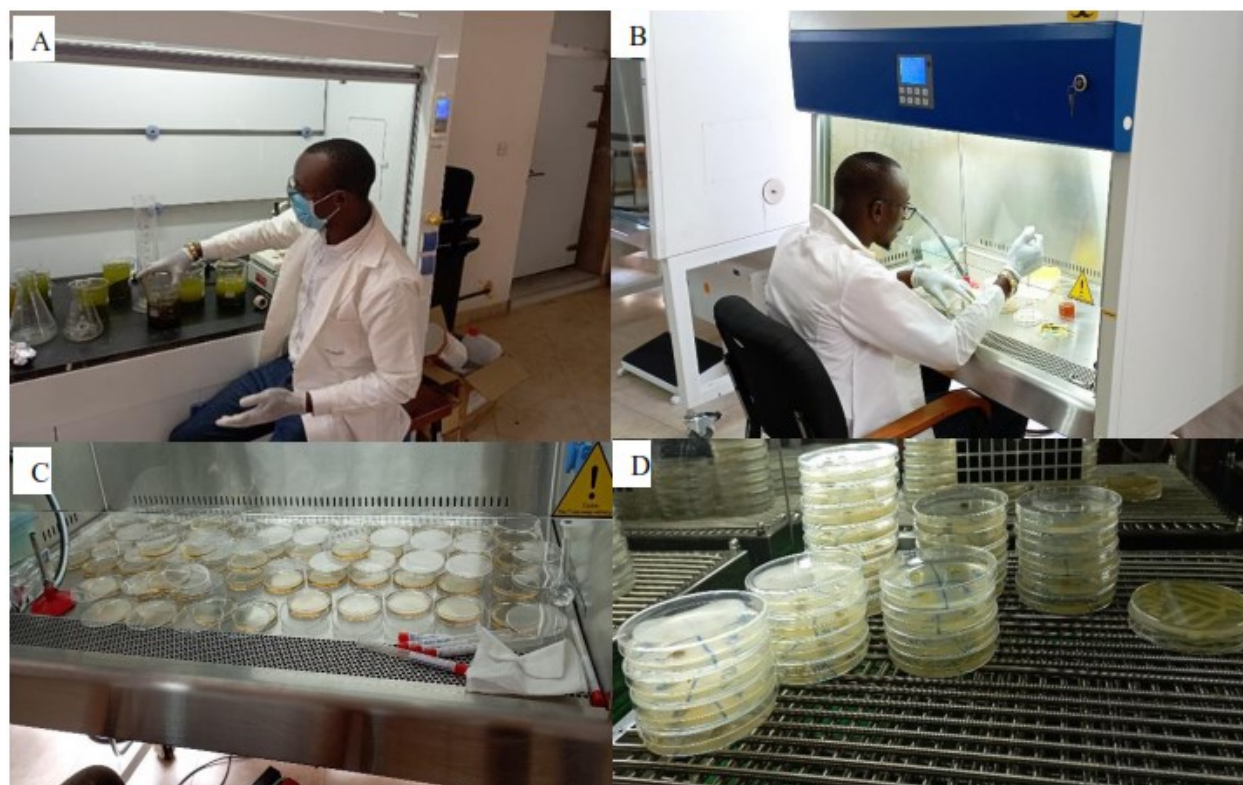
Figure 5: **Some laboratory procedures, (A) extraction procedure of plant leaf crude extracts, (B) treatment of the inoculated petri dishes with leaf extracts and honey samples, (C) Petri dishes with nutrient agars prior to inoculation and treatment, (D) Incubated Petri dishes of different microorganisms and treatments**

3.12 Statistical Analysis

For plant diversity, the Shannon-Wiener diversity index (Yeom & Kim, 2011) of plant species was calculated for each established quadrat. Shapiro–Wilk test for normality was performed on the generated indices. The independent sample t-test was used to determine if there was a significant difference in plant diversity between study areas within a particular season. The paired

sample t-test was used to determine if there was a significant difference in plant diversity in the same study area during different rain seasons. The statistical software used was R version 4.1.1 (2021), with a significance level $\alpha < 0.05$. In addition, for antimicrobial assay, the Shapiro-Wilk test was used to test for the normality of the data, and for normally and non-normally distributed data, One-way Analysis of Variance (ANOVA) and the Kruskal–Wallis tests were used, respectively. Pearson's correlation relation was employed to determine the relationship in antimicrobial activity between honey samples and plant species in different rain seasons. The statistical analysis software used was JAMOVI version 2.3.18 (2022), with significance set at $\alpha < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Plant Diversity and Foraging Patterns

4.1.1 General Observations for Plant Diversity and Foraging Patterns

In Vumari ward, 42 and 47 plant families were recorded in long and short rainy seasons, respectively; in contrast, for Kisiwani ward, a total of 52 and 41 plant families were recorded during short and long rainy seasons, respectively. During the short rainy season, the most dominant plant families in both study areas were *Poaceae, Malvaceae, Commelinaceae, Acanthaceae, Amaranthaceae, Polygonaceae,* and *Asteraceae* (Fig 6. A and B). Likewise, during the long rainy season, the most dominant plant families in both study areas were *Poaceae, Malvaceae, Acanthaceae, Commelinaceae, Amaranthaceae, Lamiaceae,* and *Asteraceae* (Fig. 6 C and D).
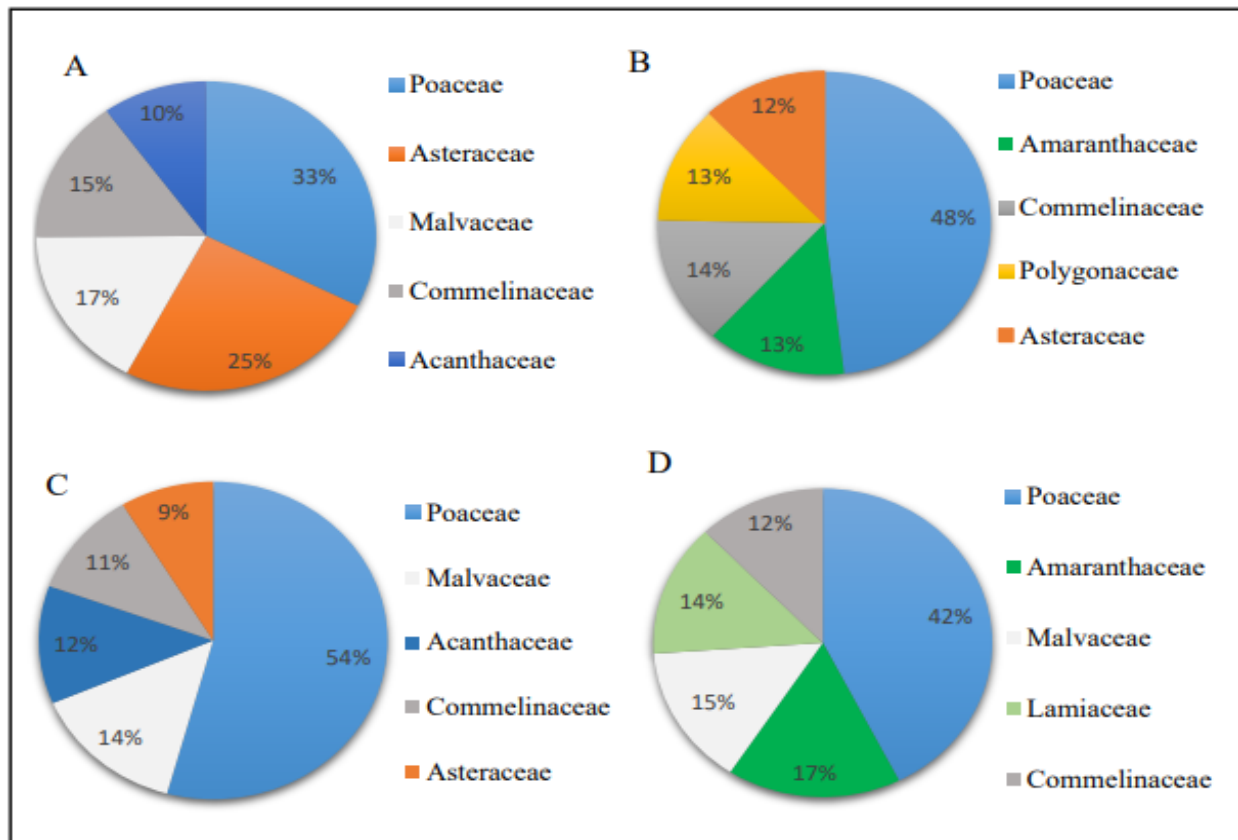
Figure 6: **The most dominant families in Vumari (A) and Kisiwani (B) during the short rain season and Vumari (C) and Kisiwani (D) during the long rain season of 2021/2022**

A total of 6638 and 7017 plant species abundances were recorded during the short rainy season in both Vumari (study area I) and Kisiwani (study area II) wards respectively. *Triumfetta rhomboidei, Bidens Pilosa, Cynodon dactylon, Commelina Africana, Commelina benghalensis, Crabbea velutina, Brachiaria deflexa, Oxygonum sinuatum, Achyranthes aspera* and *Acanthospermum hispidum* were the most dominant plant species (Fig. 7).
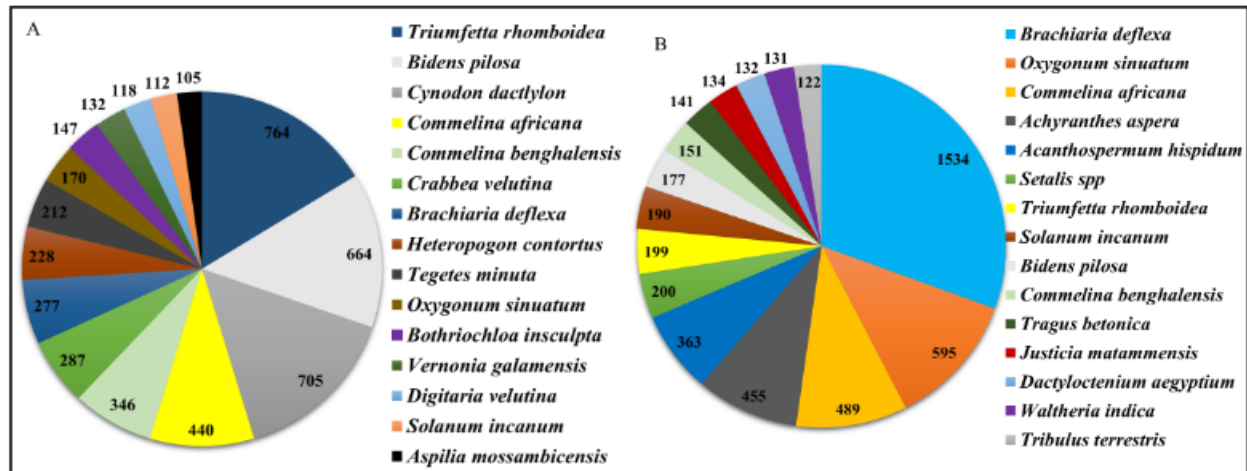
Figure 7:   **The most abundant plant species (top fifteen) in Vumari (A) and Kisiwani (B) during the short rain season**

During the long rainy season, a total of 16816 and 19790 species abundances were recorded in Vumari and Kisiwani wards respectively. Where, *Aristida kenyensis, Digitaria macroblephara, Triumfetta rhomboidea,* Heteropogon contortus, *Commelina benghalensis, Crabbea velutina, Eragrostis superba, Cynodon dactylon, Achyrathes aspera, Ocimum basilicum, Brachiaria deflexa, Bidens Pilosa* and *Cyathula arcantha* were the most dominant plant species (Fig. 8).
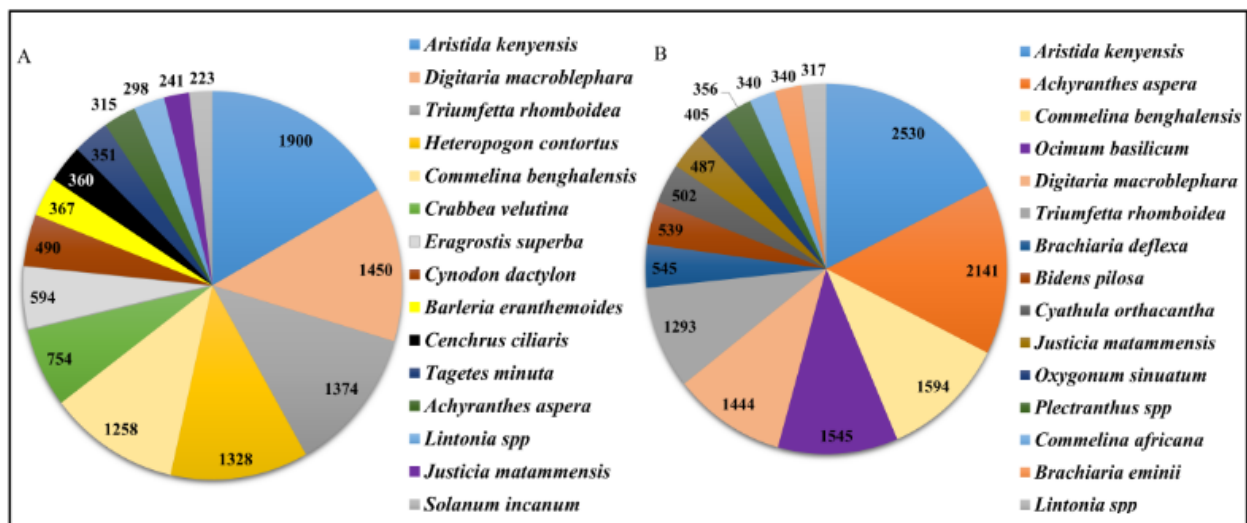
Figure 8:   **The most abundant plant species (top fifteen) in Vumari (A) and Kisiwani (B) during the long rain season**

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The most visited plant families in both study areas during the short rainy season were *Malvaceae, Combretaceae, Rhamnaceae, Lamiaceae, Asteraceae, Cordiaceae, Poaceae,* and *Polygonaceae* (Fig. 9 A and B). During the long rainy season, the most visited plant families in both study areas were *Fabaceae, Lamiaceae, Euphorbiaceae, Poaceae, Malvaceae,* and *Amaranthaceae* (Fig. 9 C and D).

Figure 9: **The most visited families in Vumari (A) and Kisiwani (B) during the short rain season and Vumari (C) and Kisiwani (D) during the long rain season of 2021/2022**

4.1.2 Plant Diversity in the Study Areas

While there were significant differences in plant diversity between short and long rainy seasons in both study areas (t = 2.60, p = 0.01, and t = 2.27 p = 0.03) (Table 1), no significant difference in plant diversity between study areas was observed during the same rain season for both short and long rain (t = 0.47, p = 0.64 and t = 0.58, p = 0.57, respectively). Plant species diversity in both study areas was higher during the long rainy season than in the short rainy season (Table 1).

Table 1: Plant diversity between short and long rain seasons of the year 2021/2022 in study areas

	Vumari		Kisiwani	
	Mean	SE of the mean	Mean	SE of the mean
Short rain	1.96	0.07	1.92	0.06
Long rain	2.09	0.05	2.04	0.06
T-test	$t = 2.60, p = 0.01$		$t = 2.27, p = 0.03$	

*SE= Standard Error

4.1.3 Honeybees' Flower Visitation in Study Areas During the Short and Long Rainy Seasons

While a total of 7902 and 4201 honeybees' visitations were recorded during the short rainy season of 2021/2022 in Vumari and Kisiwani, respectively (Table 2), a total of 2099 and 2568 visits were recorded in Vumari and Kisiwani, respectively, during the long rainy season (Table 3).

Table 2: Honeybees' flower visitations during the short rain season of 2021/2022

Vumari		Kisiwani	
Plant species	No. of visitations	Plant species	No. of visitations
Grewia bicolor	2761	*Combretum schumannii*	1163
Terminalia brownii	2528	*Grewia bicolor*	1082
Ziziphus mucronata	1966	*Cordia monoica*	662
Ocimum gratissimum	295	*Oxygonum sinuatum*	461
Aspilia mossambicensis	233	*Urochloa panicoides*	445
Oxygonum sinuatum	50	*Acacia nilotica*	118
Ocimum obovatum	30	*Clerodendrum spp*	89
Lantana camara	16	*Waltheria indica*	82
Commelina benghalensis	10	*Combretum hereroense*	39
Hypericum revolutum	4	*Justicia matammensis*	21
Eragrostis superba	3	*Brachiaria deflexa*	18
Justicia nyassana	3	*Justicia eranthemoides*	13
		Digera muricata	4
		Digitaria abyssinica	3
		Barleria eranthemoides	1

Table 3: Honeybees' flower visitations during the long rain season of 2021/2022

Vumari		Kisiwani	
Plant species	**No. of visitations**	**Plant species**	**No. of visitations**
Acacia mellifera	1638	*Acacia mellifera*	1527
Ocimum basilicum	340	*Hoslundia opposita*	788
Acalypha fruticosa	38	*Ocimum basilicum*	146
Heteropogon contortus	26	*Acacia nilotica*	77
Triumfetta rhomboidea	19	*Achyranthes aspera*	14
Vernonia galamensis	11	*Acalypha fruticosa*	7
Commelina benghalensis	11	*Indigofera brevicalyx*	5
Justicia matammensis	6	*Eragrostis superba*	3
Ocimum obovatum	3	*Triumfetta rhomboidea*	1
Panicum maximum	3		
Indigofera arrecta	3		
Gnidia eminii	1		

Grewia bicolor and *Combretum schumannii* were the most visited plants, with 2761 and 1163 visits, in Vumari and Kisiwani, during the short rain season (Fig. 10 A and B). On the other hand, during the long rain season, the most visited plants in the study areas were *Acacia mellifera and Hoslundia opposita* (Fig. 10 C and D).
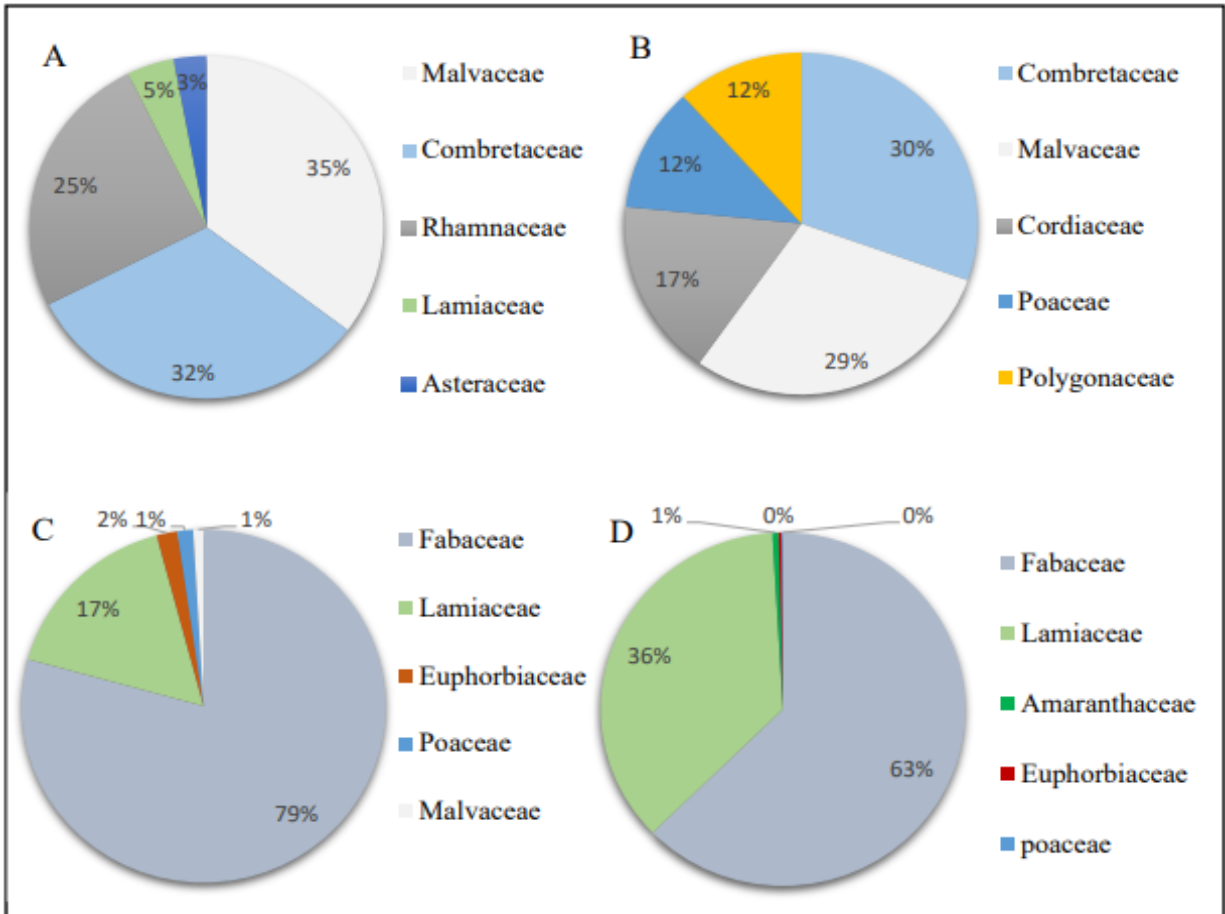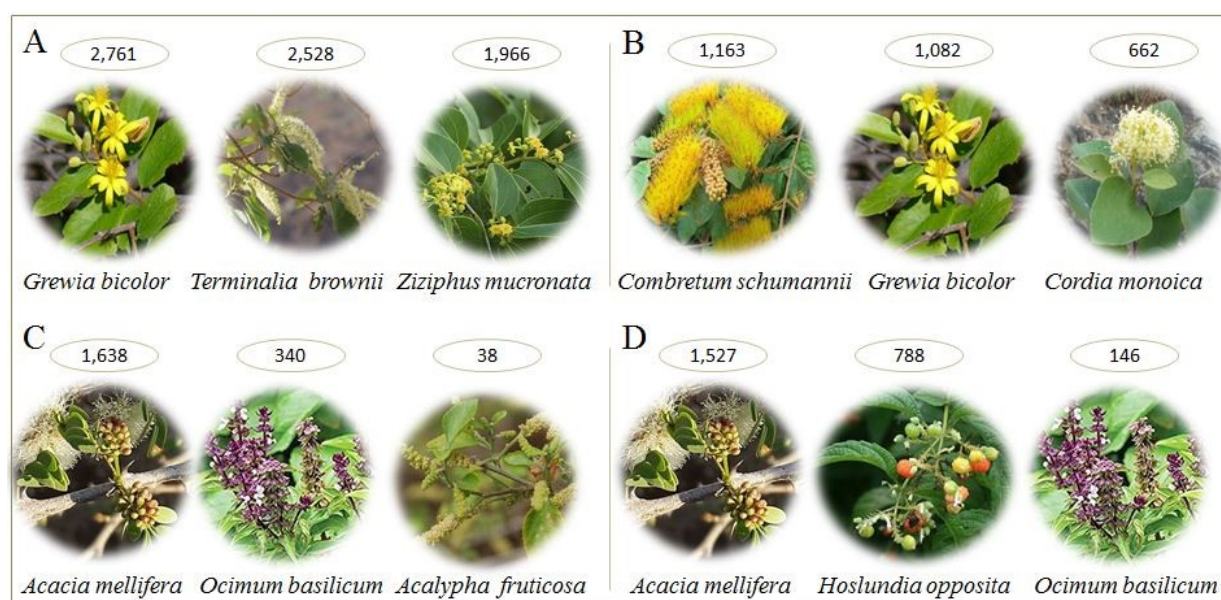

Figure 10: The most visited plant species in Vumari (A) and Kisiwani (B) during the short rain season and Vumari (C) and Kisiwani (D) during the long rain season of 2021/2022

The numbers above a picture represent the total number of honeybees visiting per plant

4.1.4 The Antimicrobial Activities of Honey Samples

The zones of inhibitions for different honey samples ranged from 10 mm to 19 mm (Table 4). Honey samples A and D exhibited higher antimicrobial activity against the tested microorganism than other samples (Table 4). The least honey sample inhibiting microorganisms' growth was sample B, with the lowest zone of inhibition for all tested microorganisms ranging from 10 mm to 11 mm (Fig. 11). The highest susceptible test microorganisms were *Escherichia coli* and *Staphylococcus aureus,* while the fungi *Candida albicans* was the least inhibited microorganism by all tested honey samples. Moreover, there was a significant difference in the antimicrobial activity of honey samples harvested in different rain seasons to pathogenic organisms (F=28.5 $p <$ 0.001).

Table 4: Antimicrobial activity of honey samples on different test pathogenic microorganisms

Honey Sample	Inhibition zone for different microbe (mm) Mean ± SD				
	Bacillus subtilis	*Candida albicans*	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*
A	12.0±0.0	11.0±0.0	11.7±1.2	17.3±1.2	12.7±1.2
B	10.3±0.6	10.0±0.0	10.7±0.6	11.0±1.0	10.0±0.0
C	14.7±1.2	10.3±0.6	14.3±0.6	15.0±0.0	12.3±0.6
D	14.3±0.6	11.7±0.6	15.7±0.6	19.0±0.0	13.0±0.0
Chloramphenicol/ Fluconazole	18.3±0.6	21.3±1.2	20.0±0.0	22.0±0.0	16.3±1.2

*SD- Standard deviation
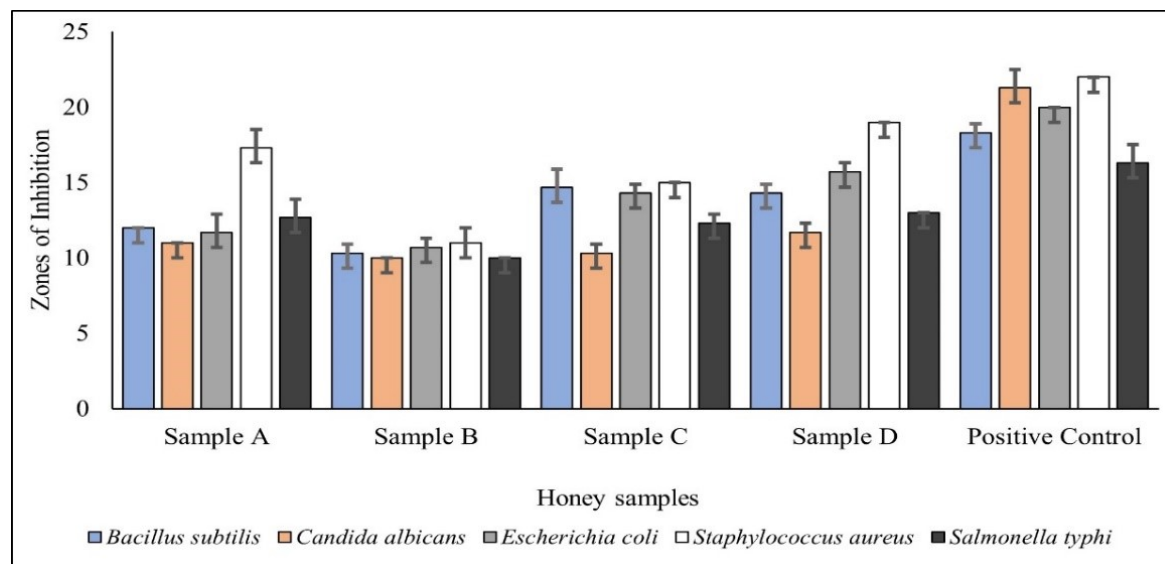
Figure 11: Zones of inhibition of honey samples against test pathogenic microorganisms

4.1.5   The Antimicrobial Activity of Plant Extracts

The ethanolic extracts exhibited higher antimicrobial activity than petroleum ether extracts for all the plant species assayed (Table 5). *Combretum schumannii*, *Hoslundia opposita,* and *Terminalia brownii* showed higher antimicrobial activity against test microorganisms than other plant extracts with inhibition zones ranging from 11.3 mm – 17.7 mm, 14 mm to 16.7 mm and 11.7 mm to 19 mm, respectively (Table 5, Fig. 6). While *Acacia mellifera* was the most diminutive plant in inhibiting microorganisms' growth, the zone of inhibitions ranged from 10 mm to 12 mm (Table 5, Fig. 12). Further, *Bacillus subtilis* and *Staphylococcus aureus* were the most susceptible microorganisms to tested plant extracts, followed by *Escherichia coli*. Whereas *Candida albicans* were the least inhibited microorganism with the lowest recorded zones of inhibitions (Table 5). There was a significant difference among plant extracts in antimicrobial activity against the test microorganisms (F= 15.9, p <0.001).

Table 5: Zone of Inhibition of plant extracts against tested pathogenic microorganisms

Plant extracts	Zones of Inhibitions (mm) of plant extracts against microorganisms, Mean ± SD				
	Bacillus subtilis	*Candida albicans*	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*
S1-EE	15.3±0.6	12.0±1.7	14.0±0.0	16.7±0.6	16.3±1.2
S2-EE	17.7±0.6	11.3±1.2	14.7±0.6	15.7±0.6	16.0±1.0
S3-EE	16.0±1.0	11.7±1.5	10.7±0.6	13.3±0.6	10.7±1.2
S4-EE	13.7±1.5	13.7±0.6	13.7±1.2	19.0±1.0	11.7±1.2
S5-EE	14.3±0.6	12.3±0.6	16.7±0.6	14.0±1.0	10.0±0.0
S6-EE	11.0±0.0	11.3±0.6	10.3±0.6	12.0±0.0	10.0±0.0
S7-EE	11.0±0.0	14.7±0.6	14.3±1.2	12.0±1.7	10.0±0.0
S1-PE	12.3±2.1	10.7±1.2	11.7±1.5	11.7±0.6	10.7±0.6
S2-PE	11.7±1.5	10.7±1.2	10.7±1.2	13.3±2.5	10.3±0.6
S3-PE	13.3±1.5	10.0±0.0	12.3±0.6	12.3±0.6	10.0±0.0
S4-PE	11.0±0.0	11.7±1.2	12.7±1.5	12.3±1.5	11.3±1.5
S5-PE	12.0±1.7	10.7±1.2	11.7±1.2	13.7±1.5	11.0±0.0
S6-PE	11.0±1.7	10.3±0.6	10.3±0.6	10.7±0.6	10.0±0.0
S7-PE	11.3±1.5	12.0±0.0	13.0±1.7	12.3±0.6	10.0±0.0
Chloramphenicol/ Fluconazole	18.3±0.6	21.3±1.2	20.0±0.0	22.0±0.0	16.3±1.2

*S1-*Hoslundia opposita*, **S2**-*Combretum schumannii*, **S3**-*Ocimum basilicum*, **S4**-*Terminalia brownii*, **S5**-*Grewia bicolor*, **S6**-*Acacia mellifera*, **S7**-*Cordia monoica*, **PE**- Petroleum ether extract, **EE**- Ethanolic extract, SD-standard deviation.

Figure 12: Zones of inhibition (ZI) of some plant extracts against the test microorganisms, where (a) T. brownii against B. subtilis (b) C. schumannii against B. subtilis, (c) H. oposita against B. subtilis

4.1.6 Comparison between Honey Samples and Plant Species in Different Rain Seasons

The honey samples A and D harvested at the end of the short rain season were compared with individual plants that flowered during the same rain season; *Combretum schumannii*, *Grewia bicolor*, *Cordia monoica,* and *Terminalia brownii* similar to samples B and C were compared with *Ocimum basilicum*, *Acacia mellifera,* and *Hoslundia opposita* for the long rainy season. There was a significant correlation in antimicrobial activity between honey sample A with *Terminalia brownii* (Pearson's r +0.756, *p* = 0.139) and *Cordia monoica* (Pearson's r +0.732, *p* = 0.159), while honey sample D strongly correlated with *Terminalia brownii* (Pearson's r +0.836, *p* =0.078), and *Cordia monoica* (Pearson's r +0.732, p =0.159) (Table 6). In addition, Table 7 shows the correlation relation of sample C with *Hoslundia opposita* (Pearson's r +0.660, *p* = 0.226).

Table 6:   **Pearson correlation in antimicrobial activity between plant extracts and honey samples**

Plant species	Honey sample A		Honey sample D	
	Pearson's r	*p-value*	Pearson's r	*p-value*
Combretum schumannii	+0.506	0.384	+0.487	0.405
Grewia bicolor	+0.586	0.299	+0.541	0.349
Terminalia brownii	+0.756	0.139	+0.836	0.078
Cordia monoica	+0.732	0.159	+0.732	0.159

Table 7:   **Pearson correlation in antimicrobial activity between plant extracts and honey samples**

Plant species	Honey sample B		Honey sample C	
	Pearson's r	*P* value	Pearson's r	*P* value
Ocimum basilicum	+0.203	0.744	+0.477	0.416
Hoslundia opposita	+0.424	0.477	+0.660	0.226
Acacia mellifera	+0.497	0.394	+0.170	0.785

4.2   Discussion

4.2.1 Plant Diversity and Foraging Pattern

Based on study findings, the family *Poaceae* was observed to dominate all study sites and had more species than other families during both rain seasons. Similar observations were reported in other studies conducted in Western Africa, Eastern Africa, Northern and Southern America (Bao *et al*., 2018; Moges *et al*., 2017; Peterson, 2013; Zerbo *et al*., 2016). In Northern Tanzania, studies conducted by Barboni (2014), Mseja *et al*. (2020), and Courtney-Mustaphi *et al*. (2021) likewise reported the dominance of the family *Poaceae*. *Poaceae* is the most prominent plant family in different landscapes; it comprises many plant species with historical and evolutionary dominance characteristics, covering over 40% of all plant species globally (Strömberg, 2011). The dominance of this plant family could be attributed to several factors (Aboulaich *et al*., 2013), including the ability of the family members to grow and sustain minimum rainfall ranging from 50 mm per month and can survive in harsh environment (Linder *et al*., 2018; Prins & Loth, 1988); their allelopathic mechanisms favor their growth and colonization (Favaretto *et al*., 2018). Most plant species found in the family *Poaceae* are significant in the management of honeybees; for instance, they save as food sources by providing pollen resource (De França Alves & De Assis Ribeiro DosSantos, 2014; Pangestika *et al*., 2017). In addition, the family *Poaceae* plays a significant role in protecting and improving soil fertility by retaining soil moisture (Wang et al., 2012) and preventing soil erosion (Fullen, 1998), which favors the survival of other plant species that are important for honeybees' survival.

Other plant families were further observed, which are essential in beekeeping, and help conserve honeybees by providing food and shelter. For instance, the family *Asteraceae* saves the honeybees as a source of nectar and resin used in making honey and propolis (Çelemli & Sorkun, 2012). Plants from the families *Amaranthaceae, Malvaceae, Lamiaceae, Polygonaceae, Acanthaceae* and *Commelinaceae* have also been reported as potential sources of honeybees' resources (Addi & Bareke, 2019; Akunne *et al*., 2016; Frankie *et al*., 2005). Therefore, it is essential for areas with such plants that play a significant role in beekeeping to be conserved so that they can help to ensure sustainable beekeeping for both honeybees' conservation and societal development through revenue generated from honey and other beekeeping-related products.

4.2.2 Plant Diversity

The results from the study indicate a difference in plant diversity in study areas across the rainy seasons. These findings are consistent with other studies done to assess plant diversity in different seasons (Hassler *et al*., 2010; Tonkin *et al*., 2017). This variation in plant diversity could be attributed to several factors, such as variations in rainfall and other environmental factors within and between these seasons (Hatfield & Prueger, 2015; Smith *et al*., 2016). Moreover, compared to the short rain season, the long rain season is often characterized by having consistent precipitation that favors the growth and development of various plant species and their composition (Aronson & Schmida, 1992; Knapp *et al*., 2002). The higher plant diversity during the long rainy season favors the persistence of honeybees due to increased food resources, it provides honeybees with a broader selection of essential resources such as nectar, pollen, and resin (Sutter *et al*., 2017). Decreased plant diversity in a landscape has been associated with several negative implications for honeybees and other potential pollinators' survival (Mensah *et al*., 2017). Given the rapid decline in managed and wild bee populations, there is a great demand for programs to improve plant diversity purposely for honeybee conservation (Donkersley, 2019; Goulson *et al.,* 2015). Results from this study alert beekeepers in Northern Tanzania and elsewhere that the long rain season is crucial in the beekeeping calendar as the season of the primary honey flow because of diverse plants, especially honeybee fodders bloom, compared to the short rain season.

Moreover, similarities in plant diversity were found in study areas within the same rainy season; similar results were reported previously (de Maçaneiro *et al*., 2016; Jiang *et al*., 2007). This similarity in plant diversity in the study areas within the same season could be due to several factors; for instance, de Carvalho *et al*. (2014) reported that soil physical-chemical characteristics

in certain areas could contribute to the similarity in plant diversity. In addition, the level of disturbances and conservation initiatives of areas determine plant species diversity (de Maçaneiro *et al*., 2016). Therefore, if different areas receive the same level of conservation, they will most likely have uniformity in plant diversity. Jiang *et al*. (2007) and Vasconcelos *et al*. (2020) reported that the altitude of different areas, evolutionary lines, and genetic factors also have a countable impact on similarity or variation in plant species diversity across different areas. Therefore, having similar plant diversity across the landscape in the same season facilitates easy adaptation of honeybees to areas within a landscape, especially for migratory beekeeping, which in turn ensures continued production of honeybees' products for both revenue accruing and honeybees' conservation, as their survival depends on the plants (Carreck *et al*., 1997; Rodolfo & Irene, 2009).

4.2.3 Honeybees' Flower Visitation and Foraging Patterns

It was found that honeybees highly visited some plant species over others. In both Vumari and Kisiwani wards during the rainy seasons, most of the highly visited plant species were less abundant; this phenomenon reveals the fact that bees' foraging pattern goes beyond individual plant's abundance (Irene, 2009; Sinu & Bronstein, 2018; Williams *et al*., 2011), and that honeybee seeks out plants that have their preferred floral morphological features, and composition (Aronne *et al*., 2012; Hawkins *et al*., 2015). Similar findings were observed in other studies (Akter *et al*., 2017; Dafni *et al*., 1988; Lázaro & Totland, 2010; Sajwani *et al*., 2014). The increased honeybee flower visits observed on the reported plant species could be contributed to several factors, including the number of flowers per plant (Akter *et al*., 2017) and floral color (Kevan, 1972; Miller *et al*., 2011). Flowers with bright and ultraviolent colors have been reported to attract more pollinators (Kevan, 1972b; Miller *et al*., 2011). Besides, flowers' colors enhance the visibility of honeybees and other plant depending on insects (Whitney & Glover, 2007). In this study, it was found that most of the identified plants as most honeybees' foraged fodders had flowers with bright colors. In addition, the quality and quantity of nectar and other plant resources offered by plants to pollinators similarly contribute to plant choice by honeybees; this feature of plant resources varies among plant species (Abrol, 2006; Vere *et al*., 2017). Most of the visited plants found in this study were also reported in other studies conducted to have adequate quantity and quality of nectar and pollen that attract honeybees and plant-depending insects, respectively (Adgaba *et al*., 2017; Martins, 2004). The presence of amino acid (proline) in nectar triggers honeybees and other pollinators to visit and forage particular plants (Carter *et al*., 2006), of which some of the identified plants as most honeybees' visited plants reported by

several studies to contain a large amount of proline-amino acid in their nectar. For instance, (Elaloui *et al*., 2015) revealed the availability of high proline in the nectar content of *Acacia* species and *Ziziphus mucronata*. The large size of flowers and the architecture of identified plants contributed to an increase in foraging preference by honeybees (Whitney & Glover, 2007) reported that plants with large flowers or many congested small-sized flowers are more likely to be visited than others. The identified plants, especially from the family *Lamiaceae,* contain smell-producing volatile compounds that stimulate honeybees to visit them (Díaz-Maroto *et al*., 2004; Pichersky & Gershenzon, 2002). These features determine honeybees' foraging pattern and solve the tradeoff of which plant to visit, especially when most plants bloom simultaneously (Butler & Station, 1944). Plants' features and characteristics that attract pollinators benefit plants and honeybees as well (Härtel & Steffan-Dewenter, 2014; Hung *et al*., 2018).

4.2.4 Antimicrobial Assay

The results from this study indicated that all assayed honey samples have potential bactericidal and fungicidal activities against the selected pathogenic microorganisms. These findings were similar to other studies conducted elsewhere and exhibited the antimicrobial activities of honey against ranges of pathogenic drug-resistant microorganisms in both *in vitro* and clinical trials (Khalil *et al*., 2014; Sherlock *et al*., 2010; Smaropoulos & Cremers, 2020). However, the antimicrobial activity of honey varies in different samples against tested microorganisms (Kwakman & Zaat, 2012; Wasihun & Kasa, 2016); honey samples harvested at the end of short rai season were found with higher antimicrobial activity to tested pathogenic microorganisms than that of the long rain. This variation in antimicrobial activities among honey is accounted by the difference in their chemical and biological properties that are highly determined by plant sources from which honeybees collect resources, which are used as primary raw material during honey production (Adgaba *et al*., 2020; Mandal & Mandal, 2011; Mensah *et al*., 2017). Regarding the honeybees-plant interaction, the medicinal properties of foraged plants have reported to influence and relate to that of produced honey (Roby *et al*., 2020). Similar observations were found in this study where there was a positive correlation in antimicrobial activities between honey samples and assayed extracts from honeybees' fodders which bloomed during the same season of harvest. Moreover, these plant species showed higher antimicrobial activities when assayed on similar pathogenic microorganisms, which could be due to their synthesized secondary metabolites, as also reported in other studies conducted (Engl *et al*., 2013; Salih *et al*., 2017). However, the results on variation in antimicrobial activities among plant species are in agreement with the findings from studies conducted elsewhere (Gonelimali *et al*., 2018; Masalu *et al*., 2020; Mujovo *et al*., 2008),

and the antimicrobial potentials of these plants assessed purposely to evaluate the antimicrobial correlation relation of them and that of honey samples regarding their respective seasonality.

It was found that honey samples harvested during the end of the short rain season had higher antimicrobial activity than those harvested at the end of the long rain season. These findings align with other studies evaluating honey antimicrobial activities across the seasons (Boanerges *et al*., 2013; Mahmood *et al*., 2021; Mandal & Mandal, 2011). This could be due to physiological responses by plants toward environmental stress during this period (Yang *et al*., 2018). The short rain season in the study areas was characterized by unpredictable and little rainfall that was stimulated by its semi-arid characteristics (Charles, 2013; Prins & Loth, 1988), which could have triggered competition for resources and survival amongst plants and led to the formulation of more and concentrated Phyto-compounds by plants as a competition avoidance strategy (Harborne, 1990; Yang *et al*., 2018). The higher formulated Phyto compounds by plants during this period could have contributed to the increased antimicrobial activity in honey.

On the other hand, the low antimicrobial activity observed on honey samples harvested at the end of the long rain season could be due to the dilution of nectar and the small content of Phyto-compound, which play a significant role in nectar antimicrobial potential (Lawson & Rands, 2019). Any alteration in nectar contents affects honey's chemical and biological properties (Mandal & Mandal, 2011). Likewise, seasonality influences the variation in different plant secondary metabolites (Prinsloo, 2018), and it has been reported that during the period of rain shortage, the diversity, concentration level, and complexity of the secondary metabolites produced by some plants are higher compared to long rain period (Rodrigo *et al*., 2017). Additionally, regardless of available plants with flowers in the long rainy season, regular and consistent rainfall during these days negatively affects the honeybees' foraging paradigm and flower visitation (Lawson & Rands, 2019), which in turn hinders honeybees from exploiting and benefiting from the available flowers' resources compared to the short rain season (He *et al*., 2016; Riessberger-Gallé & Crailsheim, 1997).

The susceptibility of the tested microorganisms to honey and plant extracts varied, in which some microbes were more susceptible to almost all honey and plant samples than others. These results are similar to findings from previous conducted studies (Abdallah & Hamed, 2019; Boorn *et al*., 2010; Đogo *et al.*, 2019; Lusby *et al*., 2005; Wasihun & Kasa, 2016). The discrepancies among tested microorganisms' responses to honey and plant extract samples might be attributed to the fact that microbes differ in their cellular organization (Elbanna *et al*., 2014; Wasihun & Kasa,

2016). Besides, the resistance of some microorganisms, especially the Candida species towards the antimicrobial substances/products could be due to their molecular mechanisms (Dymova *et al.*, 2021), including the antifungal resistance phenotypes found in different species of fungi (Niimi *et al.*, 2010).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Honeybees' existence and the sustainability of the beekeeping industry depend much on a healthier and diversified ecosystem. Identification and availability of honeybees' preferred plant species in an area trigger their existence and persistence. This necessitates establishing conservation initiatives and managing landscapes to ensure honeybees' fodder availability, which is crucial for the sustainable conservation of honeybees and the continued supply of their products and services.

Nonetheless, the study highlighted the variation in antimicrobial activities among honey harvested in different rain seasons where honey harvested in the short rainy season offers a higher antimicrobial potential than honey harvested in the long rainy season and that there is antimicrobial relation between honey and honeybees' plant forages. Accordingly, the antimicrobial ability of the honey depends much on the plant species where the honeybees were foraging. Therefore, beekeepers can include specific plants with medicinal content to increase the antimicrobial activity of the produced honey, which is advantageous in human health and increasing market potential of the produced honey.

5.2 Recommendations

Based on findings from this study, it is recommended that:

(i) The information on the botanical origin and harvest period should be included in honey labeling, particularly by stakeholders in the honey business. Given that floral sources and the season of harvest greatly influence the antimicrobial potential of harvested honey, doing so will aid consumers in deciding whether to use that honey, mostly for those who require honey for medicinal purposes.

(ii) The management initiatives should be emphasized to increase and maintain the abundance of the most preferred plant species in the study areas, as it was found that most of the frequently visited plant species were less abundant; conservation of such plants will in turn favor honeybee persistence, by ensuring the availability of forages that will consistently supply of their required resources.

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RESEARCH OUTPUTS

(i)   Publication

Rikohe, I. F., Mlozi, S. H., & Ngondya, I. B. (2023). Seasons and bee foraging plant species strongly influence honey antimicrobial activity. *Journal of Agriculture and Food Research*, 12 (2023), 100622.

(ii)   Poster Presentation